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Short Communication

Microwave-assisted alkaline modification of red seaweed galactans

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Abstract

The production of 3,6-anhydrogalactose units from galactose 6-sulfated residues by alkaline treatment is an important reaction undergone by many red seaweed galactans, used commercially to enhance their gelation behavior, and thus their industrial value. A novel technique utilizing microwave irradiation to carry out the alkaline modification of three structurally different galactans is described. The reaction is accelerated several times with respect to that performed with conventional heating. The effect of the sodium hydroxide concentration was investigated, showing a higher rate around a 2 M base concentration.

Keywords: Carrageenan; Agaran; Red seaweed; Microwave irradiation; Alkaline treatment

1. Introduction

Red seaweed galactans are widely used as texturizing, viscosity-building and gel-forming ingredients in food and pharmaceutical industry. They represent a multi-million dollar market that grows every year (Van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002). These galactans have structures based mainly on linear chains of alternating 3-linked β-D-galactopyranosyl residues and 4-linked α-galactopyranosyl residues. Whereas the β-linked residue always belongs to the d-series, the α-linked residues may be either D- or L-galactose units, partly occurring as 3,6-anhydrogalactopyranosyl moieties (Rees, 1969; Stortz & Cerezo, 2000). These polysaccharides are generally substituted with sulfate ester groups and other substituents. Depending on the configuration of the α-galactose units, red seaweed galactans were classified into carrageenans and agarans, whereas the whole sulfation pattern and the presence or absence of 3,6-anhydrogalactose permits further classifications (Rees, 1969). The last factor is also related with their industrial applicability: thus, non-gelling (or weak gel-forming) polysaccharides may be transformed into gelling ones by an alkaline modification which increases the proportion of 3,6-anhydrogalactose. This reaction occurs when 6-sulfated α-galactose units are present: by heating the polysaccharide in strong alkaline media, the 3-OH group is ionized, and produces an intramolecular nucleophilic displacement of the sulfate group in position 6 (Ciancia, Noseda, Matolewicz, & Cerezo, 1993; Rees, 1961) (Fig. 1). The reaction is highly specific, as no other sulfate group is affected (Rees, 1961; Stortz & Cerezo, 2000). This reaction is widely used in the lab (Ciancia et al., 1993; Navarro & Stortz, 2003), and at industrial level in order to improve the gelling properties of carrageenans (Ciancia, Matolewicz, & Cerezo, 1993; Stortz & Cerezo, 2000) and agarans (Freile-Pelegrín & Murano, 2005).

Previous work showed that conventional-heating alkaline modification follows pseudo first-order kinetics (Ciancia, Maulewicz, & Cerezo, 1997) with half-lives (at 80 °C, in 1 M NaOH) depending on the sulfation pattern of the β-galactose units: they ranged from a few minutes for 4-sulfated polysaccharides (Ciancia et al., 1993), to about 20–40 min in polysaccharides from the agar or carrageenan families containing non-sulfated β-galactose units (Noseda & Cerezo, 1995; Noseda, Viana, Duarte, & Cerezo, 2000), up to almost 3 h for 2-sulfated polysaccharides (Ciancia et al., 1993). An increase in either NaOH concentration or ionic strength accelerates the reaction (Ciancia et al., 1997).

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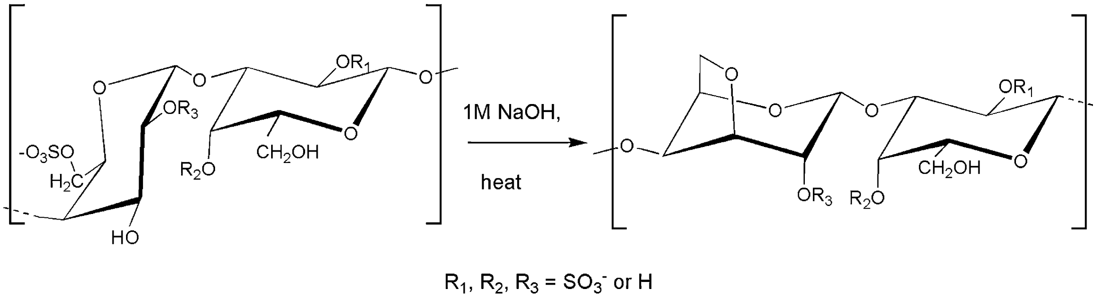

Fig. 1. Scheme of the reaction under study (applied to carrageenans).

In recent years an increasing number of papers reporting the use of microwave irradiation to speed up chemical reactions have appeared (Gabriel.et al., 1998; Lidström et al., 2001; Stadler et al., 2002)some of them applied to polysaccharides (Kunlan et al., 2001; Singh et al., 2003; Zhou et al., 2004). It was shown that the heating mechanisms are different in conventional and microwave ovens (Gabriel et al., 1998; Lidström et al., 2001). Recently, an extraction procedure for carrageenan aided by microwave heating was published (Uy, Easteal, Farid, Keam, & Conner, 2005). In the present work, we report a study of the microwave-assisted alkaline modification (with a domestic microwave oven) of three different red seaweed galactans, achieving a complete cyclization of 6-sulfated units very rapidly and with no signs of degradation.

2. Materials and methods

2.1. Microwave oven

A Sharp domestic microwave oven (model R353EA, 1200 W, operating at 2450 MHz) was used for all the experiments. Closed-vessel reactions were carried out in home-made threaded Teflon 50 ml tubes with screw caps, manufactured from 42 (tube) and 52 mm (cap) rods, with tube walls 5 mm thick. All the reactions were carried out at full microwave power, using the desired steps of microwave heating (15 s) followed by opening the vessel and cooling shortly in an ice-bath to relieve the pressure. When necessary (longer reactions), water was added in order to keep constant volumes and concentrations. In a separate experiment with porphyran, it was shown that four pulses of 15 s gave similar results to one pulse of 1 min, though the later procedure carries higher risk. With the current procedure, risks of accidents are minimized.

Temperature increase measurements were carried out using 15 s-microwave pulses on 10 ml solutions, starting at room temperature.

2.2. Polysaccharide materials

The cystocarpic and tetrasporic carrageenans from *Iridaea undulosa* were obtained as described elsewhere (Stortz & Cerezo, 1993). The porphyran was retrieved from the seaweed *Porphyra columbina* as reported (Navarro & Stortz, 2003;; Noseda et al., 2000). The polysaccharides were characterized by reported procedures (Stortz & Cerezo, 1993, 2000; Stortz, Bacon, Cherniak, & Cerezo, 1994). In order to proceed with the reactions, the polysaccharides were reduced with $NaBH_4$ and recovered by dialysis and lyophilization. In a typical reaction, 20 mg of reduced polysaccharide in 10 ml of NaOH of the desired concentration were allowed to react for the period indicated above. No additional sodium borohydride was added to the reaction vessel to avoid production of hydrogen inside the oven. The modified polysaccharides were recovered after dialysis (molecular weight cut-off 6000–8000) and lyophilization.

2.3. Following the course of the reaction

The tube was carefully opened, and aliquots were taken, neutralized and hydrolyzed in two steps (Jol, Neiss, Penninkhof, Rudolph, & De Ruiter, 1999;; Navarro & Stortz, 2003; Stevenson & Furneaux, 1991), in order to keep the acid-labile 3,6-anhydrogalactose units, and then derivatized to the alditol acetates. The proportion of 3,6-anhydrogalactose was determined by GLC using a Hewlett Packard 5890A apparatus equipped with a FID, with a Supelco SP 2330 capillary column (30×0.25 mm, thickness 0.20 μm) using a program starting at 200 °C, then at 2 °C/min up to 230 °C, and finally held at 230 °C. The injector and detector were set at 240 °C (Navarro & Stortz, 2003).

3. Results and discussion

The microwave response under alkaline conditions of three different red seaweed galactans was studied: (a) the cystocarpic carrageenans from *I. undulosa* (fraction C, Stortz & Cerezo, 1993), consisting of partly cyclized μ/ν-carrageenans (Stortz et al., 1994) which contain all of the β-galactose units sulfated on C-4; (b) the porphyran from *Porphyra columbina* (Navarro & Stortz, 2003;; Noseda et al., 2000), a 6-*O*-methoxylated agar-like polysaccharide with no sulfate on C-2- or C-4 of the β-galactose unit, and (c) the purified λ-carrageenan obtained from *I. undulosa*

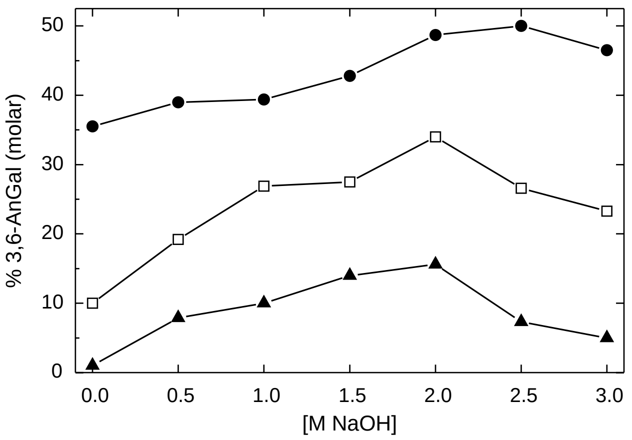

Fig. 2. 6-Anhydrogalactose generated by microwave heating at different concentrations of sodium hydroxide, at constant times of reaction. Key: (▲) λ-carrageenan T$_1$ after 60 s reaction; (□) porphyran after 30 s reaction, and (●) partly cyclized μ/ν-carrageenan C after 15 s reaction. The reaction times were regulated to achieve the production of 10–20% of additional 3,6-anhydrogalactose in the best conditions.

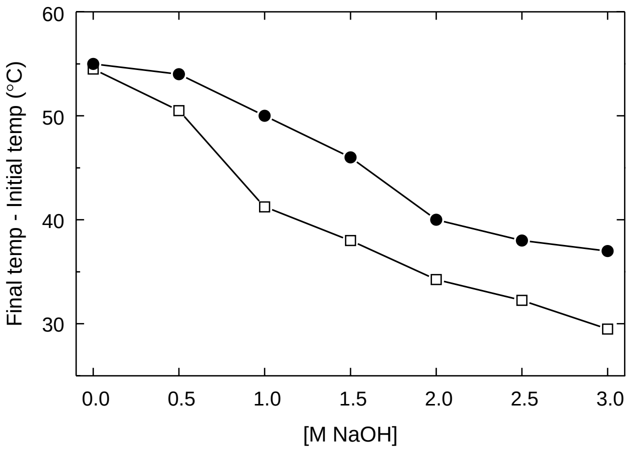

Fig. 3. Differences in temperature achieved by microwave heating solutions (10 ml) with different concentrations of sodium hydroxide, at constant times of reaction (30 s), without (□) and with (●) the addition of 10 mg of porphyran.

(fraction T$_1$, Stortz & Cerezo, 1993;; Stortz et al., 1994) which carries the β-galactose units sulfated on C-2.

Preliminary tests showed that a pre-reduction of the polysaccharide is needed to avoid side reactions (at least with porphyran), as sometimes occurs in the conventional reaction (Rees, 1961). They also showed that the alkaline modification reactions could be carried out with microwave heating in much less time than with conventional heating, though following the same rate order: 4-sulfated polysaccharides faster than those non-sulfated, and these faster than 2-sulfated ones (Ciancia et al., 1997).

The effect of the concentration of sodium hydroxide on reaction rates was studied for the three polysaccharides (Fig. 2). The speed of reaction increases all the way with alkali concentration up to 2 M, as occurs with conventional heating (Ciancia et al., 1997), but then the rate decreases with concentration of sodium hydroxide. This is probably a temperature effect: in a separate experiment (Fig. 3) we have observed that the temperature increase in microwave-treated aqueous solutions of sodium hydroxide is inversely proportional to their alkali concentration (at least above 0.5 M), either with or without the polysaccharide. The alkali effect is probably related with a shift in the relaxation time of water (Gabriel et al., 1998), not occurring for other neutral salts. The polysaccharide effect is probably caused by a decrease of the frequency necessary to cause dielectric dispersion because of the water bound to the polysaccharide chains (Gabriel et al., 1998).

The results (Fig. 2) show that a 2 M concentration of sodium hydroxide is optimal for the alkaline modification of all variants of red seaweed galactans. The reaction proceeds smoothly, giving rise to treated polysaccharides in nearly quantitative yields (Table 1). Fig. 4 shows the time effect of the treatment: for polysaccharides of the κ-family (sulfated on position 4- of β-galactose unit) after 30 s of reaction the maximum concentration of 3,6-anhydrogalactose is reached. For porphyran, about 1 min is needed, whereas for the more resilient λ-carrageenan (sulfated on position 2 of the β-galactose unit), cyclization can be considered completed only after 8 min. Taking into account the reported half-lives of the preceding polymers for conventional heating (Ciancia et al., 1993, 1997; Noseda & Cerezo, 1995; Noseda et al., 2000), and that at least three half-lives should be spent to complete the reaction, it can be considered that

Table 1
Microwave-assisted alkaline modification of several red seaweed galactans

	β-Gal sulfated on	Yield (%)	Final ratio A/G[a]	Microwave reaction time (min)	Half-life[b] (min)	Conventional reaction time (min)[c]
C[d]	C-4	82.9	1.00	0.5	4.5	120–180
Porphyran	–	96.4	0.90	1.0	23	180–300
λ-Carrageenan	C-2	87.6	0.85	8.0	117–170	960

[a] Ratio 3,6-anhydrogalactose/galactose in the final product. For porphyran, the ratio 3,6-AnGal/(Gal + 6-*O*-Me-Gal) is reported.

[b] Half-life by conventional heating at 80 °C in 1 M NaOH (Ciancia et al., 1993; Noseda et al., 2000).

[c] Usual reaction time by conventional heating (Navarro & Stortz, 2003).

[d] Partly cyclized μ/ν-carrageenan (Stortz & Cerezo, 1993).

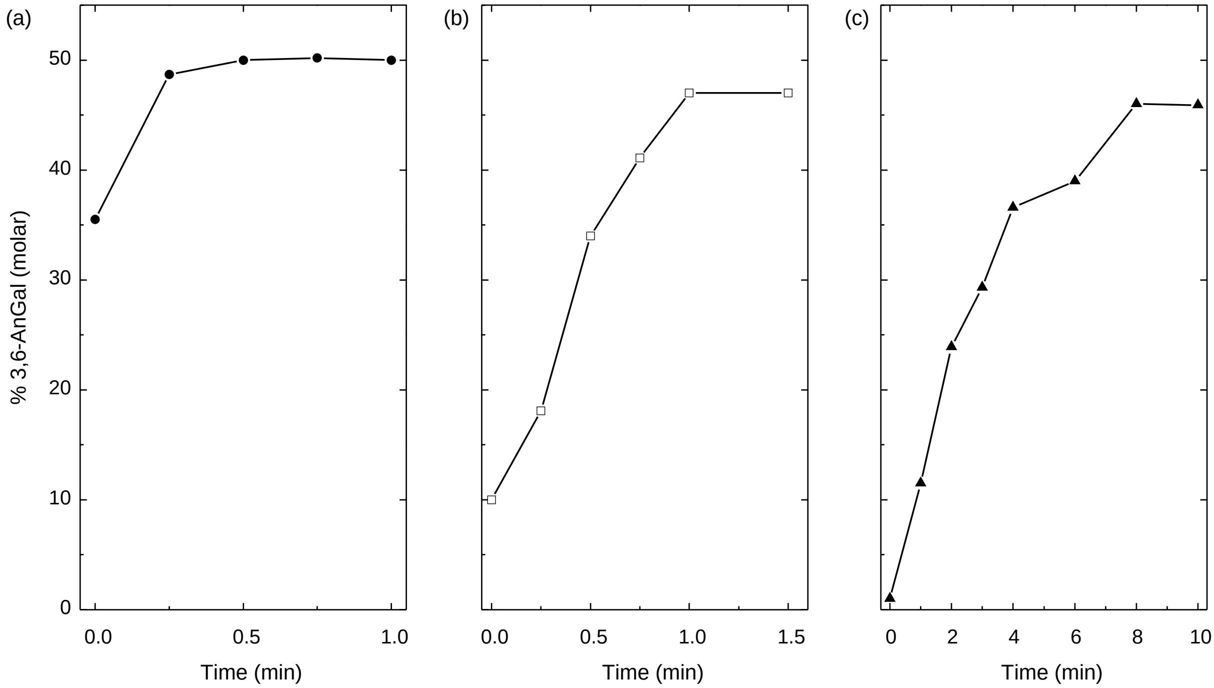

Fig. 4. 3, 6-Anhydrogalactose generated by microwave heating at different times of reaction using 2 M NaOH. (a) Partly cyclized μ/ν-carrageenan C, (b) porphyran, and (c) λ-carrageenan T_1.

microwave heating accelerates 30–60 times the rate of the reaction. Actually, the reaction can be speeded up 100–300 times (Table 1) with respect to the usual conditions of conventional heating (Navarro & Stortz, 2003).

The use of potassium hydroxide instead of its sodium counterpart gave the same results for porphyran. However, the use of potassium salts should be avoided for polysaccharides which can become gelling after the alkaline treatment (like μ/ν-carrageenans), as they may give rise to a separate phase (Stortz & Cerezo, 1993). The reaction of porphyran with 1 M sodium carbonate for 15 s gave a product with 16.3% of 3,6-anhydrogalactose, against 21.8% produced by the same concentration of sodium hydroxide. This diminished reactivity is consistent with the decreased basicity of sodium carbonate, in spite of the increase in ionic strength (Ciancia et al., 1997). Besides, the use of sodium carbonate generates pressure, thus endangering the whole reaction unless carried out for short periods.

The yields (Table 1) and analytical data of the polysaccharides treated with sodium hydroxide are consistent with a reaction without noticeable side effects. Extraction of carrageenans from seaweeds was already shown to be aided by microwave heating in a continuous-flow system (Uy et al., 2005). The possibility of applying a similar procedure to an alkaline polymer solution should not be overlooked as an economical and simple way of improving its gel properties.

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