Microwave-assisted hydrolysis of Alginic Acid

T. Bouanati[a,b,c], J-C Cabrera[c], F. Laoutid[b], J-M Raquez[b], P. Gerbaux[a]

[a]Organic Synthesis and Mass Spectrometry Lab (S²MOs), University of Mons (UMons)
[b]Laboratory of Polymeric and Composite Materials (SMPC), University of Mons (UMons),
[c]Materia Nova Biotech – Ghislenghien

Introduction

Oligosaccharides are extensively studied for their potential biological activities and they are developed, for example, as prebiotics, drugs carriers, biostimulants,…

Only few oligosaccharides are naturally produced and therefore some strategies need to be developed to prepare them either by organic synthesis or degradation of naturally occuring polysaccharides.
However, the synthesis of oligosaccharides is more complex than other polymers due to the numerous combinations, the anomericity and the configuration of the carbohydrates.
Since the past decades, differents methods for producing oligosaccharides by depolymerization have thereby been investigated [1],[2]:

- Chemical degradation → acid hydrolysis or depolymerization by hydroxyl free radicals
- Physical degradation → microwave, radiolysis (UV light or γ irradiation), ultrasonic or thermal treatment
- Enzymatic degradation

Methods	Advantages	Drawbacks
Chemical methods	Quickness Low cost Simple process	Non-specific cleavage Release of substituents Chemical products are mandatory
Physical methods	Quickness Simplicity process Not require chemicals products	Cleavage non specific High installation cost
Enzymatic methods	Specific cleavage	Microbiologicals contaminants Rigourous controls are required High cost

Experimental section

Physical methods, particularly microwave treatments appear to be promising and fulfill the fundamental rules of green chemistry.
Hydrolysis of carrageenan in aqueous solution by microwave treatment was achieved by G.Zhou [3] and oligocarrageenans were obtained after 2 and 10 minutes at 10 or 15 bar, corresponding respectively to temperatures at about 175°C and 200°C.
For testing the hydrolysis of polysaccharides in water by microwave treatment, we select alginate, a commercially available polysaccharide of 100,000g/mol extracted from algae.

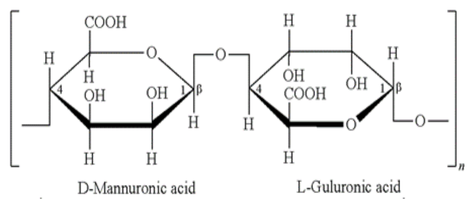

Figure 1 : structure of alginate

An aqueous solution of alginate at a concentration of 1mg/ml under stirring was prepared. Alginate being hardly soluble in water, continuous stirring was applied to keep alginate in suspension. After 24h of stirring, 1 ml of solution was placed and sealed in a specific vial for microwave treament.

The experience is repeated several times at different temperatures and reaction times :
130°C (2 bar) → 2min, 5min, 10min, 20min, 30min
150°C (4 bar) → 2min, 5min, 10min, 20min, 30min
175°C (9 bar) → 2min, 5min, 10min, 20min, 30min
200°C (14 bar) → 2min, 5min, 10min, 20min, 30min

➔ The best results are obtained for microwave treatment at 150°C

Results and discussion

1 Dosage of the reducing ends of saccharides

This method was established by Nelson and Somogoyi in 1944 and allowed the dosage of the reducing end of saccharides by reaction with the Nelson reagent to form molybdenum blue, which presents a high absorbance at 595 nm.

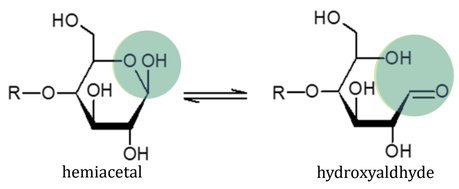

Figure 2 : reducing end of saccharides

We observe an increase of the absorbance over time, which demonstrates that smaller molecules are present, attesting for the efficiency of the hydrolysis.

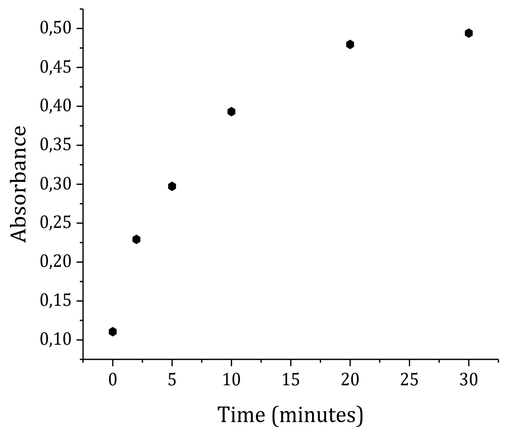

Figure 3 : Evolution of absorbance at 595nm over time after microwave treatment at 150°C

2 Viscosimetric measurements

The viscosity of each solution, treated by microwave, is measured by the viscosimeter μVis RHEOSENSE. Note that the starting alginate solution is not measured due to the lack of solubility of alginate in water.

We observe a decrease of the viscosity over time, which confirms the hydrolysis efficiency.

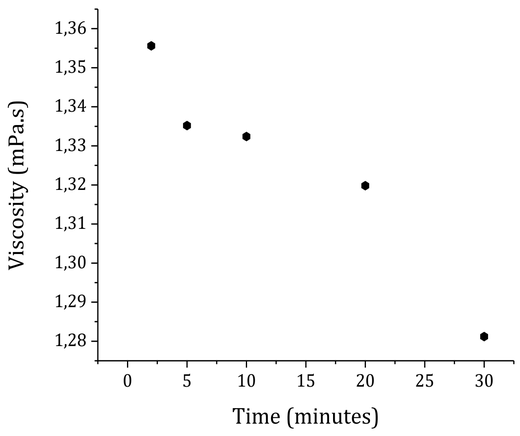

Figure 4 : Evolution of viscosity over time after microwave treatment at 150°C

3 Matrix-assisted Laser Desorption/Ionisation (MALDI)

MALDI-ToF mass spectrum is obtained by using dihydroxybenzoic acid/dimethylaniline (DHB/DMA) as the matrix and by adding NaI as the source of cationization.

The mass spectrum of each solution was measured immediately after the microwave exposition. Again no data were generated for the parent alginate due to the (i) the lack of solubility in water and (ii) the high molecular mass.

For solutions treated at 150°C during 2 minutes or 5 minutes, we detect the presence of oligosaccharide mixtures with DP_{max} up to 10. From 10 minutes to 30 minutes, signals for the highest DP oligosaccharides disappeared and after 30 minutes, DP_{max} = 4 was obtained.

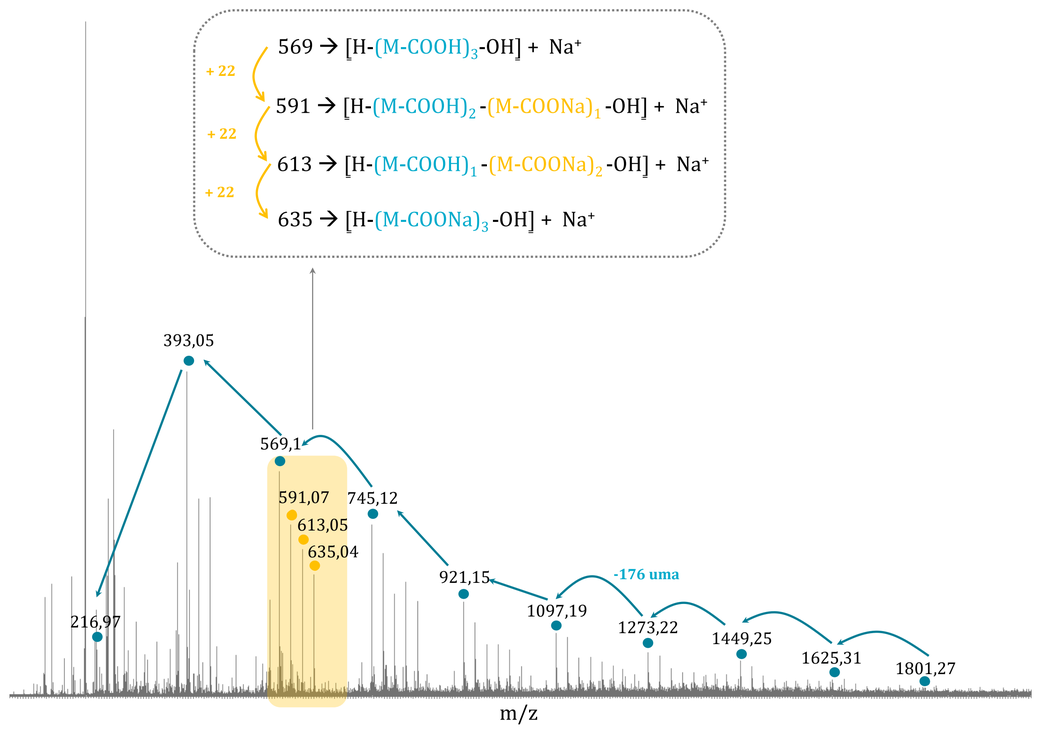

Figure 5 : MALDI-ToF mass spectrum of alginate treated at 150°C during 2 minutes

Conclusion

We produced oligosaccharides by microwave treatment at 150°C in 2 minutes from polysaccharides in water. This method seems really promizing already because no chemical reagents are required. The hydrolysis reaction is achieved in short reaction time. The hydrolysates are characterized by common methods, such as viscosimetry and reducing sugar analysis. MALDI-ToF measurements are clearly adding a great analytical values, when determining the composition and the structures of the oligosaccharide mixtures. Those preliminary results pave the way to our further investigations that intend to prepare tailor-made oligosaccharidic mixtures starting from algae-derived polysaccharides. For an example, we will focus on polysaccharides such as fucoidans, for which no enzyme is to date identified for the production of oligosaccharides.

Acknowledgments

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