

Targeted functionalization of spruce galactoglucomannans with aid of galactose oxidase (FunMan)

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ABSTRACT

The project focuses on spruce galactoglucomannan (GGM), which is a new potential product from forest biorefineries. The key technologies studied are targeted and controlled oxidation of GGM with galactose oxidase (GO), and further chemical functionalization of the oxidized GGM. The usefulness of GGM in various applications is evaluated aiming to develop novel innovative applications for a side-product from the processing of spruce.

GGM and a selection of other polysaccharides were oxidized with GO in optimized reaction conditions and the products analyzed by a GC-MS technique specifically developed for the analysis of the reactive aldehyde derivative. The method utilizes NaBD₄ reduction of the product to result deuterium labelling of the oxidized galactose prior to acid methanolysis and GC-MS analysis. The oxidation was controlled and the conversion to aldehyde was 70% at best (GGM).

The further chemical modification of oxidized GGM was first tested using oxidized methyl- α -D-galactopyranoside as a model compound. Allylation with halides and a mediating metal, such as zinc, indium and tin, was done in aqueous solution directly after oxidation. The most promising indium mediated reaction was already done on oxidized GGM and will be continued by using GGM batches with varying oxidation degrees.

The physico-chemical properties of oxidized GGM were investigated by some preliminary experiments. No apparent difference was observed between the viscosity values of GGM and oxidized GGM. Oxidized GGM formed films, but the properties of the films did not differ notably from films formed of unoxidized GGM.

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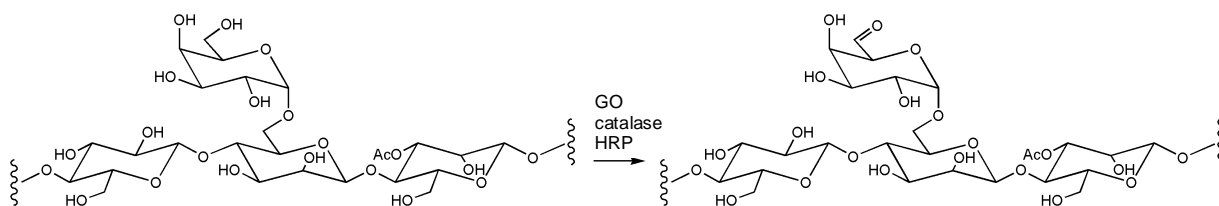
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RESULTS

1. University of Helsinki

Controlled oxidation of GGM

Galactose oxidase (GO) is a copper metalloenzyme which catalyzes the oxidation of primary alcohols to corresponding aldehydes with high selectivity for galactose. The oxidation conditions of GO-catalyzed reaction were optimized for the production of aldehyde during the first year, using methyl- α -D-galactopyranoside as a substrate. During the second year, the optimized reaction conditions were applied to the oxidation of spruce galactoglucomannan (GGM, Scheme 1). GGM is a substrate of GO as it contains terminal galactose units attached to the mannose backbone (ratio of monosaccharide units Gal:Glc:Man = 1:2:8). In the optimized conditions, three enzymes (GO, catalase and horseradish peroxidase) catalyze the reaction, which is performed in water at room temperature or at 4°C. In the case of GGM, the best yield of the aldehyde derivative was obtained when the reaction was performed at rt. The conversion also depended on the purity of starting material and was ca. 70% at best after a 48 h reaction. GGM derivatives with lower oxidation degrees were obtained by quenching the reaction after shorter reaction times, such as 1-2 h.



Scheme 1. Oxidation of GGM. GO = galactose oxidase, HRP = horseradish peroxidase

Oxidation of other polysaccharides

The GO-catalyzed reaction was also applied to the oxidation of several polysaccharides with varying structure, such as guar gum, locust bean gum, arabinogalactan, corn fiber gum and xyloglucan. These polysaccharides contain varying amounts of terminal galactose from very high levels (e.g. guar gum, 40% of all monosaccharide units) to very low levels (e.g. corn fiber gum, 6%). There were considerable differences in the obtained oxidation degrees of the products without clear dependence on the structure of the polymer. The best oxidation degree (86%) was obtained with xyloglucan where the terminal galactose units are positioned at short branches of a few monosaccharide units from the polymer backbone.

Determination of oxidation degree

The analysis of the oxidized product by NMR was problematic due to its poor solubility. Thus a GC-MS method was developed for the analysis of the products. A special method had to be used as the reactive aldehyde was not stable in the derivatizing and depolymerizing procedures. The oxidized GGM (ox-GGM) was first reduced with NaBD₄ back to the polysaccharide with only hydroxyl groups. The galactose C-6 positions that had been oxidized were thus deuterated (-

CHDOH). The GGM derivative was then degraded by acid methanolysis⁴ and derivatized. The oxidation degree was calculated from the difference in the mass spectra of unreacted and oxidized galactose (oxidized galactose carrying D label). The analysis of the other polymers was performed in the same way.

Properties of oxidized GGM

Carbonyl groups in the polysaccharide structure might alter its physical properties, such as viscosity, due to the formation of inter- or intramolecular hemiacetal bonds. The effect of the oxidation on the physical properties of GGM was investigated by some preliminary experiments. Intrinsic viscosity of GGM and ox-GGM (ca. 60% oxidation degree) was measured with an Ubbelohde capillary viscometer. However, the viscosity of the ox-GGM did not differ notably from unoxidized GGM. A film-forming experiment was done comparing GGM to ox-GGM samples with ca. 10% and 30% oxidation degree. All the samples formed films but again the properties, such as tensile strength and elongation at break, of GGM and ox-GGM did not differ significantly from each other.

2. Åbo Akademi

Modification of oxidized methyl- α -D-galactopyranoside

Oxidized methyl galactopyranoside (ox-Gal) was used as model substance for derivatization of ox-GGM. The type of reaction, as well as the reaction conditions, was chosen based on that they could later be utilized for the derivatization of ox-GGM. According to the literature, the metal mediated reaction of aldehydes and ketones with allylic halides is an efficient method for producing the corresponding homoallylic alcohols, typically in high yields. Since the reaction can be done in aqueous media, ox-Gal can be modified directly without the need of drying after the enzymatic oxidation or derivatization. Zinc, indium, and tin were tested as mediating metals. Pre-screenings showed that indium was the most promising metal. Different solvent systems were tested. Since ox-GGM remains dissolved in water:THF (1:1), this solvent system was chosen for further experiments. Four different allylating agents, allyl bromide, cinnamyl chloride, crotyl chloride, and benzyl bromide were tested for incorporating new functional groups to the molecule. Of these, the three first mentioned seemed to be the most efficient.

Modification of oxidized GGM

Using the reaction conditions optimized for ox-Gal, indium mediated allylation reaction was done on ox-GGM. Allyl bromide, cinnamyl chloride and, crotyl chloride were tested. Because of the initial low degree of oxidation of GGM, the identification and quantification of derivatized galactose units by NMR was not possible. A new ox-GGM batch with higher degree of oxidation has recently been tested, but the processing of the results is still under work.

Evaluation of the analytical methods

The analysis of the products was again challenging since the depolymerization (needed when analyzing GGM) and derivatization steps that are required for GC-analysis destroy both the aldehyde and the homoallylic alcohols. Thus the GC-MS method developed for the

⁴ Sundberg, A., Sundberg, K., Lillandt, C., Holmbom, B. Nordic Pulp and Paper Research Journal 11 (1996) 216-219.

determination of the degree of oxidation of ox-GGM was also applicable when determining the amount of unreacted aldehyde groups in allylated ox-GGM. HPLC does not require any derivatization step prior to analysis, and it was possible to separate the aldehyde and the corresponding hydrate from the derivatized sugar monomers. Identification of the separated compounds could be done with MS. Thus LC-MS, combined with a depolymerization step that does not destroy the formed homoallyl alcohol (e.g. enzymatic hydrolysis), might also be applicable for the analysis of polymeric samples and will be tested.

Physico-chemical properties of modified GGM

The average molecular weight of unoxidized, oxidized, and derivatized GGM was determined by MALLS. The average molecular weight is slightly increased after oxidation and after the addition of different allylic groups to the molecule. NMR studies need to be done to confirm whether this increase arises from agglomeration or chemical bonds, e.g. crosslinking caused by the allylic double bond. The viscosity of both GGM and ox-GGM was shear rate -dependent at a concentration of 10 mg/ml. Both solutions exhibited a shear-thinning behaviour. The viscosity decreases with shear rate at low values, but was kept constant when shear rate went over 10 S⁻¹. No apparent difference was observed between the viscosity values of GGM and ox-GGM.

PROGRESS

The research and collaboration between the partners has proceeded as planned.

ACTIVITIES

Participation in COST meetings in Bratislava and in Turku.

PUBLICATIONS

Articles

Parikka, K. and Tenkanen, M. Oxidation of methyl α -D-galactopyranoside by galactose oxidase: products formed and optimization of reaction conditions for production of aldehyde. *Carb. Res.* 344 (2009) 14-20.

Abstracts

Leppänen, A.-S., Parikka, K., Tenkanen, M., Reunanen, M. and Willför, S. Determination of aldehyde groups in oxidized O-acetyl-galactoglucomannan. Poster presentation, COST E41 Final seminar, 19.-21.5.2008, Turku, Finland.

Parikka, K., Leppänen, A.-S., Reunanen, M., Eklund, P., Sjöholm, R., Willför, S. and Tenkanen, M. Oxidation of spruce galactoglucomannans with galactose oxidase. Oral presentation, COST 868 Meeting, 16.-19.4.2008, Bratislava, Slovakia.

Manuscripts

Parikka, K., Leppänen, A.-S., Reunanen, M., Willför, S. and Tenkanen, M. Aldehyde derivatives of polysaccharides by selective oxidation with galactose oxidase.