

# Pectic rhamnogalacturonan II is present as a dimer in bilberries and their juices

Hauke Hilz, Henk A. Schols, Alphons G.J. Voragen

## Introduction

Bilberries are an important crop in northern Europe and are mainly processed to juice. Common enzymatic treatment releases enzyme resistant polysaccharides into the juice [1]. Rhamnogalacturonan II (RGII), a very complex pectic polysaccharide with 12 different sugars and 20 different linkages, might be the major part of these resistant polysaccharides, since it is present in many other fruit and vegetable juices and wine [2]. Two RGII molecules can be cross linked by a boron ester resulting in a cross linking of pectin molecules [3]. The role of this ester *in muro* is not clear, but it can have an effect on processing and gelling of pectins.

Aim of this study was to investigate the role of boron ester between two RGII molecules during bilberry processing. In juice and after enzymatic degradation of pectins, it was determined if RGII was present as a monomer or a dimer.

## Materials and Methods

After preparation, alcohol insoluble solids (AIS) were sequentially extracted with different buffers [1].

Neutral sugars were determined as alditol acetates after TFA hydrolysis [2]. Monomer and dimer of RGII were determined by HPSEC after acid hydrolysis and neutralisation in presence of boric acid [3].

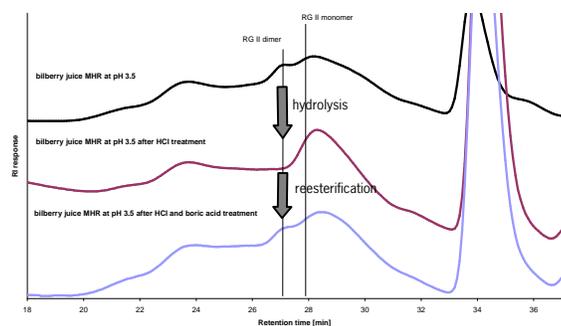


Figure 1. Hydrolysis and reformation of dimeric RGII in juice polysaccharides in HPSEC.

## Results and discussion

In all pectic fractions of bilberries (hot buffer soluble, chelating agent soluble, diluted alkali soluble solids) RGII was quantified via the amount of their diagnostic sugars. It is present up to 9 % of total cell wall polysaccharides (CWPS) in the fraction. In juice, which was extensively treated with enzymes, RGII contributes to app. 33 % of total CWPS.

The boron cross linked RGII dimer can be hydrolysed by 0.1 N HCl to the RGII monomers. In pH 3.5 acetate buffer containing 50 mM boric acid RGII is reesterified to the boron cross linked dimer. Using HPSEC, this approach can be used as an unambiguous prove for the presence of monomeric or dimeric RGII. In juice polysaccharides of bilberries, RGII is mainly present as a dimer (Figure 1).

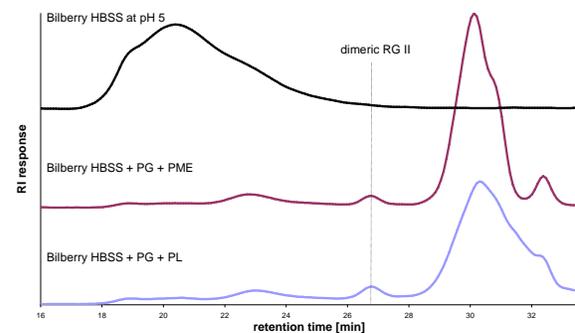


Figure 2. HPSEC-chromatogram of bilberry HBSS before and after incubation with PG/PME and PG/PL.

After degradation with polygalacturonase (PG) in combination with pectin lyase (PL) or pectin methyl esterase (PME), RGII was released from intact pectin molecules. From AIS and hot buffer soluble solids (HBSS; Figure 2) RGII was released as a dimer, while it was released as both monomer and dimer from chelating agent soluble solids and diluted alkali soluble solids, the fractions treated with chelating agent. That chelating agents are able to partly hydrolyse the boron ester of RGII was shown before [4]. The native form of RGII is, therefore, the dimer.

## Conclusions

Rhamnogalacturonan II is present in all pectic fractions and is always present as a dimer *in muro*. Since RG II is covalently linked to homogalacturonan, it is an important cross linker in most pectic molecules and can, therefore, play an important role for the structure of the pectin network of the cell wall and during food processing similar to calcium complexes.

## References

- Hilz, H.; Bakx, E.J.; Schols, H.A.; Voragen, A.G.J., *Carbohydr. Polym.* **2005**, 59, 477-488.
- Doco, T.; Williams, P.H.; Vidal, S.; Pellerin, P., *Carbohydr. Res.* **1997**, 297, 181-186.
- Matoh, T.; Ishigaki, K.I.; Ohno, K.; Azuma, J.I., *Plant Cell Physiol.* **1993**, 34, 639-642.
- Ishii, T.; Matsunaga, T.; Pellerin, P.; O'Neill, M. A.; Darvill, A.; Albersheim, P., *J. Biol. Chem.* **1999**, 274, 13098-13104.

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