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A multivariate approach for high throughput pectin profiling by combining glycan microarrays with monoclonal antibodies

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ABSTRACT

Pectin—one of the most complex biomacromolecules in nature has been extensively studied using various techniques. This has been done so in an attempt to understand the chemical composition and conformation of pectin, whilst discovering and optimising new industrial applications of the polymer. For the last decade the emergence of glycan microarray technology has led to a growing capacity of acquiring simultaneous measurements related to various carbohydrate characteristics while generating large collections of data. Here we used a multivariate analysis approach in order to analyse a set of 359 pectin samples probed with 14 different monoclonal antibodies (mAbs). Principal component analysis (PCA) and partial least squares (PLS) regression were utilised to obtain the most optimal qualitative and quantitative information from the spotted microarrays. The potential use of microarray technology combined with chemometrics for the accurate determination of degree of methyl-esterification (DM) and degree of blockiness (DB) was assessed.

1. Introduction

Pectin represents a wide and complex family of plant cell-wall polysaccharides that play a significant role in the growth and development of land plants and green algae.[1] It also has important applications as a functional food ingredient by virtue of its functional properties—texture, rheological characteristics or juice extractability.[2] Three major pectic polymers are generally described as constituting the heterogenic bio-macromolecule, one un-branched domain identified as homogalacturonan (HG), and two branched domains rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II).[3] The predominant side chains contain either linear or branched α-L-arabinfuranosyl (Ara*f*), and/or β-D-galactopyranosyl (Gal*p*) residues. It has become an industrial trend to characterise pectin according to its degree of methyl-esterification (DM) as this is a major parameter in terms of gelling properties.[4] In relation to this, highly methyl-esterified (HM) pectin with DM >50% will form connection zones between HG chains by hydrogen bonds and hydrophobic interactions.[5,6] This type of gelling is important in a variety of commercial applications of pectin as for example some jams and jellies.[7] On the other end the formation of intermolecular associations between low methyl-esterified (LM) pectin (DM <50%) is mainly dependent on the presence of Ca^{2+}, more precisely between non methyl-esterified junction zones of HG domains. Besides the number of Gal*p*A units being methyl-esterified it is also important to consider the distribution pattern of methyl-esterified Gal*p*A residues—degree of blockiness (DB).[8]

Here we show the feasibility of characterising the chemical properties of pectin using monoclonal antibodies (mAb) combined with microarray based screening. It has been established that glycan microarrays can provide information about the interactions between mAb and a diverse range of carbohydrates with wide applications in glycomics.[9] In fact, the first carbohydrate microarrays produced in 2002 encompassed the study of pectin.[10] The required components for such a study are a set of targeted molecules (pectin) a set of probes (mAbs) and a surface where the two compounds are immobilised (membranes). The main aim is the identification of specific components found in a mixture that best represents the entire pectin population in a particular biological sample.[7] This has become increasingly possible with the availability of pectin-specific mAbs as reviewed previously.[11,12] The application of this methodology has revealed that there is diversity within the

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plant cell wall components and that besides being remarkably complex, pectin components also appear to be related with the plant's developmental stage.[13]

For our investigation a total amount of 359 pectin samples from different origins (lemon, lime and orange), were spotted and analysed for the binding of a set of 14 mAbs. Samples were obtained using a diverse set of extraction conditions with varying pH, type of acid used, addition of enzymes, temperature and time of extraction. Ultimately, the extraction conditions used played a role in both the final functional and chemical characteristics of pectin.[14] The possible correlation between the mAbs binding pattern with previously determined DM and DB was assessed using efficient multivariate analytical methods, principal component analysis (PCA) and partial least square (PLS) regression. PCA is a simple algebraic data manipulation technique, which uses orthogonal transformation to convert a set of variables into a smaller number of factors named principal components (PC).[15,16] The number of new PCs is smaller than the initial number of variables, and the algorithm is developed in a such way that the first PC has the highest possible variance, while the following PCs in turn have the highest variance possible under the constraint that it is orthogonal to, or uncorrelated with, all the preceding PCs. Using this approach it is possible to probe datasets with many variables in a way that otherwise human pattern recognition wouldn't permit. On the other hand, PLS is a process, which enables the creation of relationships between two matrices (X and Y) in an approach to model the covariance structures in these two spaces.[17] The relationship created is such that it enables understanding the variance observed in the Y space, when one or a set of independent variables are carefully changed in the multidimensional space X. This technique is widely used for product quality assessment, on-line monitoring and industrial process control[18,19] and here it was used to predict DM and DB values based on mAb binding pattern in a pectin microarray.

2. Materials and methods

2.1. Samples

Pectin powder samples were provided by CP Kelco—Lille Skensved, Denmark. Samples were extracted using a variety of extraction methods from lemon (Le), lime (Li) and orange (Or) totalling 359. All the mAbs utilised during this work were obtained from PlantProbes, Leeds, UK with the exception of INRA_RU1 and INRA_RU2, which were kindly given by INRA, Nantes, France.

2.2. Degree of methyl-esterification

Following the method specified in the fifth edition of the Food Chemicals Codex,[20] DM was determined making use of an automated titration equipment, Metrohm Titrino 751 (Herisau, Switzerland).

2.3. Degree of blockiness

Samples were solubilised in 20 mM sodium acetate buffer pH 4.0 to a final concentration of 5 mg/mL. Endo-polygalacturonase was added to a final concentration of 1.2 U/mL and the samples left incubating for 24 h at 50 °C. Degree of blockiness was determined using a detailed analysis of mono-, di- and tri-GalpA residues, released after endo-polygalacturonase treatment as described previously.[21]

2.4. Glycan arrays and probing

Stock solutions of pectin were prepared in advance for deposition at 5 mg/mL in 0.05% sodium-azide in dionized water. A 3-fold dilution was made by mixing 50 μL stock solution with 100 μL buffer (99.9% H_2O, glycerol and Triton X), and from this solution a new 3-fold dilution was prepared. This step was consecutively repeated until a total number of 6 dilutions. A microarray robot (Sprint Inkjet Microarrayer, Arrayjet, USA) was used to spot all 359 samples (6 dilutions) in triplicate onto nitro-cellulose membranes. The final prints were made at 22 °C and with a humidity of 50% or above. The resulting arrays were blocked and probed with the mAbs listed in Table 1 and as described previously.[22] Overall the antibodies used could be assigned to either the HG and RG-I domains of the pectin structure.

2.5. Quantification

The mAb binding patterns were quantified using ImaGene 6.0 (BioDiscovery, CA, USA) microarray analysis software as described.[10] To enable that, glycan arrays were scanned and converted to 16-bit grey scale TIFFs and uploaded to the program. Semi-automatic gridding was used to create an analysis area of individual spots, leaving a 5-pixel zone around each spot for the calculation of local background signals. The individual spot signals were then defined as the mean pixel value within each spot area minus the median pixel value of the surrounding local background area.[29] For each array prepared, the mean values of the individual spot signals from the three replicates were calculated. Normalisation ensured the comparison between arrays and was accomplished by assigning 100% to the highest of the mean values while adjusting all the others accordingly. To ensure that no background signal was being used in the following analysis, a cut-off of 5% was applied and spots with lower binding intensity value than that were set at zero. From the resulting mean and normalised values, the heat maps, which allowed a visual analysis of the binding

Table 1
List of monoclonal antibodies used in the study and corresponding pectin domain and expected binding site

Probe	Pectin domain	Expected binding	Reference
2F4	HG	Ca^{2+}—dimers, 9 GalpA units	23
JIM5		Partially methyl-esterified/de-esterified HG	22
JIM7		Partially methyl-esterified HG	22
LM18, LM19		Partially methyl-esterified/de-esterified HG	24
LM20		Partially methyl-esterified HG	13
INRA_RU1, INRA_RU2	RGI	Rhamnogalacturonan I backbone	25
LM5		(1→4)-β-D-galactan	26
LM6		(1→5)-α-L-arabinan	12
LM9		Feryolated polymers	27
LM12		Feryolated polymers	28
LM13		(1→5)-α-linked Ara*f*	13
LM16		Sub-sets of processed arabinan	13