Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/bcdf

Characteristics of bacterial enzymes present during *in vitro* fermentation of chicory root pulp by human faecal microbiota

*Uttara S. Ramasamy, Henk A. Schols, Harry Gruppen**

Laboratory of Food Chemistry, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form
4 July 2014
Accepted 29 August 2014

Keywords:
Chicory root pulp
Fermentation
Arabinofuranosidase
β-Galactosidase
Exo polygalacturonase
Accessibility

ABSTRACT

Research was conducted to understand cell wall polysaccharide (CWPs) degradation in chicory root pulp (CRP) during an *in vitro* fermentation by human faecal microbiota. Enzyme extracts (EEs) from CRP fermentation liquids (2, 12 and 24 h) were tested for enzyme activities towards p-nitrophenyl-glycosides and polysaccharides. The EEs were also incubated for 24 h on polysaccharides and cell wall residues (CWRs) derived from CRP, to determine the mechanism and efficiency of enzyme action. The study showed that the presence of arabinofuranosidase, β-galactosidase, endo-arabinanase, endo-galactanase and exo-polygalacturonase increased for the 24 h EE, whereas the activity of enzymes degrading (hemi)cellulose was highest in the 12 h EE. It was hypothesised that increased levels of arabinofuranosidase, β-galactosidase, endo-arabinanase, endo-galactanase, exo-polygalacturonase, pectin de-esterifying enzymes and endo-polygalacturonase contributed to a synergy in degrading pectin in CRP from 12 to 24 h of fermentation. The higher degradability of arabinan compared to galactan in the network is due to the architecture in CRP involving more accessible arabinan than galactan.

1. Introduction

Dietary fibre encompasses polysaccharides and oligosaccharides that are resistant to absorption and/or digestion in the human small intestine and end up in the colon where they are fermented by the human microbiota (DeVries et al., 2001). The microbiota can utilise fibres for fermentation by expressing a wide array of enzymes (Flint, Bayer, Rincon, Lamed, & White, 2008). The levels and types of enzymes expressed depend on the type of polysaccharides present during fermentation (Sonnenburg et al., 2005). Till date, the activity and mechanisms of the different types of enzymes involved in degrading cell wall materials containing a mixture of polysaccharides are not fully understood.

Abbreviations: CRP, chicory root pulp; HG, homogalacturonan; RG, rhamnogalacturonan; Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; Man, mannose; Fuc, fucose; UA, uronic acid; GalA, galacturonic acid; Rha, rhamnose; CWPs, cell wall polysaccharides; PS, polysaccharides; EE, enzyme extract; CWRs, cell wall residues; WUS, water unextractable solids; CHUS, chelating agent unextractable solids; DAUS, dilute alkali unextractable solids; CAUS, concentrated alkali unextractable solids; PNP, p-nitrophenyl; BP, sugar beet pectin; LA, linear arabinan; BA, branched arabinan; PG, potato galactan; OSX, oat spelt xylan; XG, xyloglucan; M, mannan; C, cellulose

*Corresponding author. Tel.: +31 317483211, +31 317482888.
E-mail address: harry.gruppen@wur.nl (H. Gruppen).

http://dx.doi.org/10.1016/j.bcdf.2014.08.001
2212-6198/

Chicory root pulp (CRP), an agricultural by-product obtained after inulin extraction, contains a mixture of cell wall polysaccharides (CWPs) (58 w/w%) especially pectin (62%), followed by cellulose (27%) and hemicellulose (11%) (Ramasamy, Gruppen, & Schols, 2013). The latter is mainly represented by xyloglucan in CRP (Ramasamy et al., 2013). In addition to CWPs, CRP also contains remaining inulin (6 w/w%) originally present as a storage polysaccharide (Ramasamy et al., 2013). Pectin in CRP is abundant in homogalacturonan (HG) (65%) followed by arabinan (19%) and galactan (11%) (Ramasamy et al., 2013).

In vitro fermentation of CRP for 24 h, using human faecal inoculum resulted in the utilisation of 86% arabinose, 84% galacturonic acid and 64% galactose present in the polysaccharides (Ramasamy, Venema, Schols, & Gruppen, 2014). The utilisation rate of sugars from these polysaccharides increased after 12 h of fermentation. Although insoluble polysaccharides from the cell wall were degraded and utilised, no oligomers were found in the fermentation liquid as degradation products. Such fermentation characteristics could be due to the type and levels of bacterial enzymes expressed during fermentation of specific cell wall components (Jonathan et al., 2013). According to Jonathan et al. (2013), the level of specific enzymes may also go down during the course of fermentation.

Along with the expression of sufficient levels of enzymes during fermentation, the accessibility of polysaccharides within a cell wall network towards enzyme degradation is regarded to be crucial in determining the extent to which they are degraded. The accessibility of polysaccharides for degradative enzymes is in turn determined by the architecture of the cell wall network (Guillon, Auffret, Robertson, Thibault, & Barry, 1998). Sequential removal of polysaccharides from CRP using extractants of increasing severity provided information on the various polysaccharides involved in building up the plant cell wall architecture (Ramasamy et al., 2013). Removal of a polysaccharide from a cell wall network can alter the porosity of the network and the CWP arrangement, and may increase the accessibility of other polysaccharides within the network towards enzymatic degradation (van Laar, Tamminga, Williams, Verstegen, & Schols, 2000).

Since enzymes are produced by bacteria during fermentation to cause degradation of polysaccharides, the purpose of this study is to characterise bacterial enzymes as present during CRP fermentation by human faecal microbiota. As can be inferred from above, the action of enzymes may be determined by the accessibility of CWPs in a network. For this reason, the study was also aimed to elucidate if CWP degradation by bacterial enzymes is affected by the accessibility of polysaccharides in the network.

2. Materials and methods

2.1. Substrates

Residues obtained from sequential extraction of CRP were used to represent different cell wall structures of CRP (Ramasamy et al., 2013). Residues included were water

Table 1 – Constituent monosaccharide composition of chicory root pulp (CRP), fractions derived from the pulp and commercial substrates.

Substrates	Carbohydrate yield [g in fraction per100 g in pulp]	mol%								Total sugar content (% w/w)	DM (%)	DA (%)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA			
CRP	100	1	0	15	4	4	7	31	38	64	70	43
WUS	87	1	0	12	4	2	7	33	40	68	70	46
CHUS	52	1	0	10	6	3	7	53	19	59	52	91
DAUS	45	1	0	9	8	3	7	61	11	62		
CAUS	39	1	0	10	4	1	7	67	10	67		
Defined substrates	References	mol%								Total sugar content (% w/w)	DM (%)	DA (%)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA			
Beet pectin	Oosterveld, Beldman, Searle-van Leeuwen, and Voragen (2000)		1	7	1	0	11	1	74	59	57	15
Linear arabinan	Kühnel et al. (2010)	4	0	56	0	0	19	7	14	100		
Branched arabinan	Kühnel et al. (2010)	4	0	67	0	0	14	5	10	100		
Potato galactan	Megazyme	4	0	9	0	0	78	0	9	95		
Oat spelt xylan	Sigma			15	75			10		n.a.		
Cellulose	Jonathan et al. (2012)	0	0	0	15	1	0	81	1	99		

DM/DA: degree of methyl/acetyl esterification expressed as moles of methanol esters/acetyl groups per 100 mol uronic acid, n.a: not available, a: determined in this study. Rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal), glucose (Glc), uronic acid (UA).