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Integration of galacturonic acid extraction with alkaline protein extraction from green tea leaf residue

Chen Zhang[a,*], Elvira Bozileva[a], Frits van der Klis[b], Yiyuan Dong[a], Johan P.M. Sanders[a,b], Marieke E. Bruins[a,b,*]

[a] Biobased Chemistry and Technology Group, ASFG, Bornse Weilanden 9, 6708WG Wageningen, Wageningen UR, The Netherlands
[b] Wageningen UR Food & Biobased Research, Bornse Weilanden 9, 6708WG Wageningen, The Netherlands

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ABSTRACT

Leaf pectin can be used as a feedstock for galacturonic acid (GA) production, but high extraction costs limit economic feasibility. To improve the extraction efficiency, leaf pectin extraction was integrated with an already cost-effective alkaline protein extraction, focusing on high yield of GA without losses of protein. GA extraction efficiencies in NaOH, HCl, phosphate buffer solution, or with Viscozyme® L were determined using green tea residues (GTR) as model material. Most GA was extracted using Viscozyme® L, mainly due to its cellulase activity. Extraction yielded more than 95% GA with only 5% protein. Alternatively, GA-containing pectin can be extracted in a weak alkaline solution. Here, GA yield is dominated by the ratio of extraction volume to biomass weight. The profits of these two integrated processes can be higher than one step protein extraction. The Viscozyme® L integrated process is suitable for GA production for application in chemicals, and may have a profit of 142$/ton GTR when enzyme cost are sufficiently lowered. The profit of the weak alkaline integrated process is estimated at 118$/ton GTR.

1. Introduction

Pectin is a family of complex polysaccharides located in the primary plant cell wall and middle lamella (Dashek and Harrison, 2006; Somerville et al., 2004) that is commonly used as functional ingredient in food industry (Willats et al., 2006). However, leaf pectin has usually been degraded and lost its functionality during plant growth, biomass harvesting, and/or pre-processing before extraction, and may no longer be suitable for food application. Alternatively, leaf pectin can be applied as chemical building block using its predominant components, such as galacturonic acid (GA). GA can be used as the starting material for vitamin C production (Mapson and Isherwood, 1956), and can be chemically transferred into various aromatic compounds under aqueous acidic conditions (Popoff and Theander, 1972). Furthermore, GA can be oxidized to its corresponding aldaric acid (C6-sugar di-acid), which has shown to be an interesting starting material for the production of 2,5-FDCA (Knoop et al., 2013), polyaldaramides and-esters (Lavilla et al., 2012, 2011; Muñoz-Guerra, 2012), sequestering agents (Abbadi et al., 1999), and corrosion inhibitors (Koefod, 2006).

Pectin can be roughly divided into three types: homogalacturonan (HG), rhamnogalacturonan I, and rhamnogalacturonan II (Ridley et al., 2001). GA mainly originates from HG pectin, of which some of the carboxyl groups are methyl esterified (Ridley et al., 2001; Willats et al., 2006). Applying leaf pectin for GA has a lower value than its application in food, and the production costs are relatively high due to its low content in leaf. To lower extraction costs, pectin extraction can be integrated with an already cost-effective alkaline protein extraction (Zhang et al., 2015, 2016). This alkaline protein extraction can be applied with a profit of about 85 €/ton GTR excluding capital and labor cost (Zhang et al., 2014). An integrated biorefinery concept was proposed to further improve the cost-effectiveness of this protein extraction (Zhang et al., 2015). It was suggested that removal of leaf pectin prior to protein extraction can reduce alkali consumption and improve protein quality (Zhang et al., 2016). Therefore, the next logic step is to consider pectin as a side product of protein extraction.

When integrated with protein extraction under alkaline conditions, pectin extraction should rather focus on GA yield and its influence on the efficiency of alkaline protein extraction rather than on pectin functionalities. Methods that can be used for extracting high yield HG pectin (with high GA content) include use of acid or

Abbreviations: GA, Galacturonic acid; GTR, green tea residue; homogalacturonan, HG.

* Corresponding authors at: Biobased Chemistry and Technology Group, ASFG, Bornse Weilanden 9, 6708WG Wageningen, Wageningen UR, The Netherlands.
E-mail addresses: zhangchenfj@sina.com, Office.BCT@wur.nl (C. Zhang), Marieke.Bruins@wur.nl (M.E. Bruins).

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Table 1
Experimental conditions for weak alkaline pectin extraction.

No	Temperature (°C)	Extraction time (h)	V/W[a] (ml/g)	pH	[Buffer] (mol/L)	No.	Temperature (°C)	Extraction time (h)	V/W (ml/g)	pH	[buffer] (mol/L)
1	40	2	40	10	0.08	13	40	1	20	7	0.04
2	50	1.5	30	7	0.06	14	70	1.5	50	8	0.02
3	30	3	10	8	0.12	15	40	0.5	10	10	0.1
4	30	1	60	7	0.04	16	70	1	40	8	0.08
5	30	1.5	20	10	0.1	17	50	0.5	10	9	0.02
6	60	0.5	10	9	0.06	18	60	0.5	60	9	0.08
7	80	2.5	50	9	0.12	19	50	2	40	10	0.06
8	30	2.5	50	7	0.1	20	50	3	30	8	0.1
9	60	3	20	9	0.02	21	60	1	30	10	0.06
10	40	2	60	7	0.12	22	70	2.5	40	7	0.08
11	80	2.5	60	8	0.04	23	70	2	50	8	0.12
12	80	1.5	30	9	0.02	24	80	3	20	10	0.04

[a] V/W, liquid (volume, ml) to solid GTR (weight, g) ratio.

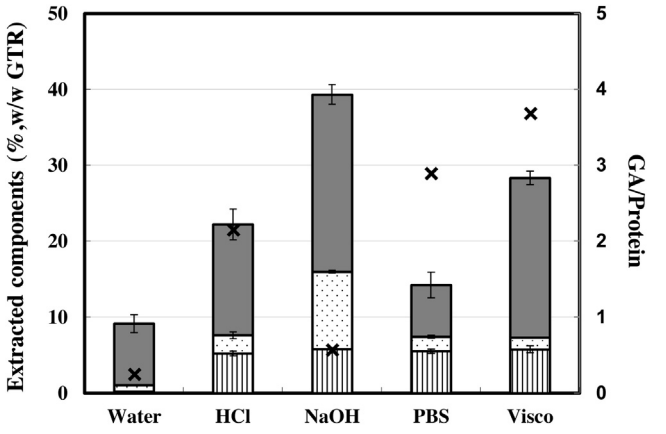

Fig. 1. Extracted components from 500 mg GTR by 20 ml water, 0.1 M HCl, 0.1 M NaOH, or 0.1 M pH 8 at 60 °C for 2 h, or Viscozyme (30 U/g GTR, in 10 ml PBS at pH 4.7) at 30 °C for 20 h. ⁘: protein; ▥: galacturonic acid; ▩: other components; ✕: ratio of galacturonic acid to protein.

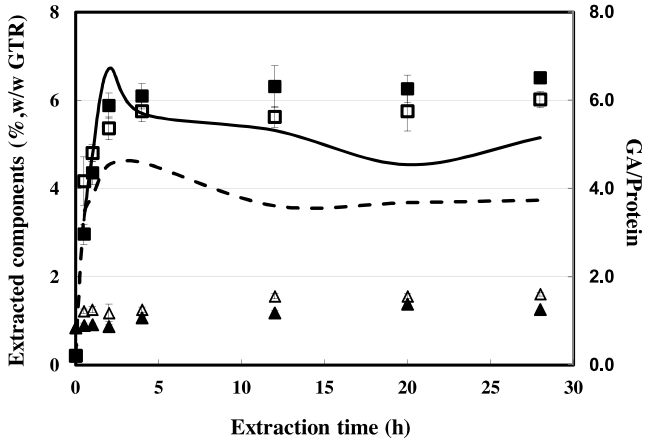

Fig. 2. Extracted components (%, w/w GTR) in time from GTR using Viscozyme (30 U/g GTR) at 30 °C. ■: GA yield without buffer; □: GA yield with buffer; ▲: protein yield without buffer; △: protein yield with buffer; —: GA/protein without buffer; ----: GA/protein with buffer.

alkali, and enzymatic methods (Lim et al., 2012; Renard et al., 1990; Seixas et al. 2014; Sengkhamparn et al., 2010; Shi et al., 1996; Wang et al., 2014; Westereng et al., 2008). Acid is commonly used for HG pectin extraction, but its integration with alkaline protein extraction will generate large amounts of salts that increase the cost of both pectin and protein extraction. HG pectin can be extracted by weak alkaline solution, but the product is rarely used due to the decrease in pectin esterification degree (Jiang et al., 2005), which influences pectin functionality (Assoi et al., 2014). However, this method may be suitable for the proposed integration, since the pH that is required for HG pectin extraction is lower compared to alkaline extraction, by which pectin product can be extracted separately with no extra salts generated. Enzymes can be used to aid GA extraction by hydrolyzing pectin into GA (Su et al., 2015) or by hydrolyzing cell wall carbohydrates (Wikiera et al., 2015). Pectate lyase and/or pectinase can be used for hydrolysis of pectin, while galactanase, arabinanase, hemi-celllulase and cellulase are often used for the degradation of cell wall carbohydrates, including rhamnogalacturonan I pectin, hemi-cellulose and cellulose (Taherzadeh and Karimi, 2008). Using an enzyme mixture such as Viscozyme® L, that contains several or even all enzymes mentioned above for cell wall degradation is popular for its high efficiency on hydrolysis (Sari et al., 2015) and relative low price compared to individual enzymes. Enzyme aided extraction is carried out under either weak acid or alkaline conditions, and may also be suitable for integration with protein extraction.

To determine the most suitable GA extraction method for integration with protein extraction, green tea residue (GTR) was used as a model material, as it was previously used for a study on alkaline protein extraction (Zhang et al., 2014, 2015, 2016). GA extraction yield in NaOH, phosphate buffer solution (PBS), and with Viscozyme® L (containing arabanase, cellulase, β-glucanase, hemicellulase, and xylanase) and its relation to protein yield were first tested using acid or water as controls. Viscozyme® L aided GA extraction was then optimized. To further investigate what enzymes determine GA extraction, extraction effects of specific enzymes, including pectinase, arabanase, galactanase, cellulase, and hemicellulase were tested individually. Conditions for weak alkaline GA extraction were optimized using a uniform experimental design. Economics of both Viscozyme® L aided or weak alkaline GA extraction with the integration of alkaline protein extraction were estimated and discussed.

2. Materials and methods

2.1. Materials

Green tea residue (GTR) is a gift from Damin Company, Fujian Province, China. This residue from tea lemonade production was collected from *Camellia sinensis* trees in Zhejiang province, China, in 2014, and it was sun-dried after soaking green tea leaves in water at 85 °C for 45 min. It contains 25% protein and around 6.7% of GA based on dry matter weight.

Viscozyme® L (Multi-enzyme mixture containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase), pectinase (EC 3.2.1.15), hemicellulase (a mixture of glycolytic enzymes containing xylanase, mannanase and other activities), and cellulase (EC 3.2.1.4)