

# Isolation of Arabinose and Galactose from Industrial Sidestreams in High Yield and Purity

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Monosaccharides such as L-arabinose (Ara) and D-tagatose (derived from D-galactose, Gal) are low-calorie sweeteners associated with improved glycaemic and insulin control compared to disaccharides such as sucrose. However, alternative sources and better sugar-sugar separation methods are needed to improve the sustainability and economics of their production. Here, these sugars were obtained from purified and ultrafiltered compression screw pressate (CSP) of thermo-mechanical pulping of softwood (*Pinus radiata*) and orange peels (OPs). Ba-substituted zeolite X (BaX) molecular sieves showed superior separation performance of Ara from other sugars compared to conventional Ca-form ion exchange resin. To facilitate subsequent separation of sugars, OP hydrolysates were fermented to leave just Ara and Gal, while OP pectin was hydrolysed to generate a Gal-rich mixture. Overall, BaX has good potential for separating Ara from Ara-rich hydrolysates containing several different sugars. It is also suited for separating Ara and another monosaccharide such as Gal or Xyl in the absence of other sugars.

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## INTRODUCTION

Low-calorie monosaccharides sugars such as the aldopentoses L-arabinose (Ara) and D-xylose (Xyl) and the ketohexose D-tagatose derived from the aldohexose D-galactose (Gal), are considered healthier options for traditional sugar sweeteners such as sucrose, glucose, and fructose due to their ability to lower glycaemic and insulin responses and their nontoxicity (Nolles and Benschop 2016; Guerrero-Wyss *et al.* 2018; Shintani 2019; Pol *et al.* 2020; Baptista *et al.* 2021; Pol and Mars 2021; Pasmans *et al.* 2022; Silva *et al.* 2023). In most cases, their polysaccharide raw materials are saccharified by chemical or enzymatic hydrolysis and the sugars are then separated and recovered from the hydrolysates by crystallisation. The sugars may be used as sweeteners as such or converted to sugar alcohols (*e.g.*, Xyl to xylitol) that are stronger sweeteners than their corresponding sugars.

Using human food ingredients to obtain low-calorie monosaccharide sweeteners is not particularly sustainable or economical, as is the case with the current industrial production of Ara by hydrolysis of edible gums such as gum arabic or locust bean gum and separation of Ara from the resulting mixture of sugars. Biorefineries using lignocellulosic products such as sugar beet pulp (the residue from sucrose extraction) (Cárdenas-

Fernández *et al.* 2018), cereal bran (Bedő *et al.* 2019), or forestry residues (Sainio *et al.* 2013) may provide widely applied and more sustainable options. Currently, the main industrial process for producing Gal is *via* hydrolysis of lactose (a by-product of the dairy industry) and separation of the resulting glucose and Gal. The supply of lactose exceeds its demand in the food industry. Regarding Xyl, the raw material of the sweetener xylitol, it is already produced industrially from nonfood raw materials (especially birch) (Silva *et al.* 2023). Thus, there is less need to identify alternative nonfood/sustainable sources for Gal and Xyl than there is for Ara.

Ara, Gal, and Xyl are among the products that may be obtained from pulp mill biorefineries using pressurised hot-water extraction of, *e.g.*, softwood (Sainio *et al.* 2013) or cereal bran (Bedő *et al.* 2019). Another potential source of these sugars is the compression screw pressate (CSP) produced during the pre-heating stage of thermo-mechanical pulping (TMP) before the wood chips or other lignocellulosic raw material are disintegrated to produce the pulp. The severity factor (SF) of the preheating stage is a parameter that includes the effects of two operational variables, namely temperature and residence time. SF determines the yield and relative proportions of the different mono-, oligo- and polysaccharides originating from the autohydrolysis of hemicelluloses and pectins in the raw material. In the case of pine, a common TMP raw material, these are galactoglucomannan (GGM), arabinoxylan, and arabinogalactan (Sainio *et al.* 2013; Smelstorius 1974). Isolating the hemicelluloses from CSP with the aim of their valorisation was recently demonstrated in a process that provides a hemicellulose-rich ultrafiltration (UF) retentate and a sugar-rich UF permeate (Widsten *et al.* 2023). However, valorising not only the hemicelluloses but also the permeate mono- and oligosaccharides would enhance the overall degree of valorisation of CSP and the starting biomass (softwood chips in this case) in general. CSP also contains non-saccharidic components such as furanic monomers and pseudo-lignin that in general do not elute together with sugars during chromatographic separation but can be removed before UF by using adsorbent resins. Such pre-treatments, often called decolourisation, are commonly applied in the production of ingredients for the food industry (Cárdenas-Fernández *et al.* 2018).

Pectin-rich fruit peels are a further potential source of valorisable monosaccharides such as Ara and Gal (Yapo *et al.* 2007; Li *et al.* 2016; Lachos-Perez *et al.* 2020; Jang *et al.* 2022; Monica *et al.* 2022; Gao *et al.* 2023; Zhou and Huang 2023). The peels of citrus and other common fruits are major sidestreams of the food industry whose disposal by dumping in the environment is linked to severe ecological impacts (Jang *et al.* 2022). While the peel pectins can be used as such in the food industry, they can also be hydrolysed in the same way as the gums used in the food industry. For example, citrus peels have a high content of rhamnogalacturonan pectins that probably have side chains of arabinan and/or arabinogalactan (Yapo *et al.* 2007), making them a rich source of Ara and Gal.

The high cost of separating Ara in high purity and yield from other monosaccharides in the hydrolysates of edible gums and other sources has hindered its wide use in functional foods. As there are no simple and efficient industrial-scale methods available to fractionate complex mixtures of monosaccharides such as those produced in biorefineries (Sainio *et al.* 2013), they need to be separated by costly stepwise removal of non-target saccharides, *e.g.*, by fermentation of glucose, fructose and rhamnose. Separation of hydrolysate monosaccharides by liquid chromatography on cation exchange resins (CERs), a method commonly used for analytical purposes in carbohydrate chemistry, has been investigated as a potential means of isolating Gal and Ara in high purity at a preparative scale using  $\text{Ca}^{2+}$  as the cation (Caruel *et al.* 1992; Saari *et al.* 2010).  $\text{Ca}^{2+}$  is the

most effective counterion among several di- and trivalent CER cations at separating monosaccharides (Caruel *et al.* 1992), but it still suffers from significant overlap of the elution profiles of the various monosaccharides (Saari *et al.* 2010). This is also the case with H<sup>+</sup>-form CER (Sainio *et al.* 2013). For example, Gal- and Ara-rich fractions were obtained from gum arabic hydrolysate containing Ara, Gal, and Rha by separation over Ca-CER resin but both fractions still contained large proportions of the other two monosaccharides (30% and 11%, respectively) (Saari *et al.* 2010). However, the little investigated separation method of monosaccharides by elution over Ba-substituted zeolite X (BaX) clay has shown better separation efficiency than Ca-CER (Chao and Sherman 1985). After the monosaccharides have been separated by any method chromatography, the monosaccharide elution fractions of sufficiently high purity can be concentrated by, *e.g.*, nanofiltration, and recovered by crystallisation (Moulik *et al.* 2015).

The aims of this study were to 1) investigate the effect of preheating severity (medium *vs.* high) on the yields of Ara, Gal, and Xyl in CSP from TMP of softwood (*radiata* pine), 2) to compare CSP and orange peel (OP) hydrolysate as sources of Ara, Gal and Xyl, and 3) to compare the ability of Ca-CER and BaX resins to separate Ara, Gal, Xyl and other monosaccharides present in the hydrolysates.

## EXPERIMENTAL

### Preparation of Compression Screw Pressates (CSPs)

Fresh debarked *radiata* pine (*Pinus radiata* D. Don) slabwood chips from a sawmill based in Rotorua, New Zealand were steamed at 80 °C for 5 min and then transferred *via* a compression screw to the preheater, where they were held at 160 °C for 20 min or at 171 °C for 72 min, corresponding to medium and high severity factors of 3.07 and 3.95, respectively (Lloyd and Murton 2016). Preheated chips were then fed through a second compression screw, which produced the pressates (CSP) for the study (stored in the dark at 4 °C until needed) (Lloyd and Murton 2016). The CSP samples (designated 160/20 and 171/72 based on the production parameters) were vacuum-filtered through wet-strength Whatman 113 filter paper to remove suspended solids.

### Preparation of Ba-substituted Zeolite X (BaX) by Cation Exchange

Granules of Na-zeolite X (NaX) molecular sieves from Guangzhou Chemxin Environmental Material Co., Ltd. (Guangdong, China) were ball-milled with ceramic balls to a fine powder and the 30 to 50 mesh fraction was sieved out. To exchange Na<sup>+</sup> to Ba<sup>2+</sup>, 100 g of this fraction was placed in a beaker with 5 L of deionized water and 264 g of BaCl<sub>2</sub> x 2H<sub>2</sub>O (≥99%, Sigma-Aldrich). The mixture was slowly stirred overnight mechanically. Fines were then filtered out through a 50-mesh sieve, a freshly prepared BaCl<sub>2</sub> solution was combined with the fines, and the mixture stirred overnight. The treatment was then performed a third time with a fresh batch BaCl<sub>2</sub> x 2H<sub>2</sub>O, after which the mixture was stored for three days at room temperature. After this the entire treatment was repeated with fresh solutions of BaCl<sub>2</sub> x 2H<sub>2</sub>O. Finally, the BaX was washed to low conductivity and dried overnight at 80 °C (yield 84.3%).

### Purification and Ultrafiltration of CSP

CSP 160/20 was prefiltered through a Nybolt 75/36 nylon mesh (pore size 75 µm; Clear Edge Filtrations, Auckland, New Zealand) to remove suspended solids and then

treated with washed Amberlite® XAD7 adsorbent resin (Sigma-Aldrich) at a resin-to-liquid ratio of 0.08, calculated on washed resin weight, to remove non-saccharidic contaminants and prevent membrane fouling during subsequent ultrafiltration (Widsten *et al.* 2023). CSP was placed in a 0.5 L or 1 L Schott bottle and the required amount of washed resin was added. The bottle was shaken at 180 rpm in an orbital shaker for the scheduled length of time (1 to 3 h) at 24 °C. After treatment the resin particles were removed by vacuum filtration through wet-strength Whatman 113 filter paper and the filtrate was stored at 4 °C in the dark until needed. Ultrafiltration (UF) of the resin-treated CSP sample 160/20 was carried out at 1.0 to 1.2 kPa using a Masterflex ultrafiltration unit (motor model 7553-75; Cole-Parmer) equipped with a 10,000 Da polyethersulphone membrane (cylindrical, surface area 0.29 m<sup>2</sup>) purchased from Smart Membrane Solutions, Rolleston, NZ. The filtration was continued until the permeate flow ceased. The monosaccharide-rich permeate (160/20-P) was stored at 4 °C until needed.

### Determination of Monosaccharides

Monosaccharides L-arabinose (Ara), D-galactose (Gal), D-glucose (Glu), D-xylose (Xyl), D-mannose (Man), and L-rhamnose (Rha) in CSPs 160/20, 171/72 and in the UF permeate 160/20-P before and after acid hydrolysis were determined by ion chromatography (IC) at Celignis Ltd, University of Limerick, Ireland. Monosaccharides in BaX and Ca-CER elution fractions, OP and pectin hydrolysates, and the BaX monosaccharide test mixture (Gal, Ara, Glu and Xyl) elution fractions were quantified by on an Agilent 1290 HPLC equipped with a 300 mm x 7.8 mm Rezex RPM-Monosaccharide Pb+2 (8%) column heated to 85 °C and a Carbo-Ca 4 mm x 3.0 mm ID guard column. The mobile phase was water, the flow rate 0.5 mL/min and the sample injection volume 10 µL. Detection was performed by a Shimadzu evaporative light scattering detector with N<sub>2</sub> gas at 300 kPa and an evaporator temperature of 40 °C. Calibration was performed using mixtures of pure monosaccharides Gal, Glu, Xyl, and Ara (Sigma-Aldrich) at concentrations of 0.0625 to 2.0 mg/mL. Samples for HPLC were first ultrafiltered to improve baseline resolution using a VivaSpin 500 5,000 Da cut-off 0.5 mL ultrafiltration device (Sartorius) and then centrifuged in an Eppendorf centrifuge (15,000 g/45 min/room temperature). The filtrate was used for the analysis.

### Preparation and Hydrolysis of Orange Peel Powder

Navel oranges were bought in a local supermarket and peeled. The orange peels (OPs) were freeze-dried and ground to a fine powder in a ball mill with ceramic balls. For hydrolysis, 300 mg of OP powder was placed in an ACE tube with 7.0 g 4% sulphuric acid and reacted at 100 °C for 1 h in an oil bath. The mixture was then allowed to cool, poured into a beaker and the pH adjusted to pH 5 to 6 with 10 M NaOH. Finally, the mixture was filtered through a 75-µm mesh and the residue washed with distilled water to give 15 mL of hydrolysate solution. The solution was frozen until used. The hydrolysis was repeated twice more on fresh OP peel batches using the same procedure.

### Isolation and Hydrolysis of OP Pectin

OP powder (50 g) was mixed with 2.5 L of deionized water adjusted to pH 1.5 with HCl and then kept in a water bath at 83 °C under mechanical stirring using a water bath oscillator for 1 h. The hot mixture was vacuum-filtered, and the filtrate was collected and stored in a refrigerator at 4 °C for subsequent purification. The crude pectin filtrate (2.0 L) was precipitated with ethanol (water:ethanol 1:2, v/v) and kept without stirring at 4 °C for

4 days. The precipitated pectin was separated by vacuum filtration on a Buchner funnel and washed with ethanol to remove the monosaccharides and disaccharides. After purification, the wet pectin was dried at 40 °C in a vacuum drying to a constant weight. The yield was 9.33 g (18.7%) o.d. (40 °C) pectin. Pectin (315 mg) was then hydrolysed using the same method as for the OP powder.

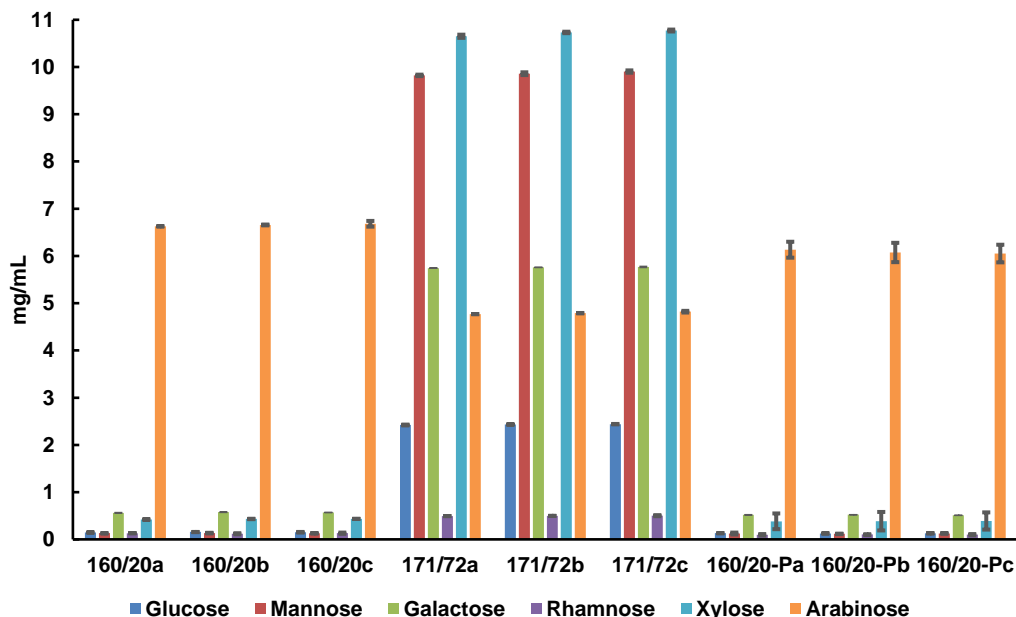
### Fermentation of OP

New Zealand sourced *Saccharomyces cerevisiae* (Baker's yeast) was used in the fermentation of OP. The seed culture was prepared in a yeast extract-peptone (YEP) medium until OD<sub>600</sub> reached 2.0 at 30 °C and 200 rpm. One percent (v/v) of this seed culture was transferred to 1% w/v of OP in a baffled flask containing 25 mL of 50 mM acetate buffer (pH 5.0) and fermented for 4 weeks at 30 °C and 200 rpm. One milliliter of liquid filtrate was sampled weekly for four weeks for monitoring monosaccharides present in the fermentation medium.

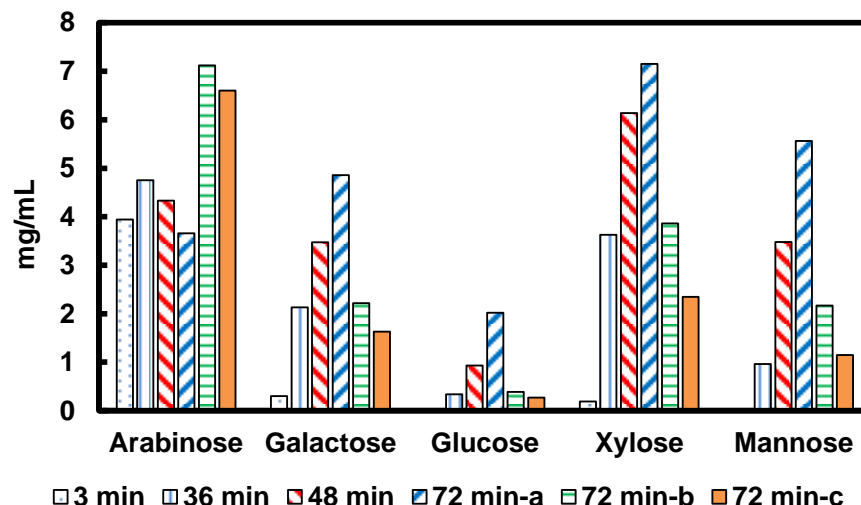
## RESULTS AND DISCUSSION

### Hydrolysis of Wood Polysaccharides During Production of CSP

CSP was produced from wood chips at different severities. As shown in Fig. 1, CSP 171/72 that was produced at a high severity had much higher total monosaccharide content than CSP 160/20 produced at a medium severity. Also, the yield of CSP solids at 14.9% of dry chip mass was much higher for 171/72 compared to the 4.0% of 160/20.



**Fig. 1.** Monosaccharide content of CSPs 160/20 and 171/72, and the permeate (160/20-P) from ultrafiltration of 160/20. Each sample was analysed in triplicate (a-c). The error bars denote standard deviation of duplicate determinations.



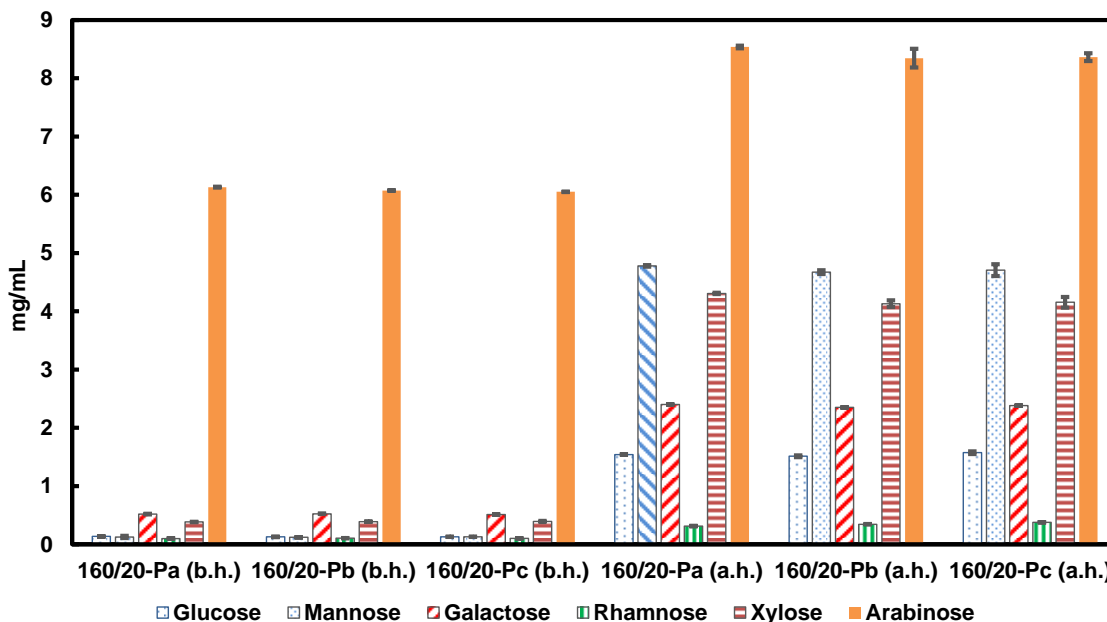
**Fig. 2.** Sugar content of CSPs produced at a pre-heating temperature of 171 °C for different times. Condition 171/72 was performed from three different batches of wood chips (a-c)

However, the Ara content of 160/20 was higher, representing nearly 80% of the total monosaccharides. This means that the Ara substituents of arabinoxylans were cleaved off the xylan backbone already at the lower severity, whereas at the higher severity, some of the Ara monomers were then converted to, *e.g.*, furfural (Gairola and Smirnova 2012), while the remaining xylan was also hydrolysed, accounting for the high content of Xyl in 171/72. Acetic acid produced by deacetylation of O-acetyl-galactoglucomannan (Song *et al.* 2013) may have acted as a catalyst for the hydrolytic reactions (Dussan *et al.* 2015). Besides Ara and Xyl, also the contents of Gal, Glu, and Man were high in 171/72 due to hydrolysis of GGM. However, obtaining Gal from GGM is undesirable for the preferred overall valorisation scheme of the CSPs, which involves isolating and valorising the GGM hemicelluloses (Widsten *et al.* 2023). To see whether reducing the pre-heating time at 171 °C would produce a less complex mixture of sugars, the time was varied from 3 to 72 min, and the 72 min was performed three times (Fig. 2). Indeed, the shortest pre-heating time produced a mixture of sugars, of which 88% were Ara. Although the other pre-heating times gave more Ara, they also resulted in a complex mixture of sugars. The sugar yields from the replicate 171/72 pre-treatments showed how much the yields of individual sugars can vary when produced at the same conditions.

Despite the high contents of Gal, Xyl, and Man in the CSPs produced at 171 °C and 36 to 72 min, it was preferred to focus further research on valorising 160/20, which contained high amounts of GGM, (arabino)xylan and Ara rather than 171/72 with a complex mixture of sugars requiring extensive separation work and little if any polysaccharides. 160/20 was also selected over 171/3 despite the higher purity of Ara in the latter because the concentration of Ara in 160/20 was 54% higher than in 171/3. The ultrafiltration at a cut-off of 10,000 Da of purified 160/20 allowed recovery of 91% the Ara in 160/20 in the permeate (160/20-P). The Ara, Gal, and Xyl contents of 160/20-P were 79.7%, 6.8%, and 5.1% of total sugars, respectively.

Besides the sugars, 160/20-P also contained oligosaccharides that could be hydrolysed to increase the yields of potentially recoverable sugars (Fig. 3). Acid hydrolysis of these oligosaccharides increased the amount of Ara by 38%. The contents of other sugars also increased substantially so that, overall, hydrolysis increased the sugar content from

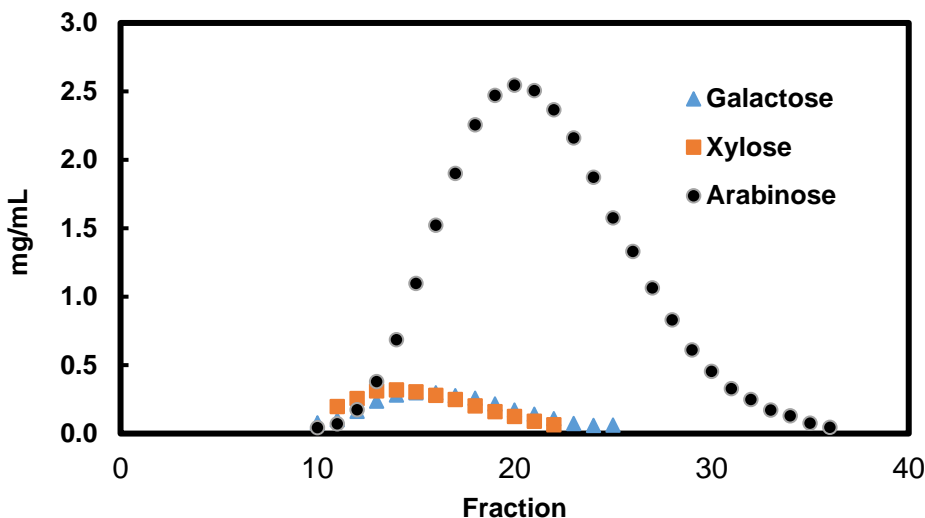
7.6 to 21.5 mg/L. The Ara-dominant unhydrolysed 160/20-P was selected for further research on sugar separation.



**Fig. 3.** Sugar content of CSP 160/20-P before (b.h.) and after (a.h.) acid hydrolysis. Each sample was analysed in triplicate (a-c). The error bars denote standard deviation of duplicate determinations.

### Separation of CSP sugars over Ca-CER

The ability of Ca-CER to separate the sugars of 160/20-P Ara from 160/20-P was investigated (Fig. 4).



**Fig. 4.** Elution curves of model sugars on Ca-CER

Co-elution of Ara, Gal, and Xyl was so severe that only 14% of Ara could be recovered at 100% purity and 30% at 97% purity (Fig. 5). In addition, there was hardly any separation of Gal and Xyl. This result agrees with those of earlier studies (Caruel *et al.*

1992; Saari *et al.* 2010) and indicates that the method could be used for separating Ara but the yield would be low. Using the method to obtain Gal when Xyl is present in significant proportion to Gal is not practical due to the similar elution curves of these sugars.

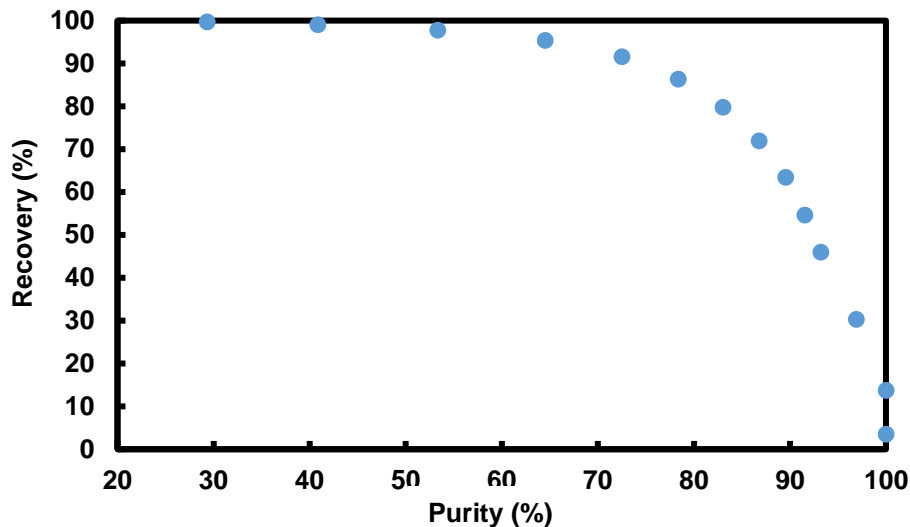


Fig. 5. Purity vs yield of Ara recovered from 160/20-P on elution over Ca-CER

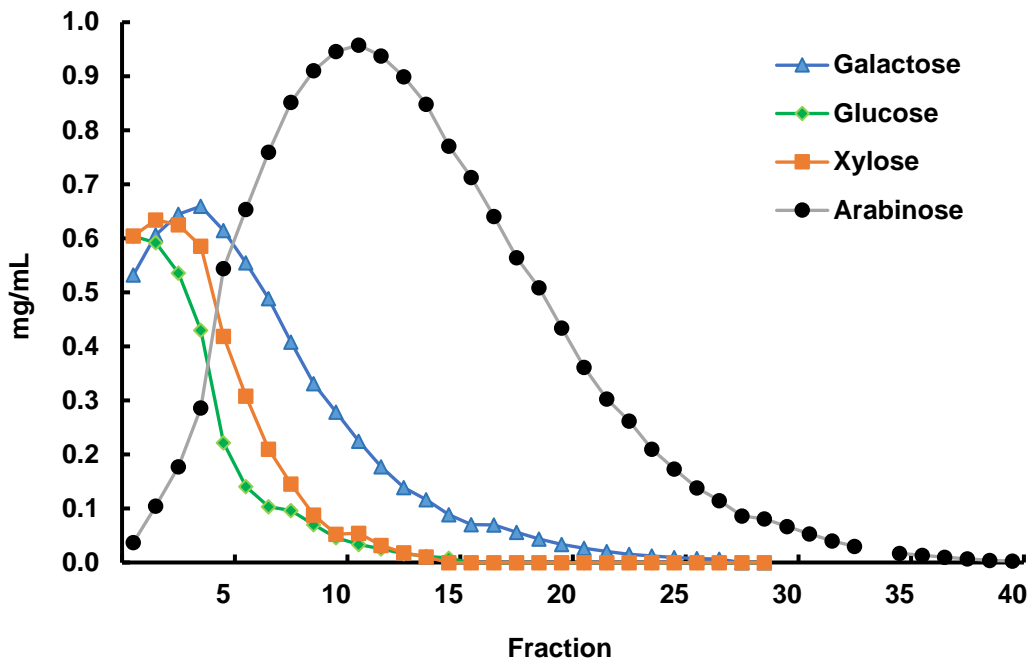


Fig. 6. Elution curves of model sugars on BaX

### Separation of CSP Sugars over BaX

To improve the separation of sugars, the elution behaviour of the four most prominent sugars in 160/20-P (Ara, Gal, Xyl, and Glu) over Ba-substituted zeolite X (BaX) was studied using a mixture of the pure sugars in which Ara was present in higher molar proportion than any of the other sugars. As shown in Fig. 6, this gave much better



separation of Ara from Gal and Xyl than Ca-CER, and the elution maximum of Glu occurred before the other sugars.

The elution of 160/20-P sugars over BaX reflected the behaviour of the model sugars (Fig. 7). The yields of Ara when isolated at different purities are shown in Fig. 8, with 100% and 99% pure Ara recovered at yields of *ca.* 25% and 50%, respectively. However, BaX did not work well for the other sugars and can be considered as a specific method for separating Ara from other sugars.

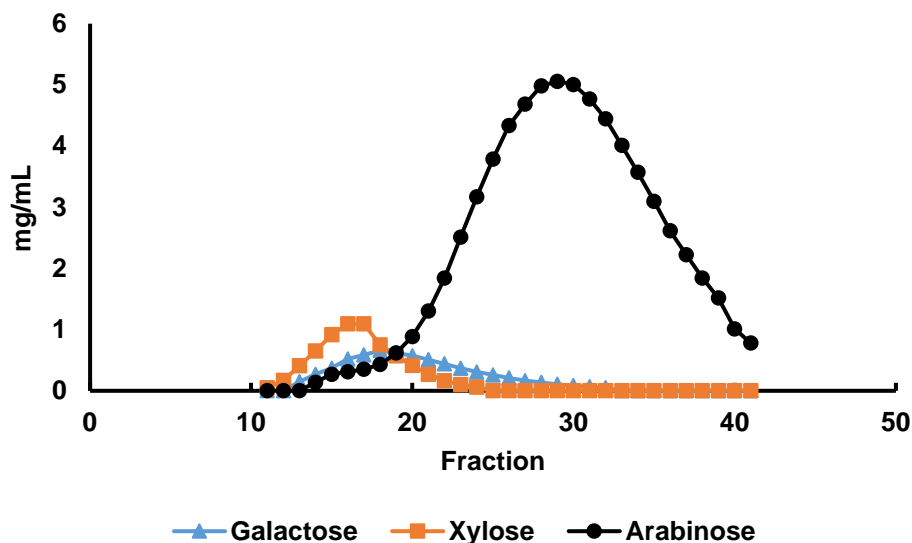


Fig. 7. Elution of the three main 160/20-P sugars on BaX

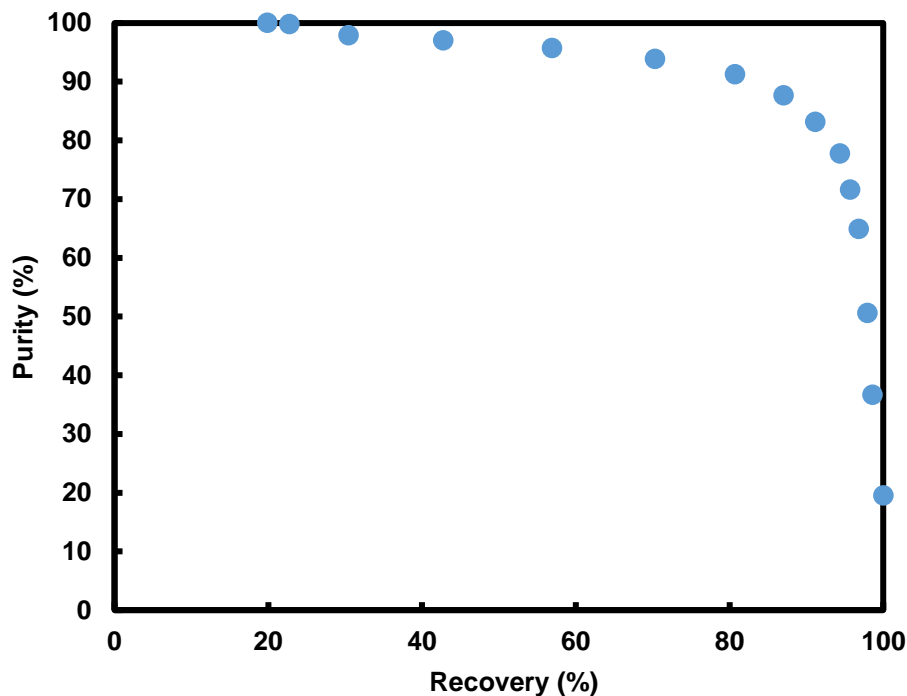
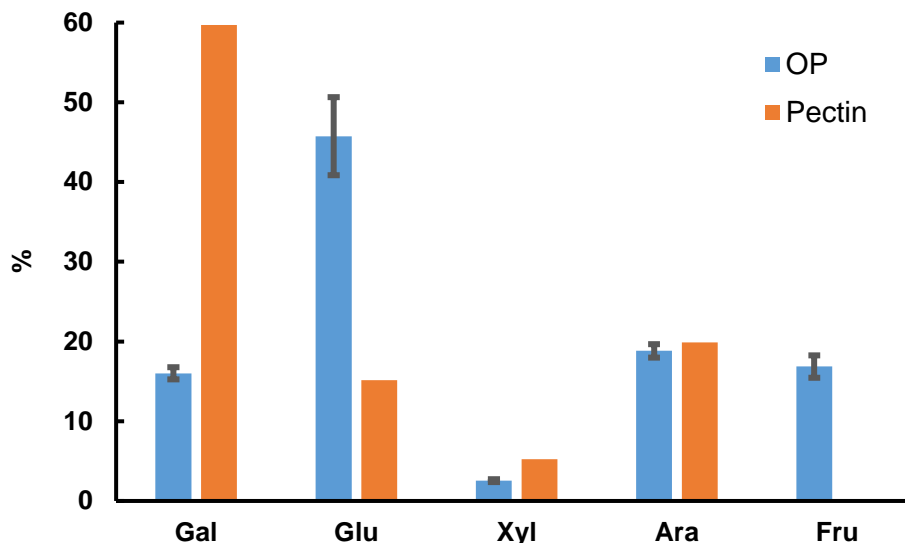


Fig. 8. Purity vs yield of Ara recovered from 160/20-P on elution over BaX

### Sugar Composition of OP and OP Pectin

Ara and Gal accounted for 15 to 20% of the sugars in the OP hydrolysate, while in the pectin hydrolysate Gal was the major sugar at 60% of the total and Ara comprised 20% of the sugars (Fig. 9).



**Fig. 9.** Sugar composition of OP and pectin hydrolysates. The error bars denote standard error of three parallel samples

### Fermentation of Orange Peel

In terms of recovering either sugar in high purity, OP can be considered as a good sidestream source of both Ara and Gal. From a valorisation point of view, it would probably be more cost-effective to obtain Ara directly from the OP rather than from the pectin isolated from it. Considering the BaX elution curves of Glu, Gal, and Ara, it can be estimated that the presence of large amounts of Glu relative to Gal and Ara would be unlikely to severely impact the separation of Ara from the other sugars, but it would significantly reduce the yield of Gal at high purity. Therefore, a fermentation pre-treatment was implemented to eliminate the low-cost and readily fermentable sugars, Glu and Fru, which had mostly disappeared after one week of fermentation, and after 2 to 4 weeks, only Gal and Ara remained (Fig. 10). After four weeks, the yields of Ara and Gal were 90% and 107% on the starting hydrolysate, respectively. The increase in the absolute amount of Gal may have been due to hydrolysis of oligomers in the pre-fermentation hydrolysate. Although Gal is a hexose like Glu and Fru, it is known to be resistant to fermentation by regular strains of *Saccharomyces cerevisiae* (Baker's yeast) and specially engineered strains are required to ferment it (Quarterman *et al.* 2016). In the current scenario, the fermentation of Gal can be a benefit or a drawback – if the only sugar to remain after fermentation were Ara, it could be recovered from the fermentation liquid without separation from other sugars. However, this would imply a loss of the other prominent and valuable OP sugar, Gal. A techno-economic analysis would reveal which strategy is better: one that provides pure Ara and bioethanol as a fermentation product, or one that requires a separation step for Gal and Ara over BaX but yields both sugars. The yield of bioethanol recoverable as a by-product of the process should also be considered in this context.

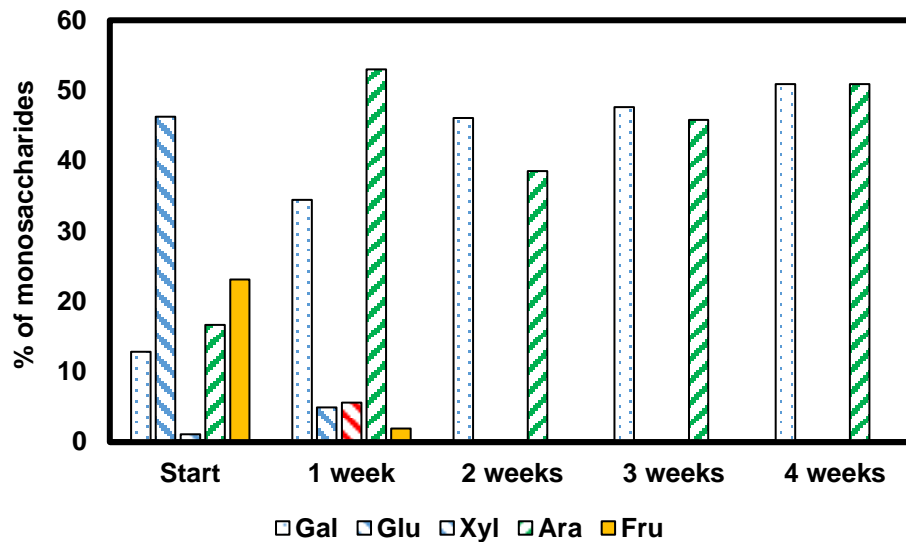


Fig. 10. Effect of fermentation with baker's yeast on sugar composition of OP as a function of time

### OP Pectin Hydrolysate

The main sugar present in the OP pectin hydrolysate was Gal at 60% of the total sugars, while the content of Ara (20% of the sugars) was similar to that of OP. In addition, it contained a much lower percentage of Glu than OP and no Fru. This reduces the need to ferment the pectin hydrolysate to improve the yield and purity of Gal on elution over BaX. However, fermentation to leave just Gal and Ara as in the case of OP might still be a good option. This option was not further evaluated in the present work.

Recovery of dry sugars from dilute elution fractions could be done by evaporation technology, *e.g.*, by spray- or freeze-drying but a more cost-effective way could be to use membrane filtration. Nanofiltration can have a high energy demand, but improvements in energy efficiency have been reported recently (Moulik *et al.* 2015).

### CONCLUSIONS

1. Production conditions (severity) of compression screw pressate (CSP) from thermomechanical pulp (TMP) of softwood could be adjusted to optimise valorisation of hemicelluloses and arabinose formed by autohydrolysis of wood polysaccharides.
2. CSP is a good potential source of arabinose. Barium-exchanged zeolite X (BaX) could be used for separating arabinose from the other prominent CSP sugars, galactose and xylose.
3. Orange peel is a good potential source of galactose and arabinose, and fermentation of orange peel hydrolysate left just Gal and Ara sugars to be separated. The hydrolysate of pectin isolated from orange peel was rich in galactose while containing little fermentable sugars compared to orange peel, so it is suited for elution over BaX to separate galactose and arabinose without fermentation.

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