A novel and integrative process: From enzymatic fractionation of wheat bran with a hemicellulolytic cocktail to the recovery of ferulic acid by weak anion exchange resin

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\textbf{ABSTRACT}

An integrated and eco-friendly process including enzymatic hydrolysis with a hemicellulolytic cocktail and a chromatographic purification step was developed to obtain ferulic acid from wheat bran. \textit{Thermobacillus xylanilyticus}, a thermophilic and hemicellulolytic bacterium, was able to produce enzymatic cocktails containing xylanase, xylosidase, arabinosidase and esterase activities. The cocktails produced were used to deconstruct destarched wheat bran, allowing the release of 6%, 20% and 37% (w/w) of monomeric arabinose, xylose and ferulic acid, respectively. A weak anionic resin under free-base form was proved successful to separate the carbohydrate fraction from the ferulate one after acidification. Ferulate was recovered at high concentration (15 g/L) during regeneration of the resin. This non-optimized purification step allowed recovering 67% of ferulic acid fixed on the resin. Global recovery of ferulic acid contained in wheat bran after both enzymatic fractionation and purification stages reached 21.8%.

1. Introduction

The bionrefinery concept embraces a wide range of technologies that aims to produce biofuels, chemicals and materials from various biomass resources (wood, grasses, corn...). Lignocellulosic biomass is a renewable feedstock abundant on earth and of interest for biorefinery. Lignocellulosic plant cell walls are mainly composed of cellulose, hemicelluloses and lignins in ratios depending on the plant, the organ and the cell type (Pauly and Keegstra, 2008). Wheat (\textit{Triticum}) bran (WB) is an abundant agricultural by-product composed of 40–50% of dry matter (DM) of cellulose, 25–35% DM of hemicelluloses and 15–20% DM of lignins (Celiktas et al., 2014).

The main hemicelluloses present in WB are arabinoxylans which represent up to 30% DM (Ebringerova et al., 2005). Hemicelluloses form a complex network composed of main chains of D-xylopyranose (xyl) residues branched by β-[1,4] bonds, which can be ramified by L-arabinofuranose (ara), D-glucuronic acid residues and acetate groups. Xylans from WB are characterized by a high ara/xyl ratio (0.72) (Rakotoarivonina et al., 2012). Arabinose residues can be esterified by phenolic compounds such as ferulic acid (Ebringerova et al., 2005) which can represent up to 0.5% DM in WB (Kroon et al., 1997). The fractionation of arabinoxylans allows the production of valuable molecules such as xyl, ara, xylo-oligosaccharides (XOs) and ferulic acid. Those molecules have diversified applications as fermentation products or bioactive ingredients (Deutschmann and Dekker, 2012). Recently, ferulic acid, already known as a precursor of vanillin, has
been used to synthesize bis- and tris-phenols (Lesage-Meessen et al., 1999; Pion et al., 2013; Priefert et al., 2001) that exhibit potent antiradical activity (Reano et al., 2015) and have been used for the preparation of biobased polymers (Ménard et al., 2017; Pion et al., 2015). Ferulic acid is produced by agricultural biomass enzymatic fractionation (He et al., 2015; Uraj et al., 2014).

Due to the biomass recalcitrance, fractionating hemicelluloses for producing the various constitutive molecules is still a challenge. The enzymatic fractionation of lignocellulosic biomass involves the use of enzymes with high selectivity and requires milder conditions than chemical fractionation, having a direct impact in the limitation of side-products production. Hemicelluloses such as endoxylanases, xylosidases and debranching enzymes (arabinosidases and esterases) are necessary to fractionate xylans into monomers. Endoxylanases catalyze the hydrolysis within xylan main chains whereas arabinosidases and esterases remove the arabinose, the acetyl and the phenolic acids substituents. Xylosidases liberate xylose from xyllooligosaccharides produced by xylanases (Dodd and Cann, 2009). These enzymes act in synergy and their presence is necessary for an optimal hydrolysis of xylans. These enzymes, mainly produced by fungi and bacteria, have been shown to efficiently produce xyl, ara, XOs and phenolic compounds such as ferulic acid by synergic actions (Aachary and Prapulla, 1999; Bonnin et al., 2002; Chapla et al., 2012). Many studies on the simultaneous release of carbohydrates and phenolic compounds have been carried out.

Thermobacillus xylanilyticus is a thermophilic bacterium able to produce an arsenal of hemicellulolytic enzymes depending on lignocellulosic substrates used for its growth (Rakotoarivonina et al., 2012). During its stationary phase of growth on wheat straw (WS) and destarched wheat bran (DWB), T. xylanilyticus produces different levels of xylanase activities as well as debranching enzymes activities. The feruloyl esterase activity produced by the bacterium was 2 folds higher when grown on WS instead of DWB (Rakotoarivonina et al., 2014). Since xylans from WS are less ramified, the ester bonds between ara and ferulic acid residues are probably more accessible, allowing a higher induction for the feruloyl esterase activity. Recently, the application of complete hemicellulolytic cocktails from T. xylanilyticus cultivated on WS at high enzyme loading produced an efficient fractionation of DWB and led to high monomerization of carbohydrates and ferulic acid (49% of monomeric xyl, 15% of monomeric ara and 50% of ferulic acid contained in DWB) (Rakotoarivonina et al., 2016).

Molecules liberated from lignocellulosic biomass fractionation must be separated and purified for further applications or transformations. Due to the DWB carbohydrates (60% DM) and ferulic acid (0.5% DM) composition, the hydrolysates obtained by using T. xylanilyticus’ cocktails contain mainly carbohydrates (Rakotoarivonina et al., 2016). The purification of ferulic acid, present in lower quantity in the hydrolysates, will bring asignificative added value to the process. The removal of phenolic compounds from hydrolysates has been largely studied due to their sometimes undesired organoleptic and nutritional effects. Soto et al. (2011) have reviewed the use of adsorption to remove them. However, few researchers have worked on recovery and purification of ferulic acid from complex hydrolysates. Studies generally focus on adsorbent resins or activated charcoal (Couteau and Mathaly, 1997; Ou et al., 2007; Tila et al., 2008). More recently, ferulic acid has been purified from corn (Zea maise) bran hydrolysates by membrane technologies combined to crystallization (Zhao et al., 2014). Besides, much work has been done on recovery and purification of aliphatic acids produced by fermentation such as acetic, lactic, malic and citric acids. Anion exchange has been widely reported in this field (López-Garzón and Straathof, 2014). With high capacities and regeneration close to stoichiometry, weak anion exchange resins display interesting features in the context of eco-friendly processes allowing highly concentrated fractions to be recovered (Fargues et al., 2010; Lameloise et al., 2015). Although weak anion exchange resins have never been studied for the purification of phenolic acids, such resins could be a cost-effective alternative to activated charcoal or absorbent resins.

Promising preliminary results were obtained with a model solution containing 0.4 g/L of ferulic acid (pH 3) and using an Amberlyst A21 (A21) resin from Dow Chemical in the free-base: a steep breakthrough occurred at 360 Bed Volume (BV) and the capacity for ferulic acid was high: 0.92 eq/Liter of Resin (LR), close to the supplier’s specification (1.2 eq/LR). Furthermore, ferulic acid was desorbed by NaOH 1 M in less than 3 BV (unpublished data). When applied to a complex hydrolysate, this strategy would allow ferulic acid-free carbohydrates to be recovered during adsorption and concentrated ferulate to be recovered during regeneration.

The aim of this study, was therefore to develop an eco-friendly integrated process using efficient enzymatic cocktails for the hydrolysis of arabinoolxans from DWB and obtaining purified fractions containing ferulic acid and carbohydrates by anionic exchange chromatography. The strategy was first to produce a hemicellulasic cocktail rich in feruloyl esterase activity by growing T. xylanilyticus on WS then test this enzymatic cocktail for the hydrolysis of DWB. Carbohydrates (mono- and oligosaccharides) and ferulic acid produced were then separated into two different fractions by implementing a purification step onto weak anionic resin.

2. Materials and methods

2.1. Material

2.1.1. Chemicals

NaOH and HCl (1 M) were purchased from Carlo Erba (Val-de-Reuil, France). Beechwood xylans were purchased from Roth (Karlsruhe, Germany), methyl-ferulate from Apin Chemicals (Abingdon, UK) and the other substrates were purchased from Sigma-Aldrich (Saint Louis, USA). Monosaccharides, anions and ferulic acid standards were purchased from Sigma-Aldrich (Saint Louis, USA). Tetradecyltrimethylammonium hydroxide (OFM-OH) was purchased from Waters (Guyancourt, France). XOs were purchased from Megazyme (Wicklow, Ireland). All other chemicals or reagents were at least of analytical grade and supplied by Sigma-Aldrich unless specified otherwise.

2.1.2. Bacterial strain and lignocellulosic substrates

T. xylanilyticus strain XE is available at the Collection Nationale de Culture de Microorganismes (France) under the number CNCM I-1007. DWB and WS are from Apache variety and were obtained from ARD (Pomacle, France). Both substrates were ground (1 mm).

2.1.3. Anion exchange resin

Anion exchange Amberlyst A21 resin was purchased from Sigma-Aldrich (Saint Louis, USA). It is a weak polystyrenic resin, with tertiary amines – NR2 representing more than 85% of the functional groups. The apparent density of the resin was 273 g/LR as measured by Fargues et al. (2010). The resin was poured in a glass column (Omnifit 25 cm × 0.78 cm i.d) purchased from Sigma-Aldrich (Saint Louis, USA). The flow was adjusted with a peristaltic pump (Minipuls 3, Gilson, Villiers-le-Bel, France) and the fractions were collected with a Gilson collector.

2.2. Methods

2.2.1. T. xylanilyticus culture and cocktails production

Medium cultures (10 mL–200 mL) were inoculated with 1% (v/v) of...
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