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Bio-conversion of apple pomace into ethanol and acetic acid: Enzymatic hydrolysis and fermentation

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HIGHLIGHTS

- Potential use of apple pomace to manufacture high value chemicals was explored.
- Enzymatic saccharification was optimized using cellulase, pectinase, β-glucosidase.
- Sugars were fermented into ethanol and acetic acid using yeast and bacteria.
- Enzymatic digestibility was enhanced by dilute acid and laccase pre-treatments.

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ABSTRACT

Enzymatic hydrolysis of cellulose present in apple pomace was investigated using process variables such as enzyme activity of commercial cellulase, pectinase and β-glucosidase, temperature, pH, time, pre-treatments and end product separation. The interaction of enzyme activity, temperature, pH and time had a significant effect ($P < 0.05$) on release of glucose. Optimal conditions of enzymatic saccharification were: enzyme activity of cellulase, 43 units; pectinase, 183 units; β-glucosidase, 41 units/g dry matter (DM); temperature, 40 °C; pH 4.0 and time, 24 h. The sugars were fermented using *Saccharomyces cerevisae* yielding 19.0 g ethanol/100 g DM. Further bio-conversion using *Acetobacter aceti* resulted in the production of acetic acid at a concentration of 61.4 g/100 g DM. The present study demonstrates an improved process of enzymatic hydrolysis of apple pomace to yield sugars and concomitant bioconversion to produce ethanol and acetic acid.

1. Introduction

Valorization of waste coming out from food processing plants is becoming an important contributor to the food industry and bio-economy. Fruit processing industry generates large volumes of biological by-products that could be used for manufacture of value-added products. In lieu of this, several thousand tons of apple processing by-products generated by fresh-cut, juice, pie and sauce manufacturing plants seek attention due to reasons such as high disposal costs and associated environmental concerns. Nevertheless, these waste by-products, especially apple pomace, are rich in both soluble carbohydrates such as simple sugars (fructose, glucose, sucrose) and polysaccharides (cellulose, hemicellulose, pectin), representing a high potential for further bio-conversion purposes (Vendruscolo et al., 2008). In this context, bio-conversion of apple pomace into bio-ethanol and organic acids provides an excellent possibility to reduce the environmental challenge posed by its vicinity. Among polysaccharides in apple pomace, cellulose (23% DW) and pectin (10% DW) present a useful substrate for enzymatic hydrolysis to yield fermentable sugars (Parmar and Rupasinghe, 2012). Commercial cellulase, pectinase and β-glucosidase are generally used for hydrolysis of polysaccharides present in fruit by-products. Cellulose and pectin, when hydrolyzed into glucose and galacturonic acid, respectively, can be converted into bio-ethanol and/or organic acids through fermentation.

The polysaccharides present in apple are marked by interactions among themselves and other polymers, which pose limitations to access of hydrolyzing enzymes. High lignin to cellulose ratio (roughly 1:1) has been reported for apple pomace as a reason for its hampered bio-processing (Parmar and Rupasinghe, 2012). In addition, previous studies have shown that cell wall of apple contains a pectin matrix, which shields cellulose and hemicellulose, thereby decreasing its enzymatic digestibility (Oechslin et al., 2003). A study by Gullón et al. (2008) investigating the enzymatic digestibility of apple pomace used cellulase and cellobiase, for optimizing the hydrolysis conditions, following a factorial, incomplete, centered, second-order experimental design for kinetics of

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sugar generation. However, apple pomace being rich in pectin, the importance of pectinase in facilitating sugar release by disrupting the pectin network around cellulose cannot be overlooked. Therefore, break down of lignin and pectin network would facilitate enzymes access to cellulose, consequently releasing more glucose. In addition, the synergistic action of carbohydrolases such as pectinases, cellulases and β-glucosidase have long known to be crucial for liquefaction and saccharification of apple pomace (Capek et al., 1995; Will et al., 2000). Pectinases are composed of four types of enzymes: (1) protopectinases (EC. 3.2.1.99) that convert insoluble protopectin into soluble pectin; (2) pectinesterase (E.C. 3.1.1.11), responsible for removal of methoxyl esters, resulting in acid pectins and methanol; (3) polygalacturonases (endo, EC. 3.2.1.15 andexo, EC. 3.2.1.67), hydrolyzing the polygalacturonic acid chain and (4) pectatelyases (endo, E.C. 4.2.2.2 andexo E.C. 4.2.2.9), that depolymerize pectic substances by a trans-eliminative split at C-4. Cellulases comprise of three types of enzymes: (1) endoglucanases (EC 3.2.1.4), which act on less crystalline regions of cellulose, resulting in free chain ends; (2) exoglucanases or cellobiohydrolases (EC 3.2.1.91), which attack on the free chain ends, resulting in cellobiose units; and (3) β-glucosidase (EC.3.2.1.21), which degrades cellobiose into glucose units.

Liquefaction and saccharification of apple pomace has been carried out to produce organic acids such as lactic acid (Alonso et al., 2009; Gullón et al., 2007, 2008), liquid polyols (Briones et al., 2011), oligomeric mixtures (Gullón et al., 2007) and releasing polyphenols (Will et al., 2000). Although the use of carbohydrolases for apple pomace hydrolysis has been reported in previous studies, to the best of our knowledge, none has attempted to standardize hydrolysis conditions of apple pomace using multi-factorial statistical designs including three major enzymes (cellulase, pectinase and β-glucosidase), varying enzyme loading, pH, temperature, and reaction time. Therefore, the present study was conducted to apply commercial pectinolytic and cellulolytic enzymes for hydrolysis of polysaccharides (cellulose and pectin) present in apple pomace and to standardize the parameters of enzyme loading, reaction temperature, working pH and duration required for maximal hydrolysis. Hydrolysis yields of the pretreated apple pomace as well as results for subsequent fermentability of the hydrolyzates into ethanol and acetic acid have also been assessed.

2. Methods

2.1. Plant material

Apple pomace used in this study was collected from a commercial juice manufacturer (J.W. Mason and Sons Ltd., Windsor, NS, Canada) during the year 2008–2009. About 120 kg of collected pomace was from 'McIntosh' cultivar of apples and was further sub sampled for experimental purposes. About 10% of the total weight of pomace contained rice husks which are commonly used by the industry as a pressing aid. Apple pomace was homogenized into a puree using a food processor (BioSpec Products, Inc. Bartlesville, OK, USA). The pH of the puree was determined using a standardized pH meter and was 3.5 ± 0.2. The compositional analysis of the raw material has been presented elsewhere (Parmar and Rupasinghe, 2012). Briefly, fresh apple pomace consisted of 21 g total dry matter (DM)/100 g fresh weight. On DM basis, apple pomace contained 3.8 g/100 g total proteins, 2.3 g/100 g total fat, 3.5 g/100 g ash, 18.2 g/100 g total reducing sugars glucose and fructose), and 44.9 g/100 g total dietary fiber (35 g/100 g being the insoluble dietary fiber). Among polysaccharides, cellulose represented 22.2 g/100 g DM, pectin represented most part of the soluble fiber content (9.9 g/100 g DM) and hemicellulose was found to be 5.5 g/100 g DM. In addition, the apple pomace contained high amount of lignin (21.2 g/100 g DM) due to the added rice hulls to enhance juice press. The reminder was reducing sugars, starch and other carbohydrates which were not analyzed.

2.2. Reagents and chemicals

Citric acid (reagent grade) and sodium citrate, sulfuric acid (reagent grade), HPLC grade acetonitrile were purchased from Fisher Scientific (Ottawa, ON, Canada). Analytical grade standards of sugars (glucose, fructose and sucrose), ethanol and acetic acid were obtained from Sigma Aldrich (Oakville, ON, Canada).

2.3. Enzymes and microorganisms

Commercial enzyme concentrates of pectinase (Pectinex 3XL®) and cellulase (Celluclast 1.5L®), were obtained from Novozymes North America Inc. (Franklinton, NC, USA), while β-glucosidase (Novozyme 188) was purchased from Sigma Aldrich (Canada). The enzymes were stored at 4 °C until use. Celluclast 1.5L, Pectinex 3XL and Novozyme 188 had 700, 3000 and 665 EU/mL activity, respectively, as declared by manufacturers. Laccase (isolated from *Trametes versicolor*) was obtained from Cedarlane (Burlington, ON, Canada). *Saccharomyces cerevisiae*, also called baker's yeast was obtained from a local winery and stored in a refrigerator at 4 °C. Mother culture of *Acetobacter aceti* was purchased from the Vinegar Centre, Pischelsdorf, Austria.

2.4. Enzymatic hydrolysis

Assessment of enzymatic hydrolysis of apple pomace was carried out according to the following conditions: (a) enzyme loading for Celluclast 1.5L as 5.3, 21.3 and 43 EU/g DM; Pectinex 3XL as 23, 91.4 and 183 EU/g DM; Novozyme 188 as 5.1, 20.3 and 40.5 EU/g DM; temperature of 40, 45 and 50 °C; pH of 4.0, 4.5 and 5.0; time of 1, 6, 12 and 24 h. The lowest level enzyme loading was selected based on their commercial application in apple mash treatment (Novozymes North America Inc., Franklinton, NC, USA). A preliminary trial (data not shown) showed that enzyme loading greater than 43, 183 and 41 EU/g DM for Celluclast 1.5L, Pectinex 3XL and Novozyme 188, respectively, resulted in no significant increment in glucose yields at constant temperature, pH and time. Therefore, these values were selected as the highest level of enzyme loading for the three enzymes used. Based on optimal activity of selected enzymes (as recommended by the manufacturer), the temperatures (40, 45 and 50 °C) and pH levels (4.0, 4.5 and 5.0) were selected. These process conditions were used to standardize the value of each independent factor as the basis of all further trials.

Enzyme hydrolysis was carried out in 125 mL Erlenmeyer flasks with working volume of 50 mL puree (equivalent to 12.5 g fresh weight (FW) apple pomace) with continuous shaking of 150 rpm (HP50 Model Apollo, San Diego, CA, USA). The pH of the puree was adjusted with 0.05 N citrate buffer. The enzyme cocktail was suspended in 25 mL citrate buffer for 10 min. prior to its addition to substrate. To prevent possible microbial spoilage, 100 μL of 2% sodium azide was added. The substrate was allowed to reach the desired operating conditions and time zero h samples were collected before addition of enzymes. A micropipette was used for sampling (1 mL), using a pipette tip with the end cut off. Samples were collected at desired time points (1, 6, 12, 24 h) and placed in micro centrifuge tubes prior to placing them in freezer (−20 °C).

2.5. Effect of dilute acid pretreatment and polyphenol degradation on enzymatic hydrolysis

Previously optimized pretreatment conditions of 1.5 g/100 mL sulfuric acid and 91 °C reaction temperature for 16 min. were used