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Simultaneous saccharification and fermentation of industrial sweetpotatoes for ethanol production and anthocyanins extraction

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ABSTRACT

A simultaneous saccharification fermentation (SSF) system was studied for ethanol production in flour industrial sweetpotato (ISP) feedstocks (lines: white DM02-180 and purple NC-413) as an integrated cost saving process, and to examine the feasibility of extracting anthocyanins from flour purple ISPs under a simultaneous extraction and fermentation (SEF) system. Furthermore, a separate hydrolysis fermentation (SHF) configuration was carried out to establish a baseline in sugar consumption and ethanol production from the ISP lines. The thermotolerant ethanol producing yeast strain *Kluyveromyces marxianus* NCYC 851 and the mesophilic *Saccharomyces cerevisiae* Ethanol Red were evaluated, using commercial alpha amylases for hydrolysis of available ISP starch to sugars. Fermentation by *S. cerevisiae* during SHF had an ethanol yield of 0.32 g/g dry ISP, a 1.1-fold increase above that produced by *K. marxianus*. Subsequent studies showed that ethanol yield could be increased in a SSF system with a maximum ethanol yield of 0.39 g/g dry ISP achieved, a 15% increase compared with using a SHF system when *S. cerevisiae* was used. Simultaneous extraction and fermentation of flour purple-fleshed NC-413 ISPs was studied to evaluate the effect of pH on extraction of total monomeric anthocyanins (TMA) and ethanol production. On average, maximum ethanol yield ranged from 0.31 to 0.34 g/g dry ISP and TMA concentration ranged from 45 to 64 mg cyanidin-3-glu/100 g dry powder (10–22 mg/100 g fresh weight) with the greatest ethanol production coming from non-adjusted pH fermentations. The highest anthocyanin recovery, 64 mg cyanidin-3-glu/100 g dry powder was obtained at 35 °C and pH 4.5 using *S. cerevisiae* Ethanol Red. This study showed the feasibility of extracting anthocyanins and producing ethanol simultaneously in one unit operation without the need of purified solvents.

1. Introduction

Demand for biodegradable and renewable resources to generate bioenergy and bioproducts has been increasing due to the necessity to rely less on petroleum and develop sustainable, eco-friendly materials and products. Bioethanol production is derived primarily from sugar crops (i.e. sugar cane) and starch-based food crops (i.e. corn). However, in the United States of America the use of cereal starches to produce bioethanol competes with agricultural land needed for food/feed production (Schenk et al., 2008; Naik et al., 2010). Therefore, it is important to find alternative renewable feedstock sources for valuable fuel and chemical commodities.

Sweetpotato (*Ipomoea batatas*) is an important starch-producing crop grown around the world primarily in the tropics and subtropics. The storage roots store energy in the form of carbohydrates representing around 80–90% of its dry matter and consisting mainly of starch and sugars (Aina et al., 2012). Sweetpotatoes bred for high dry matter content (30–40%, of which 60–90% is starch) in the US are not considered a food crop and have significant potential in industrial applications. These industrial sweetpotatoes (ISPs) are an excellent substrate for alcohol fermentation since they contain large amounts of starch, high starch yield per unit of land cultivated (12–14 MG dry starch/ha (10,926–12,297 lb dry starch/acre)), and have advantages in production including low requirements of fertilizer and pesticides, drought tolerance, and feasible growth on marginal lands (Duvernay et al., 2013; Kim and Hamdy, 1985).

Several studies have examined sweetpotato conversion to ethanol and valued-added products using enzymes (Duvernay et al., 2013; Kim and Hamdy, 1985; Bridgers et al., 2010; Zhang

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et al., 2011; Srichuwong et al., 2012). These studies have primarily focused on optimizing process conditions (e.g. substrate loading, enzyme loading, temperature, and yeast concentration) and on reducing sweetpotato viscosity during processing to improve ethanol production yield. Separate hydrolysis and fermentation (SHF) is a common process used to convert sugars to ethanol because optimal temperatures can be set in each unit, but hydrolysis product (glucose) is accumulated in the reactor and can lead to enzyme inhibition as glucose concentration increases (Drissen et al., 2009; Ohgren et al., 2007). Challenges with enzyme inhibition in a SHF system could be resolved using a simultaneous saccharification fermentation system (SSF). SSF is an alternative approach to produce ethanol and offers advantages of minimizing enzyme inhibition (reduction of glucose accumulation) and investment cost (single unit operation and vessel), and supports shorter processing times (Ohgren et al., 2007; Wyman, 1994; Isci et al., 2008; Olofsson et al., 2008).

Recent studies in SSF on sweetpotatoes have focused on evaluating process conditions, such as liquefaction temperature, enzyme loading, substrate loading, for ethanol production using sweetpotato lines with low gelatinization temperatures (line Kyushu 159) and the use of cellulases in very high gravity sweetpotato mashes to allow viscosity reduction prior to liquefaction (Zhang et al., 2011; Srichuwong et al., 2012; Cao et al., 2011). The primary objective of these research efforts was to decrease energy consumption during liquefaction, but little work has been done to assess performance of yeast and SSF operating temperatures to maximize rate of starch hydrolysis, improve sugar consumption rates, and enhance ethanol production.

Since the optimal operating temperature for glucoamylase enzymes used for hydrolysis is around 60 °C, it would be advantageous to use a temperature tolerant microorganism capable of growth and ethanol production at higher temperatures. *Kluyveromyces marxianus* is one of the most used thermotolerant yeast reported in the literature with maximum growth temperatures reported up to 52 °C and alcohol production at temperatures above 40 °C, using sugars from substrates including switchgrass, barley straw, and cellobiose (Banat et al., 1998). The use of a thermophilic microorganism in a SSF system has the potential to enhance conversion of industrial high dry matter sweetpotatoes to ethanol.

In addition, purple-fleshed ISPs produce high quantities of starch and antioxidants in the form of anthocyanins (Bridgers et al., 2010). Anthocyanins have been extensively reported to help reduce the risk of cardiovascular disease, chronic disease, and neuronal degeneration (Wu et al., 2008; Bridle and Timberlake, 1997), with industrial applications as a natural food and textile colorant and as a nutraceutical ingredient (Deroles, 2009; Wegener et al., 2009). Production and concentration of anthocyanins can be affected by environmental factors such as pH, UV radiation, temperature, and presence of nitrogen and phosphorous compounds (Wu et al., 2008; Castañeda-Ovando et al., 2009; Pascual-Teresa and Sanchez-Ballesta, 2008). Generally, at low pH (~4) the flavylium cation (red in color) is the frequent form of the anthocyanin, but the increase in pH can cause its denaturation. Extraction of anthocyanins is typically performed with solvent (acetone, ethanol, methanol, acidified methanol, and acidified ethanol) at specific pH levels (Bridgers et al., 2010; Brown et al., 2005; Steed and Truong, 2008; Cevallos-Casals and Cisneros-Zevallos, 2003). SSF on purple-fleshed ISPs under acidic pH can be further examined as an approach to simultaneously extract anthocyanins with reduced inputs and generate in a single step two value-added product streams (ethanol and anthocyanins).

This study investigated the potential fermentability of sugars from ISPs using the mesophilic yeast *Saccharomyces cerevisiae* and the thermophilic yeast *K. marxianus*. The aim was to establish SSF parameters for ethanol fermentation using flour preparations of ISPs (lines NC-413 and DM02-180) and examine the feasibility of extracting anthocyanins from flour purple-fleshed ISPs (line NC-413) during ethanol fermentation through a SEF system while considering the effect of pH.

2. Materials and methods

2.1. Substrate preparation

Two different sweetpotato (swp) clones, purple-fleshed ISP line NC-413 and white-fleshed ISP line DM02-180, were selected from the sweetpotato breeding program at NCSU (Raleigh, NC) and used in experiments. Sweetpotato line NC-413 was grown and harvested during the 2011 cropping season from the Cunningham Research Station at Kinston, NC (35.298° N, 77.575° W). Sweetpotato line DM02-180 was harvested during the 2011 cropping season from the Horticultural Crops Research Station at Clinton, NC (35.023° N; 78.278° W). After harvest, the roots were cured and stored (14 °C, 85% rh, 9 months). All roots were selected at the same time and used within 2 months. Roots were washed to remove soil, dirt, and then stored (14 °C, 2 days) before use. Sweetpotato roots were randomly selected from a stored batch and flour preparations were prepared by placing sliced (~2 mm thickness) roots in an oven at 70 °C for 60 h and dried samples were ground (using a Wiley mill) through a 2 mm mesh screen. Flour samples were stored in sealed plastic bags in the refrigerator (4 °C) until use.

2.2. Solvents, enzymes, and yeasts

Ethyl alcohol (Cat #E190, Pharmco-AAPER, 95%) used in starch alcohol insoluble solids (AIS) determination was of ACS/USP grade. Commercial sources of α-amylase and glucoamylase were used in the hydrolysis experiments. The α-amylase used for liquefaction was Liquozyme SC (Novozymes, North America, stored at 4 °C, density 1.25 g/ml) with optimal temperature of 85 °C, optimal pH of 5.5, and activity of 120 KNU-S/g enzyme. One Kilo novo unit (KNU-S) is the amount of enzyme that can hydrolyze 5.26 g of soluble starch per hour. The glucoamylase used in the saccharification studies was Spirizyme Ultra (Novozymes, North America, stored at 4 °C, density 1.15 g/ml) with optimal temperature of 65 °C and activity of 900 AGU/g protein. One amyloglucosidase unit (AGU) is the amount of enzyme able to hydrolyze 1 μmol of maltose per minute at 37 °C and pH 4.3.

S. cerevisiae Ethanol Red Yeast (Lesaffre Yeast Corp., Milwaukee, WI, optimum growth temperature 35 °C) and *K. marxianus* var *marxianus* NCYC 851 (National Collection of yeast cultures in UK, optimum growth temperature 42 °C) were used for the fermentation studies. *S. cerevisiae* and *K. marxianus* yeast culture freezer stock concentrations (liquid) were on average 1.3 mg dry cells/ml and 1.1 mg dry cells/ml, respectively and cultures were preserved in glycerol (8% salts (KH_2PO_4, $CaCl_2{\cdot}2H_2O$, $(NH_4)_2SO_4$, $MgSO_4{\cdot}7H_2O$), 50% glycerol, and 42% water) at −80 °C. Seed cultures for ISP fermentations were grown in YPD medium (Fisher BioReagents BP-2469, Fisher Scientific, Pittsburgh, PA) under the respective growth conditions until the appropriate cell density was reached for inoculation.

2.3. Experimental design and statistical analysis

2.3.1. Separate hydrolysis and fermentation in white DM02-180 and purple NC-413 ISPs

Use of sugar hydrolysates without prior sterilization can encounter contamination issues by foreign microorganisms and commonly used preservatives during hydrolysis, such as sodium