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Effect of pectinolytic and cellulytic enzymes on the physical, chemical, and antioxidant properties of blueberry (*Vaccinium corymbosum* L.) juice

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ABSTRACT

Blueberry (*Vaccinium corymbosum* L.) fruit, rich in anthocyanins, polyphenolics and antioxidant activity, has not been exploited fully for processing into juice. The objective of this study was to investigate the effect of commercial juice extraction enzymes (Cellubrix, Pectinex Ultra SPL, and Crystalzyme) on juice yield, total soluble solids (TSS), juice clarity, HPLC sugars profile, total anthocyanins (ACY), total phenolics (TPH), and antioxidant activity (assayed as FRAP). Enzyme treatments resulted in significantly ($p \leq 0.05$) higher juice yield (86.91–87.29 mL/100 g with Pectinex and Crystalzyme as compared to 79.45 mL/100 g from control), higher juice clarity, and titratable acidity, and lower extraction loss. However, enzymes treatment had no effect on total soluble solids (°Brix), fructose, glucose, and total sugars. Generally, juice Hunter color values (L, a, and b), Hue angle (h°) and Chroma (C*) were affected positively by the enzymes treatment. As compared to the control (9.69 mg/100 mL), total ACY were significantly higher (11.49–12.90 mg/100 mL) in juice from enzyme-treated macerates; whereas there was no effect on TPH, determined as gallic acid equivalent (96-89–101.30 mg GAE/100 mL) and FRAP antioxidant activity (3.87–3.97 μmol TE/100 mL). Total ACY, TPH, and FRAP were several-fold higher in the pomace than the juice.

1. Introduction

Blueberry (genus *Vaccinium* L.) is native to North America. Genotypes include wild types, often low-bush, and cultivated types, usually high-bush. (Strik & Yarborough, 2005). The high-bush blueberry (*Vaccinium corymbosum* L.) is most often cultivated commercially. The worldwide production of blueberries has increased three-fold since 1990, and the U.S. is the leading producer, contributing about three-fifth of the total crop (FAO, 2017). Almost three-fifth of the commercially produced fruit is sold as fresh and the remainder goes for processing, mainly frozen; other processed forms are canned and dehydrated blueberries (USDA, 2015).

Blueberries have a high and complex anthocyanin and polyphenolic content (Cesa, Carradori, Bellagamba, Locatelli, Casadei & Masci, 2017; Chen, Zhao, Tao, Zhang, & Sun, 2015; Skrede, Wrolstad, & Durst, 2000; Lee & Wrolstad, 2004), of which polyphenols contribute to antioxidant activity. In a USDA study, blueberries were shown to possess the high antioxidant capacity, i.e., 9135 and 6220 μmol TE/100 g for low- and high-bush blueberries, respectively (Wu et al., 2004). Low-bush blueberries contained the highest total phenolics (709 mg GAE/100 g), followed by blackberries and high-bush blueberries (660 and 531 mg GAE/100 g, respectively) (Wu et al., 2004). Interest in the role of antioxidants in human health has prompted research interventions to best retain antioxidants, such as polyphenols, during postharvest storage and value-added processing of fruits (Aaby, Grimmer, & Holtung, 2013; Kalaycıoğlu & Erim, 2017; Lee & Wrolstad, 2004; Siriwoharn & Wrolstad, 2004). According to The Institute on Medicine, a dietary antioxidant is defined as "a substance in foods that significantly decreases the adverse effects of reactive species (oxygen and nitrogen species) on normal physiological function in humans" (Prior, 2015). The researcher noted that the occurrence of reactive oxygen species or oxidative stress typically is an outcome of several degenerative diseases that are related to aging or it may also be a causative factor in the development of a number of diseases; for example, arthritis, atherosclerosis, cancer, diabetes, infection, ischemia and Parkinsonism have oxidative stress as a contributing factor for these diseases (Prior, 2015).

Development of new blueberry products that conserve the high antioxidant nature of blueberries include drum-dried powder and juice (Girard & Sinha, 2012), puree (Zorenc, Veberic, Stampar, Koron, & Mikulic-Petkovsek, 2017), osmotically and microwave-assisted dried

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blueberries (Zielinska, Sadowski, & Błaszczak, 2015), blueberry leather (Chen & Martynenko, 2018), frozen blueberries (Akharume, Singh, & Sivanandan, 2016) and blueberry jam (Zhang, Ren, Zhang, Li, Liu, Guo, & et al., 2016). The statements about the high antioxidant levels of processed fruit products is displayed prominently on the label as a marketing tool. As the single-strength blueberry juice lacks optimum sensory attributes for consumer acceptance, it is usually marketed as a concentrate, juice cocktail, or juice blend, although a niche market is emerging for single-strength blueberry juice due its health benefits. Given the additional market potential, maximizing the content of health beneficial compounds in the blueberry juice extraction process needs to be optimized.

Most of the blueberry antioxidants are located in the fruit peel Girard and Sinha (2012). These antioxidants can be released into juice if blueberry fruit is thoroughly macerated using pectinolytic and cellulytic enzymes to breakdown pectin and cell wall, respectively. Although the use of these enzymes is common for the extraction of fruit juices (Cerreti, Liburdi, Benucci, & Esti, 2016; Lee, Durst, & Wrolstad, 2002; Ribeiro, Henrique, Oliveira, Macedo, & Fleuri, 2010; Sandri, Lorenzoni, Fontana, & da Silveira, 2013), the work on blueberry juice extraction process is lacking with respect to the effect on phytochemicals characterization and antioxidant activity. A recent study used enzymes for blueberry juice extraction but anthocyanins, total phenolics, and antioxidant activity were not reported (Stein-Chisholm, Finley, Losso, & Beaulieu, 2017). Therefore, the main objectives of this study were to use selected pectinase and cellulase enzymes for the optimization of juice yield and investigate the effect of these enzymes on the physical and chemical quality of blueberry juice, including total anthocyanins, total phenolics, and antioxidant capacity. The results will have practical significance for the blueberry juice industry to not only maximize the yield but optimize the content of bioactive compounds in the juice.

2. Materials and methods

2.1. Materials

Fully-ripe blueberries (var. *Bluecrop*) were purchased from a local source in Lansing, Michigan. Juice extraction enzymes used were Pectinex Ultral SPL® and Cellubrix® (Novozyme, Denmark, distributed through Gusmer Enterprises, Fresno, CA, USA), and Crystalzyme® (Valley Research, South Bend, IN, USA). Nylon filter cloth was purchased from Day Equipment (Goshen, IN, USA). The selection of these enzymes was based on their respective activities for maximizing juice yield: Pectinex, a cocktail of pectinases, breaks down pectin; Cellubrix, a cellulase, maximizes yield by softening fruit skin; and Crystalzyme selectively breaks down pectin and complex polysaccharides. Enzymes were kept at refrigerated temperature, until used for juice extraction. All the solvents, chemicals and buffers were of reagent grade, and purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Juice extraction

Blueberries were thoroughly cleaned and washed in warm tap water to remove any dirt or debris. Two kilogram blueberries with 1.38x distilled water were blended at high speed for 2 min in a Waring blender (model 51BL32, Waring Commercial, Torrington, CT, USA). Addition of water was necessary since blended blueberries alone were too thick or viscous, exhibiting almost jelly-like consistency. The blended mass was rapidly heated to 65 °C in double jacketed stainless steel steam kettle, with constant stirring using an impeller-type stirrer to attain uniform heating. The macerate was held at 65 °C for 15 min. Heating of the macerate to this high temperature was done to significantly reduce the activity of polyphenol oxidase, an enzyme implicated in color loss. The heated macerate was allowed to cool down to < 50 °C, before the juice extraction enzymes were added separately at a ratio of 0.15 mL/kg. After holding for two hours at 45 ± 2 °C for the extraction enzymes to work, juice was extracted using a rack and cloth press. The pressed juice was finally filtered through a fine-mesh nylon cloth to remove any fruit residue. A total of four batches, using three replicates per batch were processed; one as the control (hot macerate, no added enzyme) and three treatments using commercial enzymes: Cellubrix, Pectinex Ultra SPL, and Crystalzyme). Extracted juice was batch-pasteurized in a shaking water bath at 85 °C for 1 min in 100-mL Pyrex glass test tubes with screw caps. In order to monitor the temperature during pasteurization, digital thermometers with stainless steel stems were placed in two test tubes through an opening in the caps. Unless noted otherwise, all experiments including analyses of physical and chemical properties of the juice were done using three replicates.

2.3. Juice yield and clarity

Juice yield and pomace/residue were determined as mL/100 g and g/100 g, respectively. Juice clarity or turbidity was determined according to the method of Ough, Noble, and Temple (1975). Twenty milliliters of juice samples were centrifuged at 360xg for 15 min to remove any suspended particles. The transmittance at 660 nm was measured by Genesys 10S UV–vis spectrophotometer (Thermo Scientific, Waltham, MA, USA)., and reported as percent transmittance where 100% transmittance is equal to absence of turbidity.

2.4. Juice pH, total soluble solids and titratable acidity

The juice pH was measured by pH meter (model Corning 430, Corning, Inc., Corning, NY, USA), calibrated using standard pH buffers of pH 4.0, 7.0, and 10.0. The total soluble solids (TSS) were measured using a refractometer (Abbe 3-L, Bausch & Lomb Optical Co., Rochester, NY, USA) and data reported as °Brix. Titratable acidity (TA) of the juice was determined by mixing 10 mL juice with 100 mL of deionized-distilled water and titrating with 0.1N NaOH to a final pH of 8.0. The titratable acidity, expressed as g/100 mL malic acid, was calculated from the following formula: mL 0.1N NaOH x N NaOH x 0.067 meq x (100/wt. of sample). The sugar-acid ratio was calculated by dividing TSS with TA, and is reported as unit-less measure.

2.5. Hunter color

The reflected color of blueberry juice was measured with a Hunter Color Meter, model D25 L optical sensor, Hunter Associates, Reston, VA, USA). The standard tile, supplied by the manufacturer with "L", "a", and "b" values of 94.8, −0.7, and 2.7, respectively, was used to calibrate the colorimeter. Approx. 100 mL of juice was placed in the round sample cup and color values were recorded as "L" (0, black; 100, white), "a"' (–a, greenness; +a, redness), and "b" (–b, blueness; +b, yellowness). The chroma and hue angle values were computed according to the method of Little (1975), as follows:

$$\text{Chroma} = \sqrt{a^2 + b^2}$$

$$\text{Hue Angle } (h°) = \tan^{-1(b/a)}$$

2.6. Sugars profile by HPLC

Ten mL of blueberry juice was extracted with 90 mL of HPLC grade distilled water by blending the mix for 2 min in a Waring blender. One mL extract from the above was placed into a plastic prep column containing 1 g of 100–200 mesh Bio-Rex 5 resin (the column was kept wet with HPLC grade distilled water until ready for use). Four mL of HPLC grade distilled water was added to elute sugars from this column. A stock standard sugar solution was prepared by weighing, 2 g fructose, 2.4 g glucose, 2.0 g sucrose and 2.5 g crystalline sorbitol in a 100 mL