Solid-State Bioconversion of Phenolics from Cranberry Pomace and Role of *Lentinus edodes* β-Glucosidase

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Cranberry pomace contains large amounts of phenolic glycosides, which are important sources of free phenolics that have many food uses such as antioxidants, flavorings, and nutraceuticals. Our hypothesis was that these glycosides in cranberry pomace could be hydrolyzed by β-glucosidase produced by *Lentinus edodes* during solid-state fermentation. On the basis of this hypothesis, our objective was to investigate the potential of using cranberry pomace as a substrate for the production of free phenolics and β-glucosidase through solid-state fermentation by a food-grade fungus *L. edodes*. Our results suggested that *L. edodes* β-glucosidase played a major role in release of phenolic aglycons from cranberry pomace during solid-state fermentation. After 50 days of cultivation, the yield of total free phenolics reached the maximum of 0.5 mg per g of pomace, while the β-glucosidase activity was about 9 units per g of pomace. The enzyme exhibited optimal activity at 60 °C and at pH 3.5 and was stable at temperatures up to 50 °C and between pH 3 and 6.5. The major free phenolics produced from cranberry pomace were identified by HPLC as gallic acid, chlorogenic acid, *p*-hydroxybenzoic acid, and *p*-coumaric acid. These results suggest that cranberry pomace is a potential substrate for producing food-grade phenolics and fungal β-glucosidase. The *L. edodes* β-glucosidase showed good stability and tolerance to low pH and, therefore has potential applications in wine and juice processing for aroma and flavor enrichment through enzymatic hydrolysis of glucoside precursors.

Keywords: *Cranberry pomace; glucosidase; Lentinus edodes; phenolics; solid-state fermentation*

INTRODUCTION

Cranberry pomace is a primary byproduct of the cranberry processing industry. It consists of the processed skins, seeds, and stems generated from cranberries during pressing for juice or preparation for canning, drying, and freezing. It contains a large amount of insoluble carbohydrates, smaller amounts of protein and minerals, and some remaining juice with sugars and other soluble substances. It is a poor animal feed because its protein content is extremely low. Its disposal into the soil or in a landfill poses considerable economic loss and potential environmental problems. Thus, the exploration of new uses for cranberry waste is being explored (Zheng and Shetty, 1998).

Phenolic acids, or free phenolics, are important natural compounds that can be used as natural food ingredients such as antioxidants (Hammerschmidt and Pratt, 1978; Al-Saikhan et al., 1995; Bocco et al., 1998), flavorings (Giese, 1994), and nutraceuticals (Shetty, 1997). Naturally occurring phenolics and their oxidation products are also important constituents of natural beverages, contributing to color and taste (Cliffe et al., 1994). Phenolic compounds also contribute to the color and flavor of fresh fruit and processed food products (Mazza and Velioglu, 1992). They show a broad spectrum of pharmacological activities in vitro (Glowniak et al., 1996; Meyer et al., 1998) and possibly have potential beneficiary effects on human health (Decker, 1997; Wilson et al., 1998; Meyer et al., 1998).

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Both fruit skins and seeds are an interesting source of phenolic compounds (Meyer et al., 1998; Bocco et al., 1998; Sotillo et al., 1994; Lu and Foo, 1997; Peleg et al., 1991). Cranberry pomace, consisting mainly of cranberry skins and seeds, contains many phenolic compounds, but these phenolics occur mainly in bound forms in which phenolic acids are conjugated with glucose or other sugars. Enzymatic hydrolysis of these phenolic glycosides appears to be an attractive means of increasing the concentration of free phenolic acids in fruit juice and wines to enrich taste, flavor, and aroma, also potentially increasing nutraceutical value (Schwab and Schreier, 1988; Shoseyov et al., 1990; Mateo and Stefano, 1997; Gueguen et al., 1997).

The enzyme β-D-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21), which will be referred to as β-glucosidase in this paper, can catalyze the hydrolysis of glycosidic linkages in aryl and alkyl β-D-glucosides as well as glycosides containing only carbohydrate residues (Woodward, 1982; Yan et al., 1998). β-Glucosidase can hydrolyze phenolic glycosides to release free phenolic acids and, therefore, has potential applications in certain food and beverage industries. The enzyme occurs widely in plants, fungi, animals, and bacteria (Yan et al., 1998). Many fungi have been found capable of producing β-glucosidase during solid-state fermentation on lignocellulosic wastes (Gupte and Madamwar, 1997; Martino et al., 1994; Woodward, 1982; Gueguen et al., 1994; Saha et al., 1994; Hang and Woodams, 1994). Much effort has yet to be made, however, in an attempt to find a food-grade β-glucosidase capable of hydrolyzing efficiently bound phenolics at relatively low pH with broad substrate specificity and thermal stabil-

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ity for potential applications in wine or juice processing (Woodward, 1982; Gueguen et al., 1994).

Lentinus edodes, also known as Shiitake, Black Forest, or Chinese mushroom, was successfully grown on apple pomace and other ligcocellulosic wastes (Worrall and Yang, 1992; Pettipher, 1988; Crestini et al., 1996). In addition, we found that a high level of β-glucosidase activity was produced from *L. edodes* during solid-state fermentation on cranberry pomace. The objective of this study was to examine the potential of bioconversion of free phenolic acids from cranberry pomace, by taking advantage of high β-glucosidase activity of the food-grade fungus *L. edodes* to hydrolyze phenolic glycosides in cranberry pomace and produce extractable free phenolic acids, a valuable product for both food and pharmaceutical applications.

MATERIALS AND METHODS

Microorganism. *L. edodes* CY-35 obtained from Dr. J. J. Worrall (State University of New York, Syracuse) was maintained on potato dextrose agar (PDA) slants and Petri plates at 4 °C and subcultured every 3 months. The fungus was activated by transferring onto PDA plate and cultured at room temperature for 20 days before use. The fruit bodies of *L. edodes* CY-35, which are available in the food market as Shiitake mushroom, have long been commercially cultivated and consumed as food (Worrall and Yang, 1982). Therefore, the strain is considered as a food-grade fungus.

Media and Cultivation. The 125-mL Erlenmeyer flask containing 10 g of cranberry pomace and 20 mL of water was used for solid-state fermentation. The freshly pressed cranberry pomace was obtained from Veryfine, Inc., Westford, MA, and was vacuum-dried, ground to less than 1 mm in particle size, and stored in a refrigerator before use. The water content of cranberry pomace used in the experiment was 5.8% (w/w, wet basis). The media contained in flasks with cotton plugs were autoclaved at 121 °C for 15 min. The *L. edodes* mycelium from one PDA plate was inoculated into 10 flasks. The flasks were incubated at 25 °C for 60 days. The cultivation of the fungus was also extrapolated for 100 g of cranberry pomace with proportional addition of water calculated from the 10 g level.

Crude Enzyme Extraction. A 100 mL portion of distilled water was added into fungus−pomace-containing flasks, and the culture was homogenized for 1 min using a Waring blender and then centrifuged at 15000*g* at 4 °C for 20 min. The supernatant was then filtered through a Whatman No. 1 filter paper. A 5 mL portion of the filtrate was dialyzed using Spectro/Pro membrane tubing (Spectrum Medical Industries, Inc., Houston, TX) against distilled water at 2 °C for 24 h. The resultant clear liquid was used as the crude enzyme solution after adjusting the same volume for each respective culture.

***β*-Glucosidase Activity Assay.** The enzyme activity was assayed by a modified procedure based on the methods of Gunata et al. (1990) and Hang and Woodams (1994). A standard reaction mixture contained 0.1 mL of 9 mM *p*-nitrophenol β-D-glucopyranoside (PNPG), 0.8 mL of 200 mM sodium acetate buffer (pH 4.6), and 0.1 mL of enzyme solution. After 5 min of incubation at 40 °C, the reaction was stopped by addition of 1 mL of 0.1 M Na_2CO_3, and the released *p*-nitrophenol was measured at 400 nm. The standard curve was established using pure *p*-nitrophenol (Fisher Scientific Co., Fair Lawn, NJ). One unit (U) of β-glucosidase activity is defined as the amount of enzyme that releases 1 μmol of *p*-nitrophenol per min at pH 4.6 at 40 °C under assay conditions.

Total Phenolic Acids Assay. The crude water extract of the fermented cranberry pomace before dialysis was used for total phenolics assay. The total phenolics content was determined by an assay modified from Shetty et al. (1995). One milliliter of the supernatant was transferred to a test tube and mixed with 1 mL of 95% ethanol and 5 mL of distilled water. To each sample was added 0.5 mL of 50% Folin−Ciocalteu reagent. After 5 min, 1 mL of 5% Na_2CO_3 was added, and the reaction mixture was allowed to stand for 60 min. The absorbance was read at 725 nm using a Genesys spectrophotometer (Milton Roy, Inc., Rochester, NY). Standard curves were established for each experiment using various concentrations of gallic acid in 95% ethanol. Absorbance values were converted to total phenolics, which were expressed as micrograms of gallic acid equivalent per milliliter of the extract. Each value reported in this study was an average of three replicate assays of three separate samples.

Temperature and pH Dependencies of *β*-Glucosidase Activity. The effect of temperature on β-glucosidase activity was determined by assaying the enzyme activity at pH 4.6 at temperatures from 10 to 90 °C. The thermostability of the enzyme was determined by measuring the remaining β-glucosidase activity under standard enzyme assay conditions after incubating the enzyme solution for 20 min at various temperatures from 10 to 80 °C. The effect of pH on β-glucosidase activity was determined by assaying the enzyme activity at 40 °C at various pHs from 2 to 7. The pH stability of the enzyme was determined by measuring the residual activity under standard conditions after incubating the enzyme for 24 h at 2 °C at various pHs ranging from 1.5 to 9. The following buffer systems of varying pH but fixed ionic strength (buffer capacity) were used: 0.1 M glycine buffer (glycine−HCl) for pH 1.5−3.5; 0.2 M acetate buffer (NaAc−HAc) for pH 4.0−5.5; 0.05 M Borax buffer (KH_2PO_4−$Na_2B_4O_7$) for pH 6.0−9.0.

Enzymatic Release of Phenolics from Cranberry Pomace. A mixture of 10 g of cranberry pomace and 100 mL of buffer (0.2 M sodium acetate buffer, pH 4.0) was homogenized for 1 min using a Waring blender and then transferred into a 150-mL flask. After the addition of 10 units of crude *L. edodes* β-glucosidase, the flask was incubated at 60 °C with occasional agitation for 5 h. At each 1-h interval, 2 mL of the mixture was taken out from the flask and centrifuged at 1500*g* for 15 min. The supernatant was used for total phenolics assay. The commercial β-glucosidase (from almonds, Sigma Chemical Co., St. Louis, MO) of the same amount of activity was used to compare with the crude *L. edodes* β-glucosidase for their capacity to release free phenolic acids from cranberry pomace. Distilled water was used as control.

HPLC Analysis of Free Phenolic Acids. The sample preparation procedure was modified from a method described by Onyeneho and Hettiarachchy (1992; 1993) and Cartoni et al. (1991). The procedure for water extraction of free phenolic acids from *L. edodes* fermented cranberry pomace was the same as the crude enzyme extraction as described above except there was no dialysis of the water extract. Five milliliters of the water extract filtrate was passed through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) that had previously been washed with methanol and subsequently with water. Then the cartridge was rinsed with 2.0 mL of distilled water, and the adsorbed free phenolics were desorbed from the column with 1.0 mL of HPLC-grade methanol and filtered through a Corning disposable sterile syringe filter with a cellulose acetate membrane (0.20 μm pore size). The filtrate was ready for HPLC analysis. Nine standard phenolic acids (purchased from Sigma Chemical Co., St. Louis, MO) were prepared in methanol at concentrations of 10−100 μg/mL.

High-performance liquid chromatography (HPLC) was performed with a Hewlett-Packard 1090 liquid chromatograph equipped with a 1040 diode array detector with a 10 μL, sample loop. The analytical column was a reversed-phase Whatman Ultrasphere ODS C18, 250 mm × 4.6 mm i.d., with a packing material of 5 μm particle size. The detector was set at 260 nm with an optical bandwidth of 4 nm. Absorption at 550 and 4 nm bandwidth was employed as the reference wavelength. The mobile phase consisted of two solvents: ammonium acetate buffer (0.01 M, pH 5.4) and methanol. The elution profile was a linear gradient from 100% buffer/0% methanol to 80% buffer/20% methanol at a flow rate of 1.0 mL/min over 40 min at ambient temperature. During each run, a chromatogram was recorded at 260 nm and a UV spectrum of each peak was recorded at its front, apex, and back. Phenolic standards were chromatographed singly and in a mixture. The

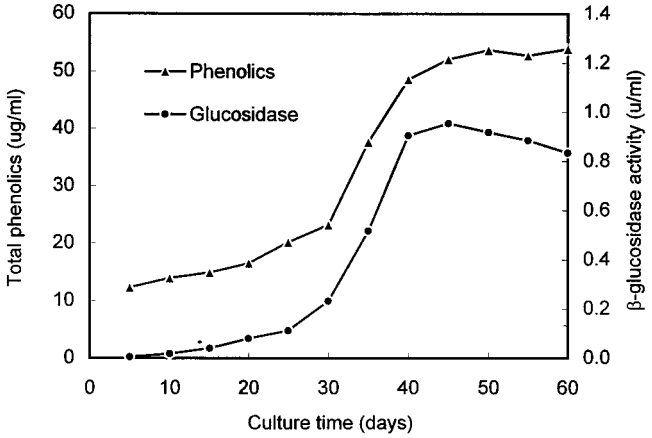

Figure 1. Production of free phenolics and β-glucosidase from cranberry processing wastes by *L. edodes*.

extract sample was chromatographed under the same conditions. The peak purity was checked by its recorded UV spectrum. Retention times and areas of the peaks were monitored and computed automatically by a Hewlett-Packard 9133 integrator. Identification of the peaks was made by comparing their spectra and retention times with standards, and was further ascertained by the standard addition method in which the corresponding peak was increased by adding a known standard phenolic acid in the extract. Free phenolic acids in the extract were quantified from their peak areas from the calculated response factors which were obtained by dividing the known concentration of a phenolic acid standard by its corresponding peak area.

RESULTS AND DISCUSSION

Production of Free Phenolics and β-Glucosidase. β-Glucosidase played an important role in the bioconversion of free phenolic acids from cranberry pomace by *L. edodes*. Figure 1 shows the yield of total free phenolic acids produced from *L. edodes* during solid-state fermentation on the substrate of cranberry pomace and the associated β-glucosidase activity. Since most natural phenolics in cranberry pomace were in bound form (insoluble) and only relatively small amounts were in free phenolic acid form, the initial total phenolics was at a low level (Figure 1). After 30 days, the free phenolics increased dramatically, as a result of the increased β-glucosidase activity (Figure 1). The enzyme activity reached a highest level on day 45 while the yield of free phenolic acids also reached nearly highest level (Figure 1). There was a clear positive correlation between the yield of free phenolics and the production of β-glucosidase activity.

The highest yield of free phenolic acids was over 50 μg/mL, and the highest β-glucosidase activity was about 1.0 units/mL. If calculated based on cranberry pomace, the highest yield of total free phenolic acids would be about 0.5 mg per gram of cranberry pomace, and this is comparable to the result of Sotillo et al. (1994) that a total of 0.48 mg of phenolic acids were extracted from 1 g of potato peel. The highest yield of β-glucosidase activity would be 10 units per gram of cranberry pomace, and this is comparable to the result of Gupte and Madamwar (1997) that a maximum of 7.65 units/g of β-glucosidase activity from *A. fumigatus*, while 11.75 units/g from *A. ellipticus*, was produced through solid-state fermentation on sugarcane bagasse substrate. The enzyme activity obtained from *L. edodes* on cranberry pomace was much higher than that from *A. foetidus* on apple pomace that only an average yield of 0.9 units/g of β-glucosidase was obtained (Hang and Woodams, 1994). There is possibility that the higher yields may be due to synergistic activity of both β-glucosidase and other enzymes such as esterase in the crude preparation.

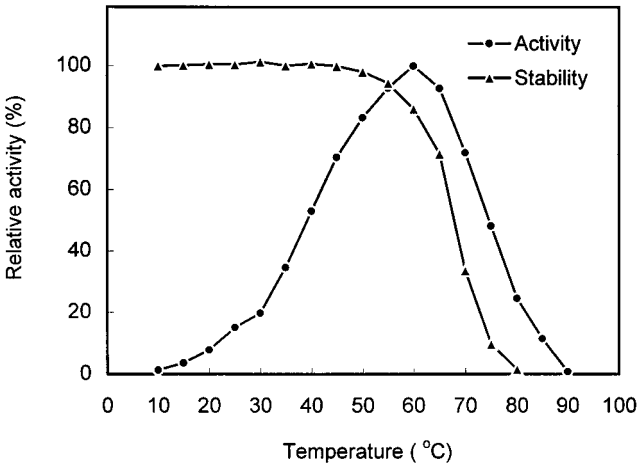

Figure 2. Effect of temperature on the activity and stability of β-glucosidase from cranberry pomace by *L. edodes*.

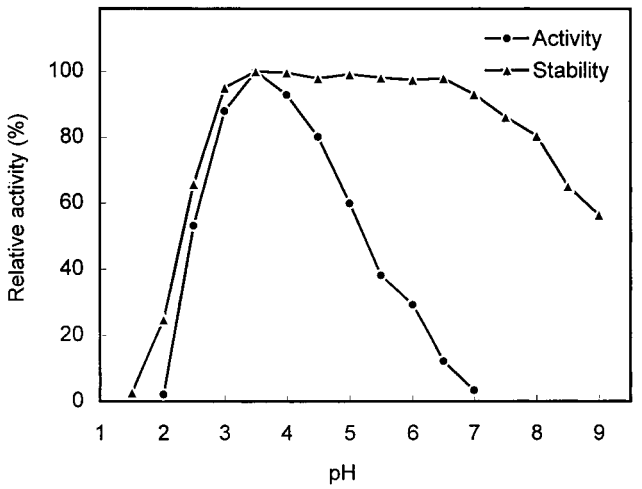

Figure 3. Effect of pH on the activity and stability of β-glucosidase from cranberry pomace by *L. edodes*.

Temperature Dependency of β-Glucosidase. The effect of temperature on the activity and stability of the crude *L. edodes* β-glucosidase is shown in Figure 2. The enzyme displayed maximal activity at 60 °C, while it retained only about 60% of the maximal activity at 40 °C which was the standard assay temperature. The enzyme was quite stable at temperatures up to 55 °C for 20 min. It was completely inactivated upon incubation at 80 °C for 20 min (Figure 2). The temperature optimum of this enzyme is similar to those of most previously reported fungal and bacterial β-glucosidases (Gueguen et al., 1994; Wei et al., 1996; Sharmila et al., 1998; Saha and Bothast, 1996; Yan et al., 1998), but lower than those of some thermostable β-glucosidases (Saha et al., 1994; Hang and Woodams, 1994). However, its heat tolerance is higher than most reported β-glucosidases and similar to thermostable β-glucosidases (Wei et al., 1996; Saha and Bothast, 1996; Saha et al., 1994).

pH Dependency of β-Glucosidase. Figure 3 shows the effect of pH on the activity and stability of the *L. edodes* β-glucosidase. The enzyme exhibited a maximum activity at pH 3.5, with 80% relative activity at pH 4.5,

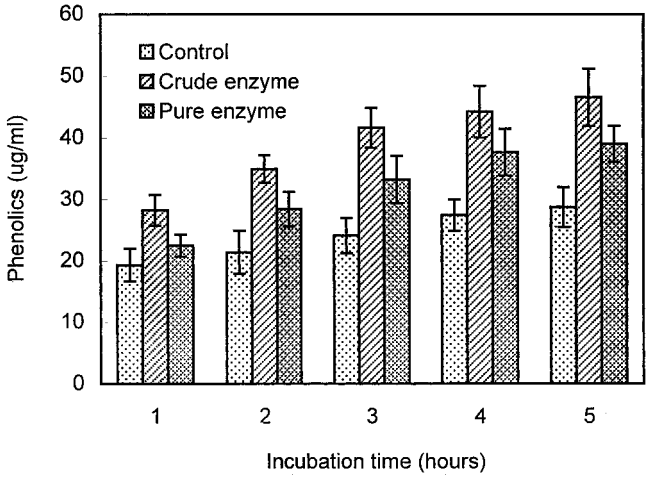

Figure 4. Production of phenolics from cranberry pomace by *L. edodes* crude enzyme and pure β-glucosidase at a concentration of 0.1 units/mL.

which was the standard assay condition. It was completely inactive at pH's below 2 and above 7. The enzyme was fairly stable at 2 °C for 24 h over a pH range of 3.0−6.5, with no activity remaining at pH 1.5 and below, but still had 60% activity remaining at pH 8.5. Interestingly, the *L. edodes* β-glucosidase showed a relatively lower pH optimum than most other fungal and bacterial β-glucosidases (Saha et al., 1994; Gueguen et al., 1994; Martino et al., 1994; Hang and Woodams, 1994; Wei et al., 1996; Saha and Bothast, 1996; Sharmila et al., 1998; Yan et al., 1998). The enzyme was also more stable than other microbial β-glucosidases at low pH, e.g., pH 3.0 (Saha et al., 1994; Saha and Bothast, 1996; Wei et al., 1996). The acid resistance property of the *L. edodes* β-glucosidase is very desirable, and the enzyme has great potential in the wine and fruit-juice industries, since most fruit juices and wines have low pH 2.5−4.0 (Gueguen et al., 1994; Woodard, 1982).

Enzymatic Production of Phenolic Acids from Cranberry Pomace. The crude *L. edodes* β-glucosidase was used for phenolic acids production from cranberry pomace and was compared with a commercial β-glucosidase purchased from Sigma Chemical Co., St. Louis, MO. The final concentration of the enzyme activity in the mixture was 0.1 units/mL for both *L. edodes* β-glucosidase and Sigma β-glucosidase. The enzymatic reaction was carried out at pH 4.0 at 60 °C, which were close to the optimal conditions for the *L. edodes* β-glucsidase activity (Figures 2 and 3). As shown in Figure 4, the yield of total phenolic acids increased significantly over incubation time as a result of *L. edodes* β-glucosidase actitity, whereas the yield increased to a lesser extent as a result of the commercial β-glucosidase activity under the same conditions. The control (water) did not show much difference over incubation time (Figure 4). With 0.1 units/mL of enzyme activity, after 5 h of incubation, the *L. edodes* β-glucosidase released nearly 50 μg/mL of free phenolic acids, which equals 0.5 mg of phenolic acids per gram of cranberry pomace; the commercial β-glucosidase produced about 40 μg/mL of free phenolic acids, which equals to 0.4 mg of phenolic acids per gram of cranberry pomace. The phenolic acids yield of the *L. edodes* β-glucosidase was higher than that of commercial enzyme with the same activity. This difference could be due to the fact that the crude enzyme solution produced from *L. edodes* was impure and probably contained other enzymes such as esterase, α-L-arabinofuranosidase, α-L-rhamnosidase, or β-D-apiosidase, which helped cleave the intersugar linkages and release the corresponding β-D-glucosides, and then the liberation of phenolic aglycon moieties could take place by the action of β-D-glucosidase (Mateo and Stefano, 1997). In addition, the reaction conditions also favored the *L. edodes* β-glucosidase activity.

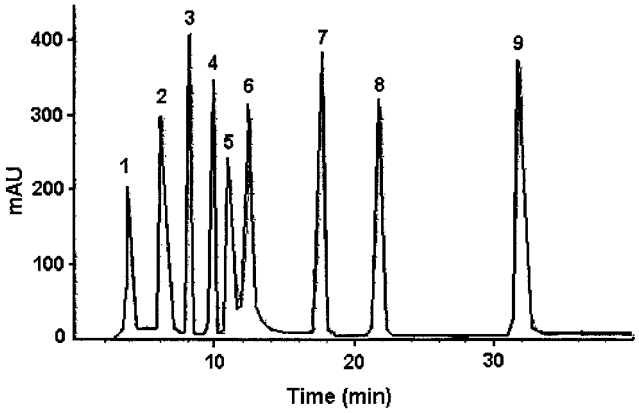

Figure 5. HPLC profiles of standard phenolic acids.

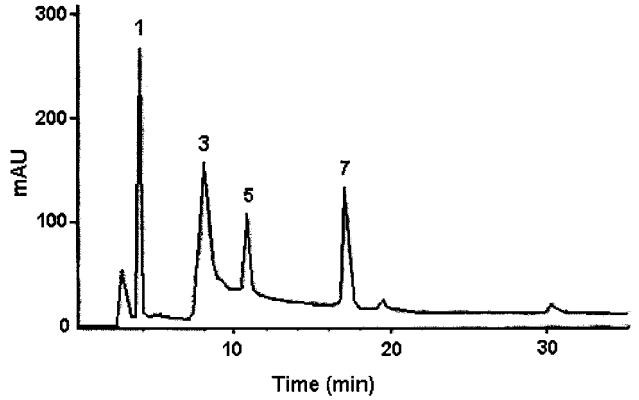

Figure 6. HPLC profiles of phenolic acids present in the water extract of *L. edodes* fermented cranberry pomace.

Table 1. Retention Times of Standard Phenolic Acids

peak	phenolic acid	retention time (min)
1	gallic acid	3.90
2	gentisic acid	6.04
3	*p*-hydroxybenzoic acid	8.10
4	vanillic acid	9.85
5	chlorogenic acid	10.90
6	syringic acid	12.48
7	*p*-coumaric acid	17.60
8	ferulic acid	21.80
9	*t*-cinnamic acid	31.86

Identification of Phenolic Acids. The free phenolic acids produced by *L. edodes* from cranberry pomace were identified by high-performance liquid chromatography (HPLC). Figures 5 and 6 illustrate the HPLC profiles of the standard mixture of phenolic acids and the phenolic acids present in the water extract of *L. edodes* fermented cranberry pomace. The retention times of individual phenolic acids are listed in Table 1. All the peaks in the extract were eluted before 30 min at a flow rate of 1 mL/min. Spiking the retention times under the same analytical conditions with the corresponding authentic phenolic acid standards was also performed to further ascertain the identity of each of the suspected phenolic acids. Gallic acid, chlorogenic acid, *p*-hydroxybenzoic acid, and *p*-coumaric acid ap-

Table 2. Major Phenolic Acid Content of *L. edodes*-Fermented Cranberry Pomace Extract

phenolic acid	content[a] (μg/mL)
gallic acid	11.3
p-hydroxybenzoic acid	14.2
chlorogenic acid	5.0
p-coumaric acid	6.7

[a] The content of phenolic acids was calculated on the basis of the original water extract.

peared to be the major phenolic acids in the water extract of fermented cranberry pomace (Table 2).

Quantification of Phenolic Acids. The retention times of all nine phenolic acid standards are given in Table 1. The response factors of individual phenolic acids were calculated by dividing their known concentrations by their corresponding peak areas obtained from the automatic HPLC integrator. The concentrations of the four major phenolic acids from the fermented pomace extract were calculated on the basis of their peak areas and their corresponding standard response factors and were translated into the content in original water extract (Table 2). The total amount of the four major phenolic acids in the extract was ~37 μg/mL (Table 2) and accounts for about 74% of the total phenolic acids, which were determined as ~50 μg/mL in the extract by the total phenolic acid assay method (Figures 1 and 4), indicating that small amounts of other free phenolic acids were also present in the extract.

CONCLUSIONS AND IMPLICATIONS

The present work shows that it is feasible to use cranberry-processing waste as a raw material for the production of phenolic acids by a food-grade fungus *L. edodes* CY-35. This fungus is able to produce high levels of extracellular β-glucosidase activity during solid-state fermentation on cranberry pomace, which is the major enzyme responsible for hydrolyzing phenolic glucosides present in cranberry pomace and releasing free phenolic acids.

The enzyme β-glucosidase produced from *L. edodes* has a relatively higher temperature optimum (60 °C) and a lower pH optimum (pH 3.5), with a good thermal stability (up to 50 °C) and a high tolerance to low pH (3.0−6.5). Considering the natural acidic pH of cranberry pomace, and the possible use of the enzyme in juice processing and wine making, the unique properties of *L. edodes* β-glucosidase would make the enzyme an potential candidate for both phenolic production from cranberry pomace and for flavor enrichment applications in fruit and wine industries. Therefore, cranberry pomace is also a potential substrate for the production of *L. edodes* β-glucosidase, which has many applications in food and pharmaceutical industries.

LITERATURE CITED

Al-Saikhan, M. S.; Howard, L. R.; Miller, J. C. Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum*, L.). *J. Food Sci*. **1995**, *60*, 341−347.

Bocco, A.; Cuvelier, M. E.; Richard, H.; Berset, C. Antioxidant acitivity and phenolic composition of citrus peel and seed extracts. *J. Agric. Food Chem*. **1998**, *46*, 2123−2129.

Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. **1976**, *72*, 248−54.

Cannel, E.; Moo-Young, M. Solid-state fermentation systems. *Process Biochem*. **1980**, *4*, 2−7.

Cartoni, G. P.; Coccioli, F.; Pontelli, L. Separation and identification of free phenolic acids in wines by high-performance liquid chromatography. *J. Chromatogr*. **1991**, *537*, 93−99.

Cliffe, S.; Fawer, M. S.; Maier, G.; Takata, K.; Ritter, G. Enzyme assays for the phenolic content of natural juices. *J. Agric. Food Chem*. **1994**, *42*, 1824−1828.

Crestini, C.; Kovac, B.; Sermanni, G. G. Production and isolation of chitosan by submerged and solid-state fermentation from *Lentinus edodes*. *Biotechnol. Bioeng*. **1996**, *50*, 207−210.

Decker, E. A. Phenolics: Prooxidants or antioxidants? *Nutr. Rev*. **1997**, *55*, 396−407.

Giese, J. Spices and seasoning blends: A taste for all seasons. *Food Technol*. **1994**, *48*, 88−92.

Gueguen, Y.; Chemardin, P.; Arnaud, A.; Galzy, P. Purification and characterization of the endocellular β-glucosidase of a new strain of *Candida entomophila* isolated from fermenting agave (*Agave* sp.) juice. *Biotechnol. Appl. Biochem*. **1994**, *20*, 185−198.

Gueguen, Y.; Chemardin, P.; Pien, S.; Arnaud, A.; Galzy, P. Enhancement of aromatic quality of Muscat wine by the use of immobilized β-glucosidase. *J. Biotechnol*. **1997**, *55*, 151−156.

Gunata, Y. Z.; Bayonove, C. L.; Cordonnier, R. E.; Arnaud, A.; Galzy, P. Hydrolysis of grape monoterpenyl glycosides by *Candida molischiana* and *Candida wicherhamii* β-glucosidase. *J. Sci. Food Agric*. **1990**, *50*, 499−506.

Gupte, A.; Madamwar, D. Solid-state fermentation of lignocellulosic waste for cellulase and β-glucosidase production by cocultivation of *Aspergillus ellipticus* and *Aspergillus fumigatus*. *Biotechnol. Prog*. **1997**, *13*, 166−169.

Hammerschmidt, P. A.; Pratt, D. E. Phenolic antioxidants of dried soybeans. *J. Food Sci*. **1978**, *43*, 556−559.

Hang, Y. D.; Woodams, E. E. Apple pomace: A potential substrate for production of β-glucosidase by *Aspergillus foetidus*. *Food Sci. Tech*. **1994**, *27*, 587−589.

Lu, Y.; Foo, L. Y. Identification and quantification of major polyphenols in apple pomace. *Food Chem*. **1997**, *59*, 187−194.

Martino, A.; Pifferi, P. G.; Spagna, G. Production of β-glucosidase by *Aspergillus niger* using carbon sources derived from agricultural wastes. *J. Chem. Technol. Biotechnol*. **1994**, *60*, 247−252.

Mateo, J. J.; Stefano, R. D. Description of the β-glucosidase activity of wine yeasts. *Food Microbiol*. **1997**, *14*, 583−591.

Mazza, G.; velioglu, Y. S. Anthocyanins and other phenolic compounds in fruits of red-flesh apples. *Food Chem*. **1992**, *43*, 113−117.

Meyer, A. S.; Jepsen, S. M.; Sorensen, N. S. Enzymatic release of antioxidants for human low-density lipoprotein from grape pomace. *J. Agric. Food Chem*. **1998**, *46*, 2439−2446.

Onyeneho, S. N.; Hettiarachchy, N. S. Antioxidant activity of durum wheat bran. *J. Agric. Food Chem*. **1992**, *40*, 1496−1500.

Onyeneho, S. N.; Hettiarachchy, N. S. Antioxidant activity, fatty acids and phenolic acids compositions of potato peels. *J. Sci. Food Agric*. **1993**, *62*, 345−350.

Peleg, H.; Naim, M.; Rouseff, R. L.; Zehavi, U. Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). *J. Sci. Food Agric*. **1991**, *57*, 417−426.

Pettipher, G. L. Cultivation of the Shiitake mushroom (*Lentinus edodes*) on lignocellulosic waste. *J. Sci. Food Agric*. **1988**, *42*, 195−198.

Saha, B. C.; Bothast, R. J. Production, purification, and characterization of a highly glucose-tolerant novel β-glucosidase from *Candida peltata*. *Appl. Environ. Microbiol*. **1996**, *62*, 3165−3170.

Saha, B. C.; Freer, S. N.; Bothast, R. J. Production, purification, and properties of a thermostable β-glucosidase from a color variant strain of Aureobasidium pullulans. *Appl. Environ. Microbiol*. **1994**, *60*, 3774−3780.

Schwab, W.; Schreier, P. Simultaneous enzyme catalysis extraction: A versatile technique for the study of flavor precursors. *J. Agric. Food Chem*. **1988**, *36*, 1238−1242.

Sharmila, T.; Sreeramulu, G.; Nand, K. Purification and characterization of β-glucosidase from *Clostridium papyrosolvens*. *Biotechnol. Appl. Biochem*. **1998**, *27*, 175−179.

Shetty, K. Biotechnology to harness the benefits of dietary phenolics: Focus on Lamiaceae. *Asia Pacific J. Clin. Nutr*. **1997**, *6*, 162−171.

Shetty, K.; Curtis, O. F.; Levin, R. E.; Witkowsky, R.; Ang, W. Prevention of vitrification associated with *in vitro* shoot culture of oregano (*Origanum vulgare*) by *Pseudomonas* spp. *J. Plant Physiol*. **1995**, *147*, 447−451

Shoseyov, O.; Bravdo, B. A.; Siegel, D.; Goldman, A.; Cohen, S.; Shoseyov, L.; Ikan, R. Immobilized Endo-β-glucosidase enriches flavor of wine and passion fruit juice. *J. Agric. Food Chem*. **1990**, *38*, 1387−1390.

Sotillo, D. R. D.; Hadley, M.; Holm, E. T. Phenolics in aqueous potato peel extract: Extraction, identification and degradation. *J. Food Sci*. **1994**, *59*, 649−651.

Wei, D. L.; Kirimura, K.; Usami, S.; Lin, T. H. Purification and characterization of an extracellular β-glucosidase from the wood-grown fungus *Xylaria regalis*. *Curr. Microbiol*. **1996**, *33*, 297−301.

Wilson, T.; Porcari, J. P.; Harbin, D. Cranberry extract inhibits low-density lipoprotein oxidation. *Life Sci*. **1998**, *62*, PL 381−386.

Woodward, J. Fungal and other β-D-glucosidase − their properties and applications. *Enzyme Microb. Technol*. **1982**, *4*, 73−79.

Worrall, J. J.; Yang, C. S. Shiitake and oyster mushroom production on apple pomace and sawdust. *HortScience* **1992**, *27*, 1131−3.

Yan, T. R.; Lin, Y. H.; Lin, C. L. Purification and characterization of an extracellular β-glucosidase II with high hydrolysis and transglucosylation activities from *Aspergillus niger*. *J. Agric. Food Chem*. **1998**, *46*, 431−437.

Zheng, Z.; Shetty, K. Cranberry processing waste for solid-state fungal inoculant production. *Process Biochem*. **1998**, *33*, 323−329.

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