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Solid-state fermentation of apple pomace using *Phanerocheate chrysosporium* – Liberation and extraction of phenolic antioxidants

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ABSTRACT

Apple pomace is a by-product from the apple processing industry and can be used for the production of value-added phenolic compounds. A study was carried out to understand the changes and liberation of phenolic compounds and improvement in antioxidant activity during solid-state fermentation of apple pomace using *Phanerocheate chrysosporium*. The solid-state fermentation of apple pomace using *P. chrysosporium* mobilised the polyphenolic compounds and improved the nutraceutical properties. The polyphenol content in acetone extract increased and the results were statistically significant ($P < 0.05$) from 4.6 to 16.12 mg GAE/g dry weight during solid-state fermentation. The effect of various solvents, temperature, time and detergents were also investigated for the extraction of polyphenolics by ultrasonication and microwave-assisted extraction methods. The polyphenol content of the extracts was found to be in the range of 5.78–16.12 mg GAE/g DW of samples, depending on the solvent, extraction time and temperature. Antioxidant activities of polyphenol extracts were tested using the 2,2-diphenyl-1-picryhydrazyl (DPPH) radical methods, where the IC_{50} ranged from 12.24 to 23.42 μg DW sample, depending on the extraction conditions and the antioxidant activities correlated well with the polyphenol concentrations.

1. Introduction

There is an increasing global trend towards the efficient utilisation of natural resources. The direct disposal of agro industrial by-products as a waste in the environment represents a major cause for environmental pollution and also an important loss of biomass which could be used for the production of different metabolites with added commercial value (Vendruscolo, Pitol, Koch, & Ninow, 2007). Sustainable food production and value-addition of wastes is the most important issue in the agro and food processing industry. Apple and apple products are one of the major fruit and fruit products consumed all over the world. Several million tonnes of apple pomace are generated during the processing of apple products, such as apple juice, jelly, cider, among others (Bhushan, Kalia, Sharma, Singh, & Ahuja, 2008), which contributes about 20–35% of the total fruit production. Apple pomace has been used as a source for several applications, such as pectin recovery (Schieber et al., 2003), enzyme production (Favela-Torres, Volke, & Viniegra, 2006), animal feed (Sehm, Lindermayer, Dummer, Treutter, & Pfaffl, 2007), organic acids production (Shojaosadati & Babaeipour, 2002), ethanol production (Paganini, Nogueira, Silva, & Wosiacki, 2005), aroma compounds (Medeiros, Pandey, Vandenberghe, Pastore, & Soccol, 2006), natural antioxidants (Foo & Lu, 1999), among others.

Diets rich in fruits and vegetables are gaining increased importance due to their significant role in reducing the risk of certain types of cancer, cardiovascular diseases and other chronic diseases (Joshipura et al., 2001; McCann et al., 2007; Suárez et al., 2010). Fruits and vegetables contain many antioxidant compounds including phenolic compounds, carotenoids, anthocyanins and tocopherols (Naczk & Shahidi, 2006). Apple is an important source of bioavailable polyphenols, such as flavonols, monomeric and oligomeric flavonols, dihydrochalcones, anthocyanidins, as well as others (Escarpa & Gonzalez, 1998). The most abundant polyphenols present in apples are chlorogenic acid, phloretin glucosides and quercetin glucosides (Wijngaard, Rȩossle, & Brunton, 2009). Other polyphenolic compounds, such as catechins and procyanidins, have also been identified, but are present in relatively small amounts (Foo & Lu, 1999). The polyphenolic compound contents vary greatly among different varieties and various parts of apples; apple peels contain a higher concentration of phenolic compounds than the flesh (Vrhosek, Rigo, Tonan, & Mattivi, 2004). Owing to the increasing interest in new natural sources of antioxidant products, apple pomace has been investigated as a potential source of bioactive polyphenols during recent years (Cetkovic et al., 2008), which have many applications in food, pharmaceutical and

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cosmetic industry by virtue of its antioxidant and antimicrobial activities.

While the potential of apple pomace as a source of polyphenols seems clear, there is less information on potential strategies for the recovery of these compounds. Most of the polyphenolic compounds are present in a bound form with carbohydrates, such as glycosides in nature. This bound nature of polyphenolics as glycosides reduces their ability to function as good antioxidants (Vattem & Shetty, 2003). The lowered antioxidant activity has a direct effect on the health functionality when these compounds are ingested into the body via food or nutraceuticals, and may have to depend on the probiotic status of the digestive system (Vattem & Shetty, 2003). Therefore, the release of free phenolics can improve the health functionality of these phytochemicals. Many ligninolytic and carbohydrate metabolising enzymes are produced by fungi during fermentation of lignocellulosic wastes. These enzymes can hydrolyse the phenolic glycosides and can release the free aglycones, potentially having high antioxidant activity, making them very useful for applications in food and beverage industries (Vattem & Shetty, 2003).

In the present investigation, the ability of white rot fungi, *Phanerocheate chrysosporium* to release phenolic antioxidants from apple pomace by solid-state fermentation has been studied. The extraction of polyphenolics from apple pomace and fermented apple pomace was carried out by ultrasonic assisted extraction and microwave-assisted extraction methods. The optimisation of solvents, extraction time, extraction temperature, power and effect of non-ionic surfactants for the extraction of polyphenolics has also been investigated.

2. Materials and methods

Apple pomace samples from the apple processing industry, Lassonde Inc., Rougemont, Montreal, Canada was collected and used as the solid substrate for the solid-state fermentation. All chemicals required for the experiments have been purchased from Fisher Scientific (Fisher Scientific Company, Ontario, Canada), VWR chemicals (VWR international, Quebec, Canada) and Sigma Chemicals (Sigma–Aldrich Canada Ltd., Ontario, Canada) and were of analytical grade.

2.1. Solid-state fermentation

Medium for fermentation: Apple pomace was used as natural substrate for the solid-state fermentation. The apple pomace solids were stored at −20 °C for its conservation prior to use. For the fermentation, apple pomace was treated with inducers, such as copper sulphate (2 mM), veratryl alcohol (2 mM) and Tween-80 (0.1%), before the pH was adjusted to 4.5 and it was sterilised in an autoclave for 30 min at 121 ± 1 °C. The moisture content in the apple pomace was 72% w/v. *P. Chrysosporium* was propagated and stored at −20 °C, according to the procedure developed by Gassara, Brar, Tyagi, Verma, and Surampalli (2010). The concentration of spore suspension used in the experiments was 2.5×10^6 spores/g of solid.

Solid-state fermentation: The fermentation was carried out in flasks. The media in flasks were autoclaved at 121 ± 1 °C for 30 min. The inoculation was performed using the spore suspension. The fermentation was carried out in a controlled environment with temperature at 37 ± 1 °C for 14 days.

2.2. Ultrasonic assisted extraction of polyphenolic compounds

Apple pomace and fermented apple pomace were accurately weighed to 1 g and 20 ml of solvents was added. The samples were placed in an ultrasonication bath (Elma Hans Schmidhauer GmbH & Co. KG, Germany). The optimisation of solvents for the extraction of phenolics by ultrasonication was carried out using different solvents, such as water; 60% ethanol; 70% ethanol, 80% ethanol; 60% acetone; 70% acetone and 80% acetone; 60% methanol; 70% methanol and 80% methanol. The ultrasonic extraction was performed for 30 min at 40 ± 1 °C. The optimisation of time for the extraction of phenolics was carried out using different solvents, such as water, 80% acetone and 80% alcohol. Duplicates of each sample were extracted at different time intervals of 20, 30 and 40 min at 40 °C.

The optimisation of temperature for the extraction of phenolics was performed using water. Duplicates of each sample were extracted at different temperatures of 30, 40, 50, 60, 70 and 80 °C for 30 min. The effect of Tween-20 as a surfactant for the extraction of polyphenolics was carried out using different concentrations of Tween-20, such as 0.1%, 1%, 2% and 5% in v/v with water.

For each of the optimisation experiments, duplicates of each sample were extracted at the same time. Each sample mixture was centrifuged at 9268*g* for 20 min to obtain the supernatant. The supernatant was used for determination of total phenolics content.

2.3. Microwave-assisted extraction of polyphenolic compounds

Apple pomace and fermented apple pomace were accurately weighed to 1 g and 20 ml of solvent was added. Each mixture was taken in a sealed 100 ml green chem Teflon reactor vessel and extracted for 10 min at 60 °C with a pressure of 692 kpa and power 400 W, using a sophisticated microwave extractor (Mars, CEM Corporation, Northcarolina, USA). The microwave extractor allowed highly accurate control of pressure, power and temperature. After 10 min of extraction, vessels were allowed to cool prior to their removal from the extractor. Duplicates of each sample were extracted and each sample mixture was centrifuged at 9268*g* for 20 min to obtain the supernatant. The optimisation of solvents, extraction time, temperature and effect of detergents for the extraction of phenolics was performed using the same protocol as for ultrasonication.

2.4. Estimation of total phenolic content

The content of total polyphenolics in the phenolic extract of apple pomace and fermented apple pomace was determined by the method of Swain and Hillis (1959). The supernatant obtained after centrifugation of ultrasonic and microwave-assisted extraction was used for the determination of total phenolics content. The absorbance was recorded at 725 nm. Gallic acid was used as a standard. The content of total polyphenolics in the extract was expressed as gallic acid equivalents (GAE) in g/dry weight of the samples.

2.5. Free radical scavenging activity of polyphenolic compounds

The effect of polyphenol extracts on DPPH radical was determined according to the method by Brand-Williams, Cuvelier, and Berset (1995). A 100 μM solution of DPPH in methanol was prepared and polyphenol extract (200 μl) was mixed with 1 ml of DPPH solution. The mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 517 nm. The control contained all the reagents except the polyphenol extract. The capacity to scavenge DPPH radical was calculated by following Equation:

$$\text{Scavenging activity}(\%) = [1 - (A_s/A_0)] \times 100 \quad (1)$$

where A_0 is the absorbance at 517 nm of the control and A_s is the absorbance in the presence of polyphenol extract. The results were plotted as the percentage of scavenging activity against concentration of the sample. The half-inhibition concentration