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## Short Communication

# Antibrowning potential of Brassicacaea processing water

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#### ABSTRACT

The effects of *Brassicacaea* processing water on polyphenol oxidases from different plant sources were investigated. Results showed that processing water (PW) prepared by cooking young *Brassicacaea* leaves with water has the capacity to inhibit both commercial tyrosinase and plant polyphenol oxidases. PW was freeze-dried (LPW) in an attempt to increase its utility and improve its efficiency at inhibition of polyphenol oxidases, however this resulted in a loss of activity, probably due to the stresses of the freezing and/or drying processes. The addition of PW to ascorbic acid resulted in complete inhibition of grape polyphenol oxidase, suggesting that the combination of the two antibrowning agents may be a promising tool to control this enzymatic activity.

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BIORESOURCE TECHNOLOGY

#### 1. Introduction

Currently, there is a growing interest in the exploitation of the residues generated by the food industry. The main strategic objective is to achieve more value with a lower amount of natural resources and reduced waste production throughout a given product/service lifecycle (Laufenberg et al., 2003; Negro et al., 2003). The agro-food industry provides interesting opportunities for the application of this concept due to crop by-products, high water consumption levels, and the wastes involved (Catarino et al., 2007). Despite the tremendous potential for using recycled or reconditioned water in food processing and preparation plants, there is relatively little published information in this area, as reported by Palumbo et al. (1997). Reuse water can be utilized in a wide range of unit operations. In one example, concentrated starch was produced as a by-product of wastewater from the potato chip industry and used as a raw material in paper manufacturing, contributing to extra business profit (Catarino et al., 2007). In another rare example of exploiting the promise of wastewater, Cancino et al. (2006) suggested that a combination of microfiltration and reverse osmosis is a suitable treatment for the recovery of starch from waste water.

The *Brassicaceae* family vegetables are commonly grown and consumed worldwide and have been reported to possess antioxidant and anticarcinogenic properties due to compounds such as vitamins, carotenoids, polyphenols and glucosinolates (Beecher, 1994; Halkier and Gershenzon, 2006). Industrial processing such as blanching, canning, and sterilization, as well as domestic cooking, affects the content of these bioactive compounds (Lo Scalzo

et al., 2008; Volden et al., 2008). In general, their concentrations and activities in processed vegetables are lower than those of the corresponding raw samples due to losses in processing water (Cartea and Velasco, 2008). Rosa and Heaney (1993) found a reduction of total glucosinolate content when boiling Portuguese cabbage, and they also reported a 40-80% leaching of glucosinolates from broccoli heads and cabbage leaves into the cooking water. Moreover, Song and Thornalley (2007) found that around 90% of the glucosinolates losses could be accounted for in the cooking water. Therefore Brassicaceae processing water may be useful as a source of bioactive compounds and natural food additives (West et al., 2008), providing a connection between the needs to reuse water, improve food quality, and reduce chemical substances. On promising application for the reuse of wastewater is the control of polyphenol oxidase (PPO, EC 1.14.18.1), which is also referred to as tyrosinase. Control of polyphenol oxidase is very critical for minimizing the browning of fruits, vegetables and seafoods (Mayer, 2006). However, few PPO inhibitors have shown potential for use in the food industry due to off-flavors and off-odors, food safety, economic feasibility, and effectiveness of inhibition. The inhibitors of enzymatic browning most frequently used in industry include ascorbic acid and various forms of sulfite-containing compounds. The latter have applications for a broad range of products and are strong antibrowning and antimicrobial agents. However, in addition to causing off-flavors, sulfites pose health risks to allergic individuals and, consequently, their application on fresh and freshcut products has been banned by Lu et al. (2006) and US FDA (1986).

With the current sensitivity to the use of synthetic food additives, inhibitors of 'natural' origin may have some advantages. Processing water may thus be useful as an antibrowning agent since it is non-toxic and has no known adverse side effects. In addition,

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Table <sup>†</sup>	1
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ICP-OES operating conditions.

Operating conditions		
Power	1420 W	
Outer gas	13.6 l/min	
Intermediate gas	0.80 l/min	
Nebulizer gas	0.92 l/min	
Sample aspiration rate	2 ml/min	
Preflush time	30 s	
Read time	24 s	
Wavelength	182.034 nm	

tyrosinase inhibitors have become increasingly important for medicinal and cosmetic products that may be used to prevent or treat pigmentation disorders (Kim and Uyama, 2005).

Due to the impact of browning caused by PPO in the food industry and its great significance in melanogenesis, research on potential inhibitory compounds continues (Kim et al., 2005). In this study, the potential of PW as an antibrowning agent is explored.

#### 2. Methods

#### 2.1. Recovery of processing water

Samples of *Brassica oleracea* L. convar. *botrytis* L. var. italica Plenck were collected from the field in February, 2009, and processed on the same day as collection. After the manual harvest, broccoli was cleaned and the older leaves were removed. Young leaves were randomly sampled from 500 g of raw material. The sample was rinsed with distilled water, weighed, and cooked in distilled water at 85 °C for 15 min using a HAAKE SWB25 Shaking Water Bath (Thermo Scientific, MA, USA). The ratio of vegetable to water was 1:4. PW (pH = 5.7) was filtered via vacuum filtration using Whatman No. 1 filter paper (Maidstone, England) and Millipore 0.22  $\mu$ m filter membranes (MA, USA). PW was stored in a dark glass bottle without headspace at 4 °C until the analytical measurements were made. PW samples were also freeze-dried at -40 °C (Edwards Mini Fast 1700, Crawley, England) and stored at -20 °C.

#### 2.2. Sulfur determination

Sulfur concentration was determined by inductively coupled plasma optical emission spectrometry (ICP-OES), using a SPECTRO CIROSCCD ICP (SPECTRO Analytical Instruments, Kleve, Germany) with axial plasma viewing. The operating conditions for sulfur ICP-OES determination are summarized in Table 1.

#### 2.3. Enzyme sources

Commercial mushroom tyrosinase was obtained from Sigma (St. Louis, MO, USA). Artichokes (*Cynara cardunculus* subsp. *scolymus* L.), pears (*Pyrus communis* L. cv. Abate), apples (*Malus domestica* L. cv Golden delicious), and grapes (*Vitis vinifera* cv. Garganega) were purchased at commercial maturity from a local store. Potato tubers (*Solanum tuberosum* L. cv. Agata) were purchased from Ghisetti 1870 Enterprise (Rovigo, Italy). Plant material was washed under running water to eliminate any surface contamination and wiped with blotting paper. Potatoes, apples and pears were hand-peeled. PPOs were extracted as reported by Weemaes et al. (1998), with some modification. Product (100 g) was immersed in 100 ml distilled water containing 1 g insoluble poly(vinylpolypyrrolidone) (PVPP; Sigma) plus 0.5 ml Triton-X-100 (BDH

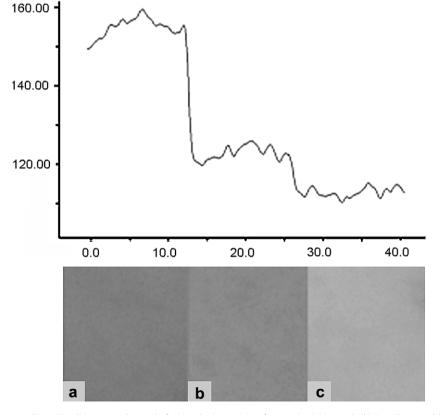


Fig. 1. Antibrowning effect on potato slices. The slices were observed after incubating at 30 °C for 30 min: (a) control; (b) PW alone; and (c) PW containing ascorbic acid (0.5 g/l).

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