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Clarification of red beet stalks extract by microfiltration combined with ultrafiltration

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ABSTRACT

Red beet stalks constitute an agro-industrial residue with potential to be utilized as betalains source. However, in order to maintain the stability of these natural pigments, it is necessary to minimize the factors that favor their degradation. The main aim of this study is the clarification of the juice extracted from beet stalks, which are rich in betalains, using microfiltration process combined with ultrafiltration. Despite the permeate flux reduction during the processes, the occurrence of fouling and betalain degradation in the permeate stream, these separation processes have been demonstrated to be efficient in clarifying the extracts. Comparing the initial extract (microfiltration feed) with the final permeate (ultrafiltration permeate), it was possible to achieve a 99.5% reduction in peroxidase activity, more than 99.9% reduction in turbidity and the color too appeared to be more intense, luminous and reddish.

1. Introduction

The waste of foods not only represents a misuse of resources but also an environmental and socioeconomic problem to society (Mirabella et al., 2014). The use of these residues can reduce the material discarded in the environment, contributing to natural resources exploration being less harmful to the planet and favoring cost-effective production by replacing more noble sources. Despite being traditionally treated as a problem, foods processing by-products have been increasingly used as sources of important products, such as natural pigments and phenolic compounds (Vulić et al., 2012).

Beet stalks constitute an example of residue scarcely explored by the food industry and has been poorly studied by the scientists. When beet stalks are used as a source of natural pigments, they are no longer considered an agro-industrial residue, but rather a high-value-added by-product.

Nowadays, the consumers are more and more rejecting the use of synthetic pigments in the food industry and the utilization of by-products of agro-industrial production is a plausible possibility to produce natural pigments (Vulić et al., 2012; Shui and Leong, 2006). However, the stability of these natural pigments is a challenge for their use.

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The natural pigments that impart reddish-violet color to beets are betalains (Stintzing and Carle, 2007). Betalains are hydrosoluble compounds and are considered more water-soluble when compared with anthocyanins (Stintzing et al., 2006). Despite presenting colours similar to the plants that contains anthocyanins, unlike these, the color of the compounds rich in betalainis not affected by the pH (Stintzing and Carle, 2007). According to Stintzing and Carle (2008), the most common betalain in red beet is the betanin.

Betalain extract obtained from red beet is widely used as natural coloring in dairy products, beverages and sweets (Gonçalves et al., 2012). The presence of betalains enables other advantages besides the red color, such as antioxidant, anti-inflammatory, hepatoprotective and antitumor activities (Gengatharan et al., 2015; Georgiev et al., 2010). Betalains become liable to degradation immediately after the extraction, due to the breakage of the vegetable tissue (Stintzing and Carle, 2008). Betalains stability is related to endogenous factors, such as enzymes activity and factors related to the conditions of extraction, such as temperature, presence of oxygen and pH (Stintzing and Carle, 2008).

Among these degradation-causing factors, there is also the activity of the peroxidase enzyme, which is the most heat stable enzymes present in vegetable tissue (Fennema et al., 2010). Since the temperature can be considered the most important factor in betalains stability they degrade when submitted to high temperatures during heat treatment for enzyme inactivation, and also during storage (Herbach et al., 2006). Thus, other technologies are being studied to replace heat utilization, causing less degradation on the pigments.

In this context, membrane separation processes (MSP) can be considered adequate for industrial utilization of heat-sensitive substances since they can be operated at room temperature and, most of them, do not need the addition of chemical products. Moreover, they constitute an alternative when it is necessary to obtain a final product free of organic solvents (Chhaya et al., 2012; Drioli and Fontananova, 2004). The microfiltration (MF) and ultrafiltration (UF) processes are the most used MSP in food industry. MF process can also be applied as a pre-treatment to remove suspended solids, polysaccharides, cellular debris and other materials present in the solution, which usually can not be removed by conventional filtration (Charcosset, 2006). UF process, in turn, is applied to purify and fractionate solutions that contain macromolecules. During MSP, it is common the occurrence of permeate flux reduction over time; this can occur due to the accumulation of components present in the feed of membrane pores (fouling), which can be estimated through of hydraulic permeance determination, as well as due to the formation of a polarized layer on the membrane surface (concentration polarization) (Mondor et al., 2000).

Considering the assignments of the MSP, this technology arise as a promising alternative for obtaining an extract rich in betalains, since, besides promoting its clarification, the utilization of a single MSP step can even replace a second treatment for enzymes inactivation.

In this context, the main objective of this study is to evaluate the use of microfiltration and ultrafiltration membranes processes in the clarification (with consequent enzyme inactivation) of an extract rich in betalains from red beet stalks (*Beta vulgaris* L.).

2. Materials and methods

Red beets (*Beta vulgaris* L.) used in this study were purchased at CEASA – Supply Food Center of Rio Grande do Sul Statein Porto Alegre – RS, Brazil.

The extracts rich in betalains, used in the membrane system feed, were obtained from cold-crushed beet stalks previously selected and sanitized. These crude extracts were, then, centrifuged, vacuum filtered and diluted with distilled water to standardize the feed solution.

During all experiments, solutions and extracts were kept between 10 and 15 °C and protected from light.

2.1. Membranes system

In order to carry out the experiments, two tubular (ZrO_2/TiO_2) ceramic membranes were used: one for MF (0.05 μm nominal pore size) and the other for UF (molecular weight cut-off (MWCO) of 20 kDa). Both membranes were provided by Andritz Separation and presented the following dimensions: 250 mm long, 6 mm of internal diameter and 10 mm of external diameter, with a permeance area of 0.0047 m^2.

Before using the membranes, both were submitted to chemical cleaning with sodium hydroxide solution (5 g L^{-1}) and citric acid (5 g L^{-1}), followed by ultrasonic heating bath (40 °C) for 1 h and, then, immersed in sodium hypochlorite solution (1 ppm). Finally, they were rinsed with distilled water. After cleaning, the membranes were compacted during 1 h and the pressures used for compaction were 1.0 and 2.0 bar for MF and UF processes, respectively.

The system utilized for the clarification process of the extract rich in betalains is shown in Fig. 1. The procedure for membrane characterisation is given below.

- Permeate Flux

Permeate flux (L m^{-2} h^{-1}) represents permeate flow rate per unit area of the membrane; permeate flux measurements were determined by direct measurement of its volumes.

- Estimation of fouling by means of hydraulic permeance

Hydraulic permeance, before and after extract filtration, was estimated by measuring permeate flux with distilled water under four different pressures. For MF membrane, the pressures used were 1.0; 0.75; 0.5 and 0.25 bar. For UF membrane, in turn, the pressures used were 2.0; 1.5; 1.0 and 0.5 bar. The experiment began with distilled water circulation under the highest pressure during 10 min; the permeate flux was measured and the pressure was reduced. Angular coefficients of each curve were used to calculate the fouling percentage using Eq. (1).

$$Fouling(\%) = \left(1 - \frac{Lp_a}{Lp_b}\right) \times 100 \quad (1)$$

where Lp_b and Lp_a are, respectively, the angular coefficient of the hydraulic permeance before and after solution filtration.

- Determination of Operating Pressure

The operating pressure was determinate using the extract as feed solution (10 mg of betanin/100 mL of extract) by measuring the permeate flux under different pressures. After 20 min operating under minimum pressure required for permeation (around 0.1 bar), the pressure was adjusted to the initial value, 0.25 bar for MF and 0.5 bar for UF, and the experiment started. The extract circulated under this pressure for 30 min, with permeate flux being verified every 5 min. If the permeate flux remained constant after 30 min, the pressure was increased 0.25 bar to the next observation point for additional 30 min. The critical operating pressure was considered the one in which the permeate flux did not remain constant