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Clarification of Stevia extract by ultrafiltration: Selection criteria of the membrane and effects of operating conditions

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ABSTRACT

Clarification of pre-treated Stevia extract using ultrafiltration is presented in this study. Performance of four different ultrafiltration membranes, namely 5, 10, 30 and 100 kDa were investigated in terms of permeate flux and permeate quality, mainly the recovery of Stevioside in the permeate. In this regard, the 30 kDa membrane was found to be most suitable. A systematic set of experiments under steady state were conducted to analyze the effects of the operating conditions, transmembrane pressure drop and stirrer speed on the permeate flux and permeate quality. Steady state was reached in between 8 and 16 min depending on the operating conditions. Steady state was reached earlier at higher stirring speed. A simple resistance in series model was used to quantify the fouling resistance. Membrane resistance was found to be negligible compared to the fouling resistance. It was a strong function of the stirrer speed but remained almost invariant with transmembrane pressure drop. The steady state permeate flux increased with pressure drop as well as the stirrer speed. 45% average recovery of Stevioside was obtained during stirred steady state experiments at lower operating pressures (276 and 414 kPa). At higher operating pressures, recovery of Stevioside in the permeate decreased remarkably.

Keywords: Stevioside; Clarification; Ultrafiltration; Permeate flux; Resistance; Recovery

1. Introduction

Demand for non-calorific natural sweeteners in the food industry has increased the importance of the Stevia plant. It is a herbaceous plant, belonging to the Asteraceae (Compositae) family, found mainly in certain regions of south America (native to Paraguay). Diterpene glycosides present in the Stevia leaves are responsible for sweet taste of this leaf. The glycosides are Stevioside, Rebaudioside A, B, D, E, Dulcoside A and B (Leung and Foster, 1996). Among these, Stevioside is generally found in maximum amount among the glycosidic constituents in the leaf. Their content varies in between 4 and 20% of the dried leaves (wt/wt) and Stevioside is reported to be approximately 300 times sweeter than sucrose (Geuns, 2003). Stevioside can be used as dietary supplement because it is heat stable, non-calorific and safe for diabetic, phenylketonuria patients and obese persons. Stability studies of Stevioside in carbonated beverages (Chang and Cook, 1983) indicated that the sweetener was stable when carbonated phosphoric and citric acidified beverages were exposed to temperature of 60 °C for a few days. Stability and interaction studies of Stevioside with different food components (organic acids, water soluble vitamins, coffee and tea and other low calorie sweeteners) were analyzed (Kroyer, 1999). It was concluded that Stevioside was stable in water solution up to temperature of 120 °C. In Stevioside sweetened tea and coffee no significant effect on Stevioside and caffeine was observed up to 80 °C. Thus, use of Stevioside as a substitute to sugar is quite promising. Therefore, extraction of Stevioside from Stevia leaves to edible quality is a subject of active research.

Between 1986 and 1999, several U.S. patents were available dealing with extraction of Stevioside using different technologies. Kumar (1986) described a process that involved various steps starting from extraction and obtaining final product by crystallization. The steps involved were, extraction by water, addition of chelating agent followed by addition of calcium containing agent (leading to precipitation) and crystallization of Stevioside (using solvent). Giovanetto (1990) disclosed

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a method in which water extracted liquor was centrifuged and then treated with calcium hydroxide. The precipitate was purified using a series of ion exchange resins. Payzant et al. (1999) proposed a method that used ion exchange columns for removal of impurities from Stevia extract and then methanol was used for separation and precipitation of Stevioside. Kutowy et al. (1998) were the first to use membrane separation process in a three stage diafiltration mode to separate the sweetening components. Shi et al. (2002) synthesized polymeric adsorbents by introducing quaternary ammonium groups into conventional resin adsorbent for purification of Stevia glycosides. The results of experiments concluded that the adsorption capacity for Stevia glycosides decreased and decolorization efficiency increased as the content of quaternary ammonium groups in the adsorbent increases. The mechanism of adsorption and decolorization was also proposed. Moraes and Machado (1999) verified that the adsorption process with Zeolite CaX not only clarified the Stevia extract but also presented a good recovery of sweeteners after the adsorption. Stevia extract (Mantovaneli et al., 2004) was clarified using calcium zeolites in fixed bed columns. The experimentation concluded that the mass transfer coefficient increased with an increase in flow rate.

The above mentioned processes are time consuming, expensive and unsuitable for use in food products due to the use of organic solvents. Removal of added chemicals and traces of solvents from the final product is also a big challenge. To overcome these problems, membrane based technologies offer attractive alternatives. Membrane technology can be used under room temperature resulting to no phase change; no chemicals are added; it is easy to scale up. Thus, membrane based processes were extensively used to process apple juice (Cetinkaya and Gokmen, 2006), mosambi juice (Rai et al., 2007), pomegranate juice (Neifar et al., 2009), carrot juice (Cassano et al., 2003), pineapple juice (de Barros et al., 2003), kiwi fruit juice (Tasselli et al., 2007), green coconut water (Jayanti et al., 2010), black tea (Evans et al., 2008), selective extraction of (−)epigallocatechin gallate from green tea leaves (Kumar et al., 2011).

Membrane technologies have been reported for extraction of Stevioside from Stevia leaves. In most of the cases, hybrid membrane separation was reported. Fuh and Chiang (1990) carried out purification of Stevioside using inorganic salts and ultrafiltration membranes having molecular weight cut-off (MWCO) of 25,000 and 100,000 Da. After purification, reverse osmosis was used for concentration and further purification was carried out using ion exchange. 45% recovery of Stevioside after ultrafiltration was reported. Zhang et al. (2000) proposed a membrane based separation process for processing sweeteners from Stevia extract. Microfiltration (using 0.35 micron ceramic membrane at 104 kPa transmembrane pressure) was used as a pre-treatment followed by ultrafiltration (using a 2500 Da molecular weight cut off membrane at 440 kPa pressure) for subsequent clarification. Finally, nanofiltration was used for further purification and concentration of Stevia extract. Modified Zeolite (NaX) was used for pre-treatment of crude Stevia extract followed by microfiltration (Silva et al., 2007). Approximately 80–100% clarification of Stevia extract was achieved with membranes of varying pore sizes and at different operating pressures. Reis et al. (2009) have used ceramic microfiltration membranes for clarification of Stevia extract. They found that 0.1 μm membranes at 4 bar pressure yielded the maximum Stevioside from the process. In another study by Vanneste et al. (2011), a comparison based on the use of commercial and tailor-made PES membrane for Stevioside purification has been reported.

The composition of permeate is the significant factor for determining the selection criteria and optimum operating conditions because the permeate is the desired product. For efficient design of industrial-scale processing unit, selection of membrane and optimum operating conditions is an important factor. Therefore, the present study was undertaken: (1) for selection of an appropriate ultrafiltration membrane (MWCO) based on the permeate flux and recovery of Stevioside in the permeate and (2) to study the effects of operating conditions on the permeate flux and quality in case of the selected membrane.

2. Materials and methods

2.1. *Materials*

Dry Stevia leaf powder was obtained from RAS Agro Associates, Maharashtra, India. Distilled water was used as the solvent for extraction process. Merck India Limited, Mumbai, India, supplied high performance liquid chromatography (HPLC) grade acetonitrile and water. Standard Stevioside of 98% purity was obtained from Sigma–Aldrich, USA.

2.2. *Extraction process*

Dry Stevia leaf powder was mixed with hot distilled water at a ratio of 1:14 (g:ml) and was held in a water bath at $78 \pm 1\,^{\circ}C$ for 56 min. These particular values have been fixed following the results of an optimized response surface methodology in extraction of Steviosides from dry Stevia leaves (Chhaya et al., submitted for publication). The above operating conditions were selected based on the optimization experiments carried out for maximum extraction of Stevioside in the liquor. Next, the aqueous Stevia extract was cooled to room temperature and cloth filtered. The filtered extract was analyzed for its color, clarity, Stevioside concentration and total solid content.

2.3. *Primary clarification*

Primary clarification of the extract was carried out in a laboratory centrifuge (Model number R-24, supplied by Remi International Ltd., Mumbai, India). The centrifugation capacity was 200 ml per batch. The operating conditions were: stirrer speed $5334 \times g$ and centrifugation time 26 min. These operating conditions were selected based on the related optimization study so that maximum clarity and recovery of Stevioside were obtained.

2.4. *Membranes*

For identification of suitable membranes, four polymeric ultrafiltration membranes of molecular cut-off, 5, 10, 30 and 100 kDa were used. These membranes were supplied by Permionics Membranes Pvt. Ltd., Gorwa, Vadodara, India. Permeance values of these membranes were measured using distilled water and are presented in Table 1.

2.5. *Experimental set up*

Two different experimental set-ups have been used. An unstirred batch cell was used to identify the suitable ultrafiltration membrane for clarification of Stevia extract. Once the