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Membrane-based separation scheme for processing sweeteners from stevia leaves☆

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Abstract

In existing processes, extraction and refining of glycoside based sweeteners from stevia leaves involves many process steps including extraction by organic solvents. The purpose of the present study was to develop a process of extraction and refining of sweeteners with reduced number of unit operations and minimization and/or elimination of chemical usage including organic solvents. It was found that water was very effective for extracting glycosides at selected pH and temperatures. It was also shown that a multi-stage membrane process was successfully able to concentrate the glycoside sweeteners. Based on the preliminary results, it appears that bitter-tasting components were washed out from the sweetener concentrate in the nanofiltration process. This work also has demonstrated that a membrane-based separation process for refining glycoside-based sweeteners could be viable and needs to be investigated further.

Keywords: Stevia; Sweeteners; Extraction; Separation; Membranes; Ultrafiltration; Nanofiltration; Microfiltration

1. Introduction

The food industry has traditionally used sugar as the sweetening agent. However, there is increasing demand for other sweeteners partially in response to consumer preference. There are synthetic as well as natural source sweeteners in the market. A significant segment of consumers is interested in sweeteners that are known to have nutritive values. Additionally the sweetener should fulfil the requirements relating to non-toxic nature, sugar-like taste profile, low calorific value, heat and pH stability. In recent years, there has been considerable interest in stevia-based natural source sweeteners that possess many of these desired qualities. *Stevia rebaudiana* is a shrub, native to Paraguay and is grown in the Far East, South America and many other areas of the world. The sweet compounds represent about 14% constituents of dried leaves and are diterpene glycosides based on the kaurene skeleton. These are mainly comprised of stevioside, rebaudioside A, B, D, E, dulcoside A and B (Leung & Foster, 1996). The most abundant glycoside is stevioside followed by rebaudioside and minor components. Sweetening potencies of various diterpenes found in *S. rebaudiana* range from 50 to 300 while non-sweet constituents mainly are labdane diterpenes, triterpenes, sterols and flavonoids. Rebaudioside A is the most desirable component due to its sweetening potency and superior taste profile (Cramer & Ikan, 1986). Responding to this challenge, new cultivars with significantly higher concentration of Rebaudioside A have recently been reported (Brandle, 1999).

There has been considerable interest in using stevia-based sweeteners in consumer products particularly in Japan. It was claimed that stevioside had a 20% of market share of low-calorie sweeteners in Japan (Kikuchi, 1985). According to some estimates, the market potential of these sweeteners at a penetration level of 4–8% in Japan and Far Eastern countries where it is approved would be in the range of US $1200 million. There is abundant published and patent literature on the stevia refining from dried leaves. For example, there are more than 150 Japanese patents on the subject. The process steps essentially are extraction, pretreatment, separation and refining. Most of the reported processes use coagulating agents and organic solvents. Some of the selected processes utilize chromatographic separation

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(Matsushita & Ikushige, 1979) and chelating agents followed by solvent extraction (Kumar, 1986). A process involving pretreatment of the extract with lime and use of a series of ion exchange column is of special mention as it has eliminated the use of organic solvents (Giovanetto, 1990). However, all of these processes have complex steps and use significant amounts of chemicals and/or generate sludge. There is a need of modifying this process to reduce the consumption of chemicals and waste streams. Furthermore, additional improvement in taste profile and colour of the final product is desirable. The present work was started to address above problems by using membrane-based process with modification in the extraction process step. This paper reports the effects of temperature and pH on extraction of sweeteners from dried leaves, pretreatment of the extract and subsequently effects of temperature on separation and refining by a multi-stage membrane process.

2. Experimental

2.1. Feed material

Ontario-grown dried stevia leaves were supplied by Agri-Canada's Delhi Research Station. These leaves were used in extraction experiment after removing twigs and branches. Concentrations of major glucosides in leave extract as determined by HPLC analysis were dulcoside, 5.6; stevioside, 58.7; rebaudioside C, 9.8 and rebaudioside A, 87.2 mg per g of dry leaves. Other plant constituents in the leave extract were not determined.

2.2. Analytical methods

Stevia leave extract was diluted appropriately by acetonitrile and water mixture (80:20) and allowed to stand overnight. In order to keep the chromatogram from cluttering by the peaks of ingredients other than glycosides, the sample for HPLC analysis was withdrawn only from the top layer. A 5 μm CSC-Sil 80 A amino column with dimensions of 255×0.46 mm was used (Chromatographic Sciences, Montreal, Canada). Standard operating conditions for HPLC analysis included a mobile phase of acetonitrile and water (80:20); flow rate 1.5 ml per min and a column temperature of 28°C. The calibration curves for stevioside, rebaudioside A and C were constructed using standards supplied by the Alberta Research Council.

2.3. Apparatus

A jacketed glass extraction column with dimensions of 600×90 mm was used for column extraction while the batch extractions were done in glass beakers. Initial membrane evaluation and characterization for separations was done using NRC cells (Sourirajan & Matsuura, 1985; Zhang, Kutowy, Kumar & Malcolm, 1997) as well as Sepacell (supplied by Osmonics, Minnetonka, MN, USA).

2.4. Procedure

A carefully weighed amount of dried leaves were placed in a standard glass column and extraction was done with reverse osmosis water at different temperature. Plant material to water ratio was maintained in the range of 0.02–0.1. The extract from this column was pretreated with a ceramic tubular membrane (US Filter, Warrendale, PA, USA) with a mean pore size of 0.35 μm. This membrane had a surface area of 0.005 m^2 and was operated at a trans-membrane pressure of 104 kPa. Permeate from above was treated with an ulrafiltration (UF) membrane (Liumar Technologies, Ottawa, Canada) in diafiltration mode. This UF membrane had a molecular weight cut-off (MWCO) rating of 2.5 kDa and was operated at a trans-membrane pressure of 440 kPa. Diafiltration was discontinued once the majority of the extracted glycosides were washed out in the permeate. This permeate was washed of lower molecular weight impurities by a

Table 1
The effects of pH on stevioside and color extraction

pH	Stevioside (mg/l)	Optical absorbency at 420 nm
2.0	8100	5.9
7.0	8000	9.0
9.0	7900	8.0

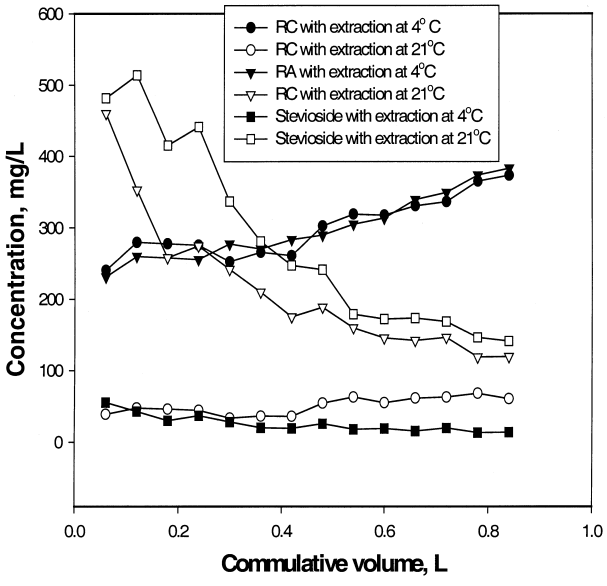

Fig. 1. Concentration of various sweeteners versus the cumulative extract volumes at different temperatures. RA and RC are Rebaudioside A and Rebaudioside C, respectively.

nanofiltration (NF) membrane (Duratherm™, Osmonics/Desalination, Minnetonka, MN, USA) that was operated at a trans-membrane pressure of 510 kPa in a diafiltration mode while feed could be maintained up to a temperature of 80°C. Finally, the NF membrane was operated in a concentration mode and retentate was recovered for further processing.

3. Results and discussion

Development of this process involves several distinct unit operations that will be discussed below.

Based on a number of preliminary experiments, it was found that dried leaves of 10–40 mm size were most suitable for column extraction. A flow rate of 24–30 ml/min was maintained through the glass column. A temperature range of 4–35°C for extracting water of varying pH was investigated. The effects of pH of extracting water are shown in Table 1. It is clear from the data that amount of stevioside in the extract remained constant while the amount of color components extracted were lower at lower pH. It was also observed that amount of extracted sweeteners as well as color components were higher at higher temperature. The rates of extraction of various sweeteners were relatively constant whereas at higher temperature the extraction rates were initially higher then gradually achieved a constant value. (Fig. 1).

In order to remove suspended fine particles and very large molecular weight components, a laboratory scale ceramic microfiltration (MF) membranes with a mean pore radius of 0.3–0.8 μm were found adequate. It was observed that about 80% of sweeteners permeated through these membranes. The rest of the sweeteners could be recovered by addition of water to concentrate followed by microfiltration.

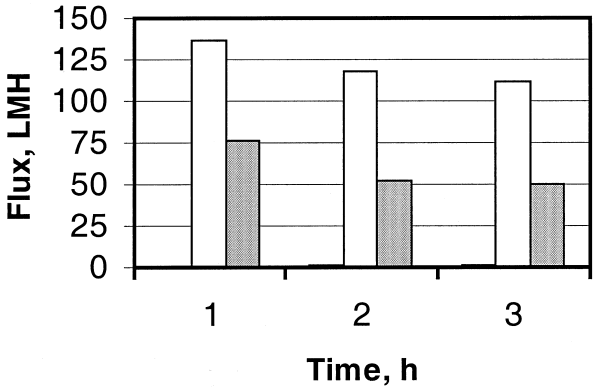

Fig. 2. Variation in ultrafiltration flux with and without chemical pretreatment.

Table 2
Stevioside concentration and permeation rates for different diafiltration volumes in ultrafiltration step

Diafiltration volume	Stevioside concentration (g/l)	Permeation rates (l m^{-2} h^{-1})
0	1.5	35
2.0	0.75	47
3.0	0.25	64
4.0	ND[a]	65

[a] ND, not detected.

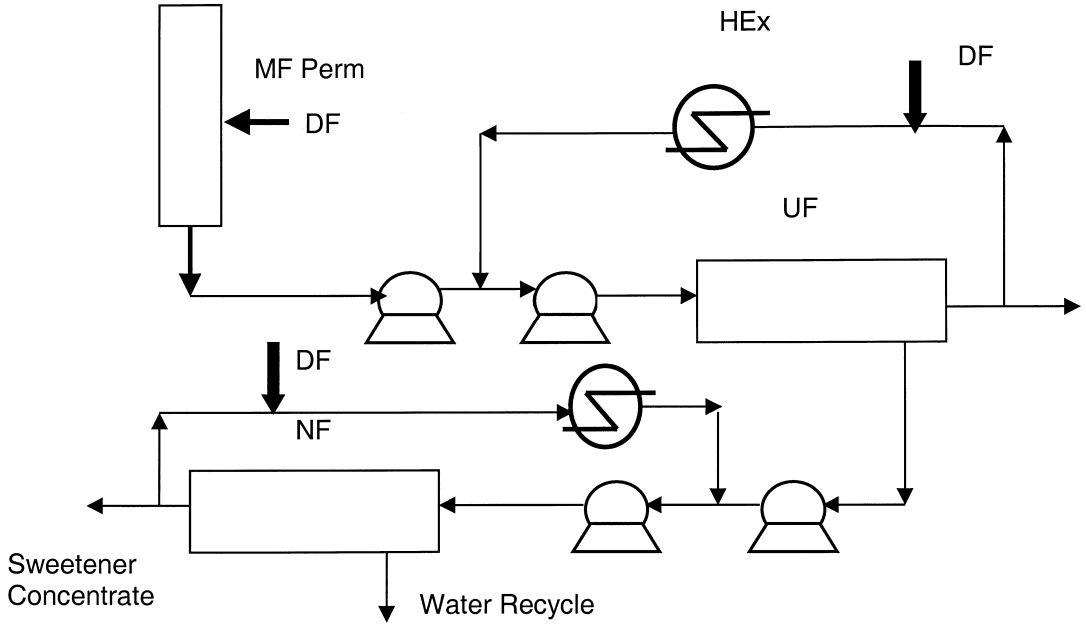

Fig. 3. A conceptual process flow diagram for producing stevia sweetener concentrate. Hex-heat exchanger, DF, UF and NF are diafiltration, ultrafiltration and nanofiltration, respectively.

The permeate stream from MF treatment contained most of the sweeteners and other natural products. This stream was treated with a selected ultrafiltration (UF) membrane. Addition of small amounts (less than 1% w/w) of flocculating agents improved the flux at this stage as shown in Fig. 2. The UF membrane with a molecular weight cut-off rating of 2.5–3.0 kDa was used. It was found that about three diafiltration volumes were sufficient to permeate sweeteners through this membrane while larger molecular weight components were rejected (Table 2).

Permeate from UF stage contained sweeteners and very low molecular weight impurities. This stream was concentrated using a nanofiltartion membrane at higher temperatures. It was observed that in addition to concentrating the sweeteners this treatment improved the taste profile of the product as determined by preliminary taste tastes and HPLC analysis. It was shown by the decline in the non-glycoside peaks in HPLC chromatograms that operation at higher temperature of 80°C reduced the impurities to 55% of original while at 50°C, these were reduced only to 89%.

Based on the above steps a conceptual flow diagram of a process involving column extraction at a suitable temperature with multiple stage of membrane treatment is shown in Fig. 3. The process could provide a relatively high purity sweetener concentrate that could be further processed by ion exchange followed by spray drying to make high purity sweetener powder. Additional membrane units could be used to recycle the process water.

4. Conclusions

It was shown that extraction of sweeteners as well as other components was dependent on temperature of the extracting water. The pH of extracting water did not affect the amount of extracted sweeteners, however, lower amounts of color components were extracted at a lower pH.

Pre-treatment with microfiltration ceramic membranes was adequate. Addition of lime and or flocculating agent to ultrafiltration feed improved the flux significantly. A diafiltration volume of 3 was found to be adequate for eluting sweeteners. The nanofiltration membranes were adequate for concentrating the sweeteners as well as removing lower molecular weight impurities. It was also observed that operation of nanofiltration membranes at higher temperatures was more effective in removing the impurities and consequently improving the taste profiles of the sweeteners.

References

Brandle, J. (1999). Genetic control of rebaudioside A and C concentration in leaves of the sweet herb, stevia rebaudiana. *Canadian Journal of Plant Science, 79*(1), 85–92.

Cramer, B., & Ikan, R. (1986). Sweet glycosides from the stevia plant. *Chemistry in Britain, 22*, 915–916.

Giovanetto, R. H. (1990). *Method for the recovery of steviosides from plant raw material*. US patent no. 4,892,938.

Kikuchi, H. (1985). *Food Chemicals. Food Science (Japan), 85*(3), 52.

Kumar, S. (1986). *Method for recovery of stevioside*. US patent no. 4,599403.

Leung, A. Y., & Foster, S. (1996). *Encyclopedia of common natural ingredients — used in food, drugs and cosmetics* (2nd ed.). New York: John Wiley and Sons, Inc. (p. 478).

Matsushita, S., & Ikushige, T. (1979). *Separation of natural sweet component from natural extract*. US patent no. 4,1717430.

Sourirajan, S., & Matsuura, T. (1985). *Reverse osmosis/ultrafiltration process principle*. NRCC no. 24188 (pp. 79–81).

Zhang, S. Q., Kutowy, O., Kumar, A., & Malcolm, I. (1997). A laboratory study of poultry abattoir wastewater treatment by membrane technology. *Canadian Agricultural Engineering, 39*(2), 99–105.