Application of tailor-made membranes in a multi-stage process for the purification of sweeteners from *Stevia rebaudiana*

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**A B S T R A C T**

In this paper the performance of a three stage process with commercial as well as tailor-made polyethersulfone (PES) membranes for the purification of sweeteners from *Stevia rebaudiana* Bertoni was evaluated. Retentions of the sweeteners for a synthetic mixture and plant extract in combination with flux decline measurements indicated that, in contrast with the lab-made membranes, on most commercial membranes a foulant layer was formed that influenced the separation performance negatively. For the plant extract, the best commercial membrane (PW010) had a selectivity and flux similar to the best lab-made membrane (27% PES), but the lab-made membrane was preferred because it showed a slightly lower retention of the sweeteners, as desired. Starting from an extract purity of 11% with the overall process (microfiltration, ultrafiltration, nanofiltration) a purity of 37% and a yield of 30% could be reached.

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1. Introduction

Stevioside and rebaudioside–A are the two main sweet steviol glycosides from the *Stevia rebaudiana* Bertoni plant (Fig. 1). They taste about 300 times sweeter than sucrose (0.4% solution). Their content varies between 4 and 20% of the dry weight of the leaves depending on the cultivar and growing conditions (Geuns, 2003). The advantages of stevioside and rebaudioside–A as a dietary supplement for human subjects are manifold: they are stable, they are non-calorific, they maintain good dental health by reducing the intake of sugar and open the possibility for use by diabetic and non-calorific, they maintain good dental health by reducing the intake of sugar and open the possibility for use by diabetic and phenylketonuria patients (Geuns, 2003). The use of solvents should be avoided as much as possible. Although the sweeteners can be extracted with water, current purification techniques still make use of methanol or ethanol (Carakostas et al., 2008). Kutowy et al. (1999) were, to the knowledge of the authors, the first to propose a membrane-based purification process. After the extraction column their process consists basically out of three membrane stages operated in diafiltration mode: a microfiltration and an ultrafiltration membrane stage, to remove impurities with a higher molecular weight than rebaudioside–A, and finally a nanofiltration membrane stage, to remove impurities with a lower molecular weight than stevioside. Zhang et al. (2000) presented some interesting lab-scale results concerning this process configuration, but the most relevant expression of the separation performance from an industrial point of view, namely the product purity and yield, was not mentioned. Although the purity can be measured easily on the basis of the sweetener content and total dry weight of a sample, it is very impractical to measure for lab-scale membrane processes, especially when a lot of different membranes and conditions should be tested to optimize the process, because a large amount of permeate is necessary in order to have a sufficiently accurate reading of the dry weight on a standard balance. As there are many different kinds of impurities present in the plant extract, it is very difficult to find another easily
measurable parameter that specifically reflects the concentration of all impurities of a sample. Moreover, if that parameter would exist, in order to determine the concentration of the impurities, standard solutions from all present compounds should be prepared for calibration. Most impurities are not available in pure form and as a result it is impossible to determine the concentration of these compounds in the plant extract. Reis et al. (2009) studied different microfiltration membranes and conditions and proposed to use the UV absorption at 420 and 670 nm as specific indicators for the impurity content as the sweeteners only absorb at 210 nm, but as Zhang et al. (2000) they do not mention the purity or yield of the product stream of the process. In this study, the process configuration as proposed by Kutowy et al. (1999) will be studied in detail. The impurity parameters, used by Reis et al. (2009) to select the most appropriate microfiltration membrane, will now be used to select the best ultrafiltration and nanofiltration membrane. But most importantly, also the evolution of the purity and the yield throughout the process will be analyzed. In addition, the effect of diafiltration on the purity and yield will be studied.

The best membrane for a certain application is often selected from a Robeson plot on which the selectivity is plotted versus the permeability of the different membranes (Mehta and Zydney, 2005; Robeson, 1991). The selectivity is undoubtedly the most important separation performance parameter of a membrane. Beside the development of new membrane materials and manufacturing techniques, tailor-made membranes could increase the selectivity for a certain application. Although it might technically be possible for a membrane company to produce for instance 10 membranes with different molecular weight cut-off (MWCO) in a certain molecular weight range, economical considerations could limit the commercially available number of membranes. As the demand for high selectivity membranes increases, so will increase the need for a flexible membrane formation platform to make a range of membranes with varying, controlled selectivity. Therefore, an important goal of this study was to prepare different polyethersulfone (PES) membranes and to compare the permeability and the selectivity of these membranes for a specific application with those of commercial ultrafiltration (UF) membranes. In this study polyethersulfone membranes were prepared using the DIPS technique (diffusion induced phase inversion) (Boussu et al., 2006b). In this process, a thin layer of the polymer dissolved in an appropriate solvent is cast on a support and phase separation is induced by a non-solvent. The most efficient way to induce the phase inversion is by immersing the polymer solution film in a non-solvent bath. By changing the preparation factors, an optimized membrane for a specific purpose can be obtained. Three parameters were varied to study the effect on permeability and selectivity: the polymer concentration, composition of the non-solvent bath (water-isopropanol (IPA) mixture) and mixture of solvents - dimethylformamide (DMF) or N-methyl-pyrrolidone (NMP). First, the selectivity was studied for a mixture of polyethylene glycols, stevioside (MW 804) and rebaudioside–A (MW 967). Although polyethylene glycols have a very specific linear structure, they are extensively used to study the separation performance of membranes (Boussu et al., 2006b; Li et al., 2009). The polyethylene glycols were added as model impurities as the real impurities were not yet identified or not available in pure form, but the molecular weight range of the different polyethylene glycols is expected to encompass the molecular weight range of the real impurities. The membranes with the highest selectivity and flux for the synthetic mixture were further studied with the real plant extract.

2. Materials and methods

2.1. Materials

A mixture of 95% pure stevial glycosides with approximately 75% stevioside and 25% rebaudioside–A was purchased from Medherbs (Wiesbaden, Germany). Pulverized stevia leaves were
provided by the same company. The polyethylene glycols (PEG $M_W$ 400, $M_W$ 1500, $M_W$ 4000, $M_W$ 10000 and $M_W$ 20000) were obtained from Sigma Aldrich (Bornem, Belgium).

2.2. Extraction method

Kutowy et al. (1999) as well as Reis et al. (2009) used cold water as a solvent to extract the sweeteners from the leaves. More sweeteners might be extracted in hot water, but large impurities could be by the heat break up into smaller compounds that are much more difficult to remove with membrane processes. A solution was made with 20 g leaves per liter and was stirred for 2 h at 5°C. After extraction a content on dry weight basis of 11% of steviol glycosides of which 7% stevioside and 4% rebaudioside–A was measured. The extract was then passed through a 63 μm sieve to remove large plant material that can otherwise foul the filtration equipment.

2.3. Commercial membranes

MP005 was used in the microfiltration membrane stage as this membrane had the same pore diameter as the best membrane from the study of Reis et al. (2009). Eight UF membranes were tested: GE GK, GE GM, UH004, GE PT, UP005, UC005, Berghof type 3705 and GE PW. Finally, three nanofiltration membranes were tested: NTR7430, NTR7450 and GE GH. The characteristics of these membranes as specified by the manufacturer are summarized in Table 1.

2.4. Membrane preparation

The membrane preparation procedure is described elsewhere (Boussu et al., 2006c). Polyethersulfone from Solvay, Belgium (PES Radel A-100) was used and dissolved in $N$-methyl-pyrrolidone (NMP) at three different concentrations: 27, 30 and 33%. One membrane with 30% PES was also made, but this membrane was only tested with the plant extract. Also a membrane with 30% PES and 50% NMP/dimethylformamide (DMF) was synthesized. As a non-solvent distilled water at 20°C was used. Isopropanol (IPA) was added to the non-solvent bath at two different concentrations: 10 and 20%. A thin film of the polymer solution with a thickness of 250 mm was made on a non-woven support (FO2471, Freudenstein, Germany) at a speed of 20 mm/s in an atmosphere with controlled relative air humidity of 40% (Boussu et al., 2006c). For each set of process parameters, three identical membrane sheets were made and tested to obtain a mean value of the flux and the retention.

2.5. Membrane performance assessment

The performance of the membranes was tested in a cross-flow set-up which is described elsewhere (Boussu et al., 2006c). The water permeability, $L_p$, can be calculated from the permeate flow rate ($Q_p$) at different transmembrane pressures ($\Delta P$) and the effective membrane area ($A_m$):

$$L_p = \frac{Q_p}{A_m \Delta P} \quad (1)$$

The effective membrane area was equal to 0.0044 m$^2$ and the permeability was measured at 293 K. The ratio of the permeate flow rate ($Q_p$) to the effective membrane area ($A_m$) is actually equal to the flux ($J$) of the membrane. The flux decline as a result of fouling can then be calculated as a function of the pure water permeability ($L_p$) as follows:

$$Flux \ decline = 1 - \frac{J}{L_p \Delta P} \quad (2)$$

The effect of the preparation parameters on the retention and selectivity was studied at 3 bar for a mixture of 1 g L$^{-1}$ of PEG with different molecular masses (400–20,000 g mol$^{-1}$) and 1.5 g L$^{-1}$ stevioside and rebaudioside–A. The retention of stevioside, rebaudioside–A and the UV absorbing impurities in the plant extract was measured after 2 h filtration at 3 bar for the microfiltration and ultrafiltration membranes and 8 bar for the nanofiltration membranes. In all cases the temperature was set at 25°C except for the final experiment with the best membranes. To inhibit growth of bacteria due to the long experiment time it was necessary to maintain a temperature of 65°C. The increased temperature had only a small influence on the retention of the compounds. The cross-flow was in all cases set to 400 L h$^{-1}$. Concentration polarization will be strongly reduced at this flow rate as with a hydraulic diameter of the module of 0.00429 m this flow rate corresponds to a cross flow velocity of 7.7 m/s. Because the concentration polarization was small and the retentions were measured in a closed loop system, the measured retention can be assumed equal to the real membrane retention. The membrane retention of a compound i in this case be calculated as follows:

$$R_{m,i} = 1 - \frac{C_{pi}}{C_{ji}} \quad (3)$$

The concentration of PEG in feed ($C_j$) and permeate ($C_f$) was measured with high-performance size-exclusion chromatography (HPSEC) on a Shodex SB-803 HQ column (300 mm × 8.0 mm i.d.)

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Manufacturer</th>
<th>MWCO (Da)</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTR7450</td>
<td>Somicon</td>
<td>600–800</td>
<td>Sulfonated polyethersulfone</td>
</tr>
<tr>
<td>NTR7430</td>
<td>Somicon</td>
<td>1000</td>
<td>Sulfonated polyethersulfone</td>
</tr>
<tr>
<td>GE GH</td>
<td>GE Osmonics</td>
<td>1000</td>
<td>Thin film</td>
</tr>
<tr>
<td>GE GK</td>
<td>GE Osmonics</td>
<td>2000</td>
<td>Thin film</td>
</tr>
<tr>
<td>GE GM</td>
<td>GE Osmonics</td>
<td>4000</td>
<td>Thin film</td>
</tr>
<tr>
<td>UH004</td>
<td>Nadir</td>
<td>5000</td>
<td>Permanently hydrophilic polyethersulfone</td>
</tr>
<tr>
<td>GE PT</td>
<td>GE Osmonics</td>
<td>5000</td>
<td>Polyethersulfone</td>
</tr>
<tr>
<td>UP005</td>
<td>Nadir</td>
<td>5000</td>
<td>Permanently hydrophilic polyethersulfone</td>
</tr>
<tr>
<td>UC005</td>
<td>Nadir</td>
<td>5000</td>
<td>Polyethersulfone, tubular</td>
</tr>
<tr>
<td>Berghof type 3705</td>
<td>Berghof</td>
<td>5000</td>
<td>Polyethersulfone</td>
</tr>
<tr>
<td>GE PW</td>
<td>GE Osmonics</td>
<td>10,000</td>
<td>Polyethersulfone</td>
</tr>
<tr>
<td>UP020</td>
<td>Nadir</td>
<td>20,000</td>
<td>Polyethersulfone</td>
</tr>
<tr>
<td>MP005</td>
<td>Nadir</td>
<td>0.05 μm</td>
<td>Polyethersulfone</td>
</tr>
</tbody>
</table>

a Ge Betz, Belgium.
b Nadir Filtration GmbH, Germany.
c Berghof, Germany.
d Somicon, Switzerland.
with a Shodex SB-G guard column (50 mm × 6 mm i.d.) from Showa Denko K.K. (Kawasaki, Japan) in combination with an evaporative light scattering detector (Alltech ELSD 2000ES, Grace Davison Discovery Sciences). Elution of the samples (20 μL) was with 25 mM ammonium acetate (pH 5, 0.5 mL min⁻¹, 30 °C) on a Kontron 325 pump system (Kontron, Milan, Italy) equipped with auto-injection. Stevioside and rebaudioside-A were separated with an Agilent 1200 HPLC system and an Altima C18 column (Alltech, Grace Davison Discovery Sciences). The concentrations were measured with a UV detector at 210 nm. The samples (100 μL) were eluted with 32% acetonitrile in water at a flow rate of 1.5 mL min⁻¹ and a column temperature of 40 °C.

For the experiments with the synthetic mixture, the selectivity ψ for stevioside (st) and rebaudioside-A (reb-A) was defined with PEG with a molecular mass of 1500 g mol⁻¹ as a reference impurity:

\[
\psi = \frac{R_{st}/R_{reb}}{1 - \frac{R_{st}}{R_{reb}}} 
\]

The retentions were calculated using Eq. (3). For the experiments with the plant extract, the selectivity was defined with the impurities absorbing at 420 nm and 670 nm as reference impurities as proposed by Reis et al. (2009):

\[
\psi = \frac{1 - \frac{R_{st}}{R_{reb}}}{1 - \frac{R_{st}}{R_{reb}}} 
\]

The absorbance was measured with a Shimadzu UV–1601 spectrophotometer. From all membrane types three sheets were tested in order to obtain a standard deviation on the flux decline and the selectivity was calculated taking into account error propagation theory. An important operating condition of a membrane module in an open system is the recovery:

\[
Rec = \frac{Q_p}{Q_f} 
\]

with Q_p and Q_f the permeate and feed flow, respectively. In practice, the recovery will be much larger than in a laboratory cross-flow set-up. Hence, the effect of the recovery on the retention should be taken into account when evaluating the separation performance of the membrane module (Mulder, 1996):

\[
R_i = 1 - \frac{1}{Rec} (1 - (1 - Rec)^{-1}) 
\]

with R_{rec} the retention of the membrane for compound i. At very low recoveries, which was the case for the lab-scale set-up operated in closed loop, Eq. (7) reduces to Eq. (3). The concentration factor CF is related to the feed concentration (C_f) by the concentration factor CF = C_p/C_f (Mulder, 1996):

\[
CF = \frac{C_{p,i}}{C_{f,i}} = (1 - (1 - Rec)^{-1}) 
\]

The purity of a product i in the permeate (P_{ip}) for a multi-component mixture is defined as:

\[
P_{ip} = \frac{C_{p,i}Q_p}{C_{p,i}Q_p + \sum_{j=2}^{n} C_{p,j}Q_p} = \frac{C_{f,i}(1 - R_i)}{\sum_{j=2}^{n} C_{f,j}(1 - R_j)} 
\]

From this formula it can be derived that the purity can only be predicted, if the retentions and the concentrations of all the compounds can be measured, which was in this case impossible. To measure the purity, however, only the total mass of the compounds of interest (numerator Eq. (8)) and the total dry weight (denominator Eq. (8)) should be known. In order to have an accurate reading on the balance it was necessary to take a sample of at least 50 ml. Due to the high sampling volume in relation to the feed volume (only 1 L in nanofiltration stage) it was not possible to take a lot of samples without interfering with the process. Hence, it was not possible to determine the standard deviation of the purity measurements. The yield of a component i in the permeate (Y_{ip}) can be predicted from the recovery and the membrane retention R_{m,i}:

\[
Y_{ip} = \frac{C_{ip}Q_p}{C_fQ_f} = (1 - R_i)Rec = 1 - (1 - Rec)^{-1} 
\]

When the product is in the concentrate, the yield can be calculated as follows:

\[
Y_{ic} = 1 - Y_{ip} = (1 - Rec)^{-1} 
\]

When the membrane process is operated in constant volume diafiltration mode, the yield in the permeate can be calculated as follows:

\[
Y_{ip} = 1 - e^{-(1 - R_{m,i})N} 
\]

with N (=V_p/V_f) the number of diaivolumes. The yield in the concentrate with diafiltration is then equal to:

\[
Y_{ic} = e^{-(1 - R_{m,i})N} 
\]

In the case of a multi-stage process without recycle as proposed by Kutowy et al. (1999), the total yield is obtained by multiplying the yield of the separate stages. As the yield can be predicted on the basis of known parameters, it can be compared with the measured yield.

3. Results and discussion

3.1. Effect of membrane preparation conditions on permeability and retention

The effect of changing the dope concentration of PES, addition of DMF to the dope solution and addition of IPA to the non-solvent bath on the permeability is shown in Fig. 2. The permeability dropped with increasing polymer concentration from 34.8 L h⁻¹ m⁻² bar⁻¹ for 27 wt.% PES to 1.4 L h⁻¹ m⁻² bar⁻¹ for 33% of PES. This trend is consistent with results from literature for different polymer substrates (Ismail and Hassan, 2006; See-Toh et al., 2007). It can be expected from the fact that a higher polymer concentration increases the viscosity of the polymer solution. A higher viscosity hampers the diffusional exchange between solvent and non-solvent which leads to a higher polymer concentration at the interface between polymer solution and non-solvent bath and hence to a lower porosity and a lower permeability of the membrane (Boussu et al., 2006a; See-Toh et al., 2007; Xiao-Lin et al., 2008). The permeability nearly halved for a 30% PES membrane by adding 10% IPA to the solvent bath. A solvent mixture of 50% NMP/DMF had a four times lower permeability. Both results are consistent with a shift of the binodal in the phase diagram to the right as predicted by model calculations and experiments which yields a denser membrane (Mulder, 1996).

From a denser membrane structure also a higher retention could be expected, but this only holds for the 50% NMP/DMF membrane for which the retention of steviol glycosides nearly doubled compared to a 30% PES membrane (Fig. 3). An increase in retention with decreasing polymer concentration seems to be in contradiction with results from literature (Ismail and Hassan, 2006; See-Toh et al., 2007). It is also difficult to explain this observation from the influence of membrane characteristics (porosity, pore size and membrane thickness) on flux and rejection. Higher porosity and pore size would lead to a lower rejection and a higher flux. An increased membrane thickness will increase rejection and decrease
flux (Bowen and Mohammad, 1998). Although the differences in retention observed here are not significant, an increased rejection in combination with an increased permeability has been observed before with addition of ethanol to the dope solution at low concentrations (Xu and Qusay, 2004). Above 10% ethanol the permeability increased and the retention decreased again with ethanol concentration. In other words a maximum retention was observed at an ethanol concentration of 10% while the permeability increased monotonously. In Fig. 3 the retention of the sweeteners seems to stabilize or even decrease slightly while the flux increased by 29% from the 27 to the 24% PES membrane (Figs. 5 and 6). Thus also here a maximum retention seems to exist, in this case as a function of polymer concentration. An opposite trend, namely a minimum in retention, was observed for the addition of IPA to the solvent-bath. At 10% IPA the retention was lower than without IPA, but at 20% IPA the retention increased again compared to 10% IPA. For all the membranes the retention of the steviol glycosides was too low to be used in a nanofiltration stage to remove impurities with a lower molecular weight, but low enough to be used in an ultrafiltration stage. A low retention of the sweeteners is necessary in the ultrafiltration stage to
minimize the loss of sweeteners to the concentrate while a high retention is necessary in the nanofiltration stage to minimize the loss of sweeteners to the permeate.

3.2. Comparison of membrane performance of lab-made and commercial membranes

The performance of the lab-made membranes was compared with that of commercial flat sheet membranes on the basis of a Robeson plot (Fig. 4). Such a plot allows to select the membranes with the highest selectivity and/or permeability for a certain application (Mehta and Zydney, 2005). A first set of membranes was selected on the basis of the highest selectivity and permeability for a synthetic mixture of steviol glycosides and different polyethylene glycols.

Fig. 4 shows that the permeability increases by a factor thirty and selectivity more than triples if polymer concentration is lowered from 33 to 27%. Addition of IPA reduced permeability and selectivity. Addition of DMF reduced permeability and did not affect selectivity. When the standard deviation is added to the Robeson plot it is also possible to compare the stability of the characteristics of the self-made membranes with that of the commercial membranes and thus evaluate the ‘tunability’ of the manufacturing process. The stability of both selectivity and permeability of the lab-scale process, where only air humidity was controlled, was very comparable with that of the fully automated industrial process. This is not so surprising as the air humidity has previously been identified to have a large influence on the stability of membrane characteristics (Boussu et al., 2006a). Most lab-prepared membranes, however, had a lower selectivity and similar permeability compared to the best commercial membranes. Only membranes prepared with low polymer concentrations (27%) combine a moderate selectivity with a high permeability, but the selectivity-gap with the best commercial membranes remains large. Further reducing the polymer concentration could yield a higher selectivity, but this was not the case for the 24% PES membrane although this membrane was only tested with the plant extract (Fig. 5).
However, when the membranes were tested on the plant extract and the selectivity was calculated on the basis of the UV absorbance at 420 and 670 nm according to Eq. (4), the selectivity-gap between commercial and lab-made membranes seems to disappear (Fig. 5). Although the selectivity is now defined differently, an important reason for the selectivity of the best commercial membranes with the synthetic mixture (UP005, UH004, GE PT) to drop so much, is that the retention of the sweeteners of the commercial membranes increased significantly with the plant extract while the retention of the sweeteners remained almost the same for the lab-made membranes (Fig. 6). Hereupon, it was hypothesized that for this application the commercial membranes were more prone to fouling than the lab-made membranes. This hypothesis is supported by the flux decline. From Fig. 6 it can be seen clearly that the membranes with a large increase in retention of the sweeteners also have a large flux decline probably due to the formation of a foulant layer. This foulant layer will act as an additional membrane impacting the separation performance and in many cases reducing the effective MWCO of the membrane. The shift of the largest selectivity towards commercial membranes with a higher MWCO confirms this (Figs. 4 and 5). For the plant extract, the PW010 membrane with a MWCO of 10 kD has now a much higher selectivity than the UP005 membrane with a MWCO of 5 kD.

For the UP020 membrane with a MWCO of 20 kD the selectivity was lower than for the PW010 membrane, but still higher than the UP005 membrane. In fact it could be hypothesized that the occurrence of a foulant layer, in this case, determines the separation performance because there was no significant difference in the selectivity of the best commercial and lab-made membranes. Of all the tested membranes, the 27% PES membrane was selected for further experiments. This membrane had a similar selectivity as the PW010 membrane and a slightly lower retention for the sweeteners (Figs. 5 and 6). From the analysis here, it is obvious that an ultrafiltration membrane with a MWCO of maximum 3000 g/mol proposed by Kutowsy et al. (1999) will probably have a low selectivity for the plant extract if a foulant layer is formed. Even if the membrane is not fouled and the selectivity is acceptable, a membrane with a cut-off of 3000 g mol\(^{-1}\) will have a much higher retention for the sweeteners than for instance the PW010 membrane, which would induce a high loss of sweeteners to the concentrate. Finally, it is also clear that, at least for this application, the behavior of the real process stream can differ substantially from that of a model mixture. Nevertheless, mixtures of polyethylene glycols are used extensively to study the separation performance of membranes and more specifically to determine the MWCO (Boussu et al., 2006b; Li et al., 2009). The MWCO determined in this way will however seldom reflect the separation performance in practice, especially when a foulant layer is formed.

### 3.3. Analysis of process performance

The raw extract contained 11% of stevioside and rebaudioside-A. After sieving the purity of the sweeteners increased to 21%. Passing the sieved extract through the MP005 microfiltration membrane further increased the purity to 27%. The purity decreased slightly as a function of time from 34 to 32% with ultrafiltration (Fig. 7).

Fig. 7 shows that the application of diafiltration did not increase the purity relative to normal filtration. However, the yield of
normal filtration increases much faster as a function of time (Fig. 8). After 6 h, the yield with normal filtration is 39% while for diafiltration only 19%. Considering both purity and yield, it can be concluded that normal filtration is to be preferred. The calculated yield values matched well with the experimental results.

In the nanofiltration stage the NTR7450 membrane was used as this membrane had the highest retention of the sweeteners from the three tested membranes (results not shown). In this stage only diafiltration was tested as not enough permeate from the ultrafiltration stage could be collected to apply normal filtration. With nanofiltration, the purity could be increased further from 32 to 37% after 6 h filtration (Fig. 9). However, this was only possible at the expense of 22% loss of sweeteners to the permeate (Fig. 10). The calculations slightly underestimated this loss of sweeteners.

Starting with an extract purity of 11%, with this process configuration, operating conditions and the selected membranes a purity of 37% can be reached with an overall yield of 30%. It can be concluded that membranes should be integrated in other process configurations or complemented with other purification techniques, like crystallization, in order to comply with the purity requirement of 95% set by JECFA (Carakostas et al., 2008). Other cultivars exist, which contain 20% steviol glycosides (Geuns, 2003), but it will probably still be impossible to reach 95% purity with this process.

4. Conclusions

In this work, the potential of an integrated process with a microfiltration, ultrafiltration and nanofiltration membrane stage for the purification of sweeteners from S. rebaudiana Bertoni was studied. For the ultrafiltration membrane stage, the performance of different commercial as well as lab-made polyethersulphone membranes was studied. Comparison of the permeability and selectivity for the separation of a synthetic mixture of sweeteners and polyethylene glycols of the lab-made membranes with that of the commercial membranes, showed that high permeability but only moderate selectivity could be achieved and only at low polymer concentrations (27% PES). However, the formation of a foulant layer with the filtration of the plant extract drastically changed the separation performance of the commercial membranes. The occurrence of a foulant layer was supported by the fact that the retention of the sweeteners increased relative to the synthetic mixture and the flux decline was in many cases larger than for the lab-made membranes. For the plant extract the best commercial membrane (PW010) had a similar selectivity and flux as the best lab-made membrane (27% PES). The stability of both selectivity and flux of the lab-made membranes, where only air humidity was controlled during manufacturing, was also very comparable with that of the commercial membranes. The lab-made membrane (27% PES) was preferred as it had a slightly lower retention of the sweeteners thus reducing the loss of sweeteners to the concentrate. The NTR7450 membrane was selected for the nanofiltration stage as it showed the largest retention of the sweeteners thus reducing the loss of sweeteners to the permeate. It was also shown that with normal filtration a higher yield could be reached faster than with the application of diafiltration in the ultrafiltration stage. With the overall process (microfiltration, ultrafiltration, nanofiltration), a purity of 37% and a yield of 30% could be reached. The use of high content cultivars (20% sweeteners) can increase the final purity of this process, but probably not up to the 95% requirement.
proposed by the Joint FAO/WHO Expert Committee on Food Additives (Carakostas et al., 2008). This process should, therefore, be seen as a useful pretreatment for other purification steps, with for instance crystallization.

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