Date Juice Decolourised by Ultra-Filtration and its Use with Stevioside Sweetener in Beverage Formulation

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Abstract
Date juice was treated by cross-flow ultra-filtration with molecular weight cut-off (MWCO) of 5 kDa. Optimized levels of feed brix, TMP and operation time were determined as 47.13, 82.11 psi and 30 min, respectively. Under such conditions, 80.94% decolourisation and 97.78% turbidity reduction were obtained while loss of glucose and fructose were estimated to be only 3.49% and 9.62%, respectively. Regarding sweetness, the results of beverage sensory indicated that samples prepared by 100% decolourised date juice, 50% sugar, + 50% stevioside, 50% decolourised date juice and + 50% stevioside did not show significant differences compared with the sample prepared by 100% sugar. From the view point of the bitter aftertaste, samples prepared by 100% sugar and 100% stevioside had the highest and lowest desirability, respectively, while there was no significant difference between colour and clarity of the samples.

Keywords: Date juice, Ultra-filtration, Decolourisation, Sugar free beverage

Introduction
Date juice obtained from low quality dates is suitable for food industry due to their high sugar content. The amount of reducing sugars in date syrup with total solids in the range of 67-72% is 85% (Al-Hooti et al. 2002). Also, in different Iranian dates, the total phenolic compounds ranged from 2.89 to 4.82, 4.37 to 6.64 and 141.35 mg gallic acid equivalents (GAE)/100 g dw (Biglari et al. 2008). The major limitation to using date juice as a sugar substitute in beverages is its dark brown colour resulting from melanoidins, Fe2+-polyphenol complex and compounds formed by degradation of reducing sugars during thermal processing (Mohamed et al. 1981). Lewandowski et al. (1999) reported that the use of resins to decolourise date juice resulted in high levels of water consumption and effluent disposal. According to Fathi et al. (2013), average decolourisation level for the membrane with MWCO of 30-50 kDa was 26% while it increased to 56% when using a membrane with 15-20 kDa pore size. Therefore, application of ultra-filtration technique in clarification and decolourisation of date juice and other fruit juices has become widespread because of its low energy consumption, simplicity of operation (Hamachi et al. 2003), and other advantages like non-thermal processing for separation, optimized use of space due to the high surface/volume ratio of membranes, and easy cleaning and maintenance of equipment used (Cassano et al. 2007b). This work intends to investigate and model the effects of ultra-filtration parameters including feed brix, Trans-Membrane Pressure (TMP), operation time on permeate flux and the efficiency of this process in complementary decolourisation of date juice. In addition, the decolourised
date juice and stevioside will be used in the formulation of carbonated beverages in order to examine the synergic effects of the sweeteners and compare sensory attributes of such products with the common carbonated beverage prepared only by sugar.

**Materials and methods**

**Materials**

Partially purified date syrup and stevioside (97%) were obtained from two Iranian companies, Sibasan and Farayandsazan, respectively. All chemicals used in this research were provided by Merck Co., Germany, unless otherwise stated.

**Ultrafiltration experiments**

A spiral wound membrane made of Poly-Ether-Sulphone (PES) with Molecular Weight Cut Off (MWCO) of 5 kDa and a filtration area of 2.5 m² (Permonics Ltd., USA) was used in this study. Experiments were carried out in batch mode using a laboratory-scale ultrafiltration setup at 25°C. Trans-Membrane Pressure (TMP) could be fixed to a desired level and calculated using Equation 1 (Girard et al. 2000):

\[
\text{TMP} = \frac{(P_{\text{in}} + P_{\text{out}})}{2} - P_{\text{permeat}} \quad (\text{Eq.1})
\]

During the ultrafiltration of date juice, permeate flux \( J_p \) was calculated using the following equation (Mirsaeedghazi et al. 2009):

\[
J_p = \frac{V_p}{A_m \cdot t} \quad (\text{Eq.2})
\]

Also, water flux was measured under equal conditions, before and after the experiments. The retentate flow rate in all experiments was 10 L/min and the permeate flux was measured while the retentate flow rate was kept constant and equal to this value.

**Analytical methods**

Total Soluble Solids (TSS) and turbidity of samples were determined using a refractometer (ATAGO DTM-1, Japan) and digital turbidimeter (Aqualytic Turbidity LAB, Germany) at room temperature. Turbidometries were reported in Nephelometric Turbidity Units (NTUs). The color of each sample was expressed as ICUMSA Units (IU). CIE L* a* b* colour parameters were determined using a spectrophotometer (HACH DR-4000, USA). The L* value is a measure of lightness and varies from 0 (black) to 100 (white), the a* value is in the range of -100 (green) to +100 (red), and the b* value varies from -100 (blue) to +100 (yellow) (Ben Thabet 2009). The reducing sugars were determined by HPLC apparatus with Vertex column 300×800 mm, according to the method described by Nasabi et al. (2013). The mobile phase was H₂SO₄ 0.01 N at a flow rate of 0.5 ml/min. The HPLC system was equipped with a Triathlon 900 auto sampler, and was connected to Refractive Index detector (Knauer K-2301, Germany). Sugars (glucose and fructose) were identified by comparison of their retention times with a standard. They were quantified to their area, obtained by integration of the peaks. The ions (i.e., Na, K, Ca and Mg) were determined using an atomic absorption spectrophotometer (Hitachi Z-6100, Japan). The pectin and ash content of the date juice were measured using the method described in AOAC (2000). The total phenolic content was also determined using the Folin-Ciocalteu method which is based on the procedure carried out by Ben Thabet et al. (2009). The antioxidant activity was estimated according to the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method used by Ben Thabet et al. (2009).

**Experimental design and statistical analysis**

Three factors including feed brix, TMP and operation time were selected to investigate their effects on the permeate flux and quality during ultra-filtration of date juice. The levels
of these variables were determined utilizing A Central Composite Design (CCD) was used to examine and model the effects of these variables. The experimental results of the central composite design were fitted with a second-order polynomial equation using a multiple regression technique. The quadratic model for predicting different levels of the dependent variables (i.e., permeate flux and reduction of colour, turbidity, sugars, ash and minerals, polyphenols and antioxidant activity) was expressed as follows:

\[ y = C_0 + \sum_{i=1}^{2} C_i x_i + \sum_{i=1}^{2} C_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j>i}^{2} C_{ij} x_i x_j \]  

(Eq.3)

The efficiency of the model to fit the experimental data was expressed using the coefficient of determination, \( R^2 \), and statistical significance was determined by F-value. The regression analysis as well as contour and surface plots were undertaken using the Minitab 14 statistical software.

**Sensory evaluation of stevioside solutions**

A trained taste panel consisting of 7 females and 8 males aged between 18 and 45 was employed to evaluate the sweetness intensity of stevioside solution. The sweetness intensity of five samples of stevioside solutions in concentration range of 300–700 mg/L were compared to that of 100 g/L sucrose solution (i.e., a standard sugar concentration in the formulation of carbonated soft drinks). Each sample was given a number ranging between 1 and 5, according to its difference to the standard value (the larger the difference, the higher the number). Finally, the sample with the lowest number (1) was selected as the stevioside solution with the sweetness intensity equivalent to that of the standard sucrose solution, and was used in the formulation of carbonated soft drinks. Each panellist used drinking water for mouth washing between intervals of different samples.

**Sensory evaluation of carbonated soft drinks**

Six samples of carbonated soft drinks (i.e. cola type) were produced in the Zam Zam Company, Iran, according to the standard method used in carbonated soft drink industry. The sensory evaluation of carbonated soft drinks was carried out according to the method described by El-Sharnouby et al. (2009). A test panel consisting of 7 females and 8 males aged 18 to 45 were employed to evaluate four factors including: sweetness, bitter, aftertaste, colour and clarity of each sample and a score between 1 (very bad) and 25 (very good) was given to each factor. The results obtained were subjected to the analysis of variance and Least Significant Difference (LSD) at 5% probability.

**Results and discussion**

**Effect of operating parameters on the permeate flux**

In all experiments, the fast permeate flux decline occurred during the initial 8 to 10 minutes. Then, the permeate flux would reach the steady state after 10 to 15 minutes of the ultra-filtration processing. Such a declining trend has been reported in other studies too. For example, Cassano et al. (2007b) reported that the fast permeate flux decline in the early period of the ultra-filtration process was due to the adsorption of material to the membrane surface and concentration polarization, as well as fouling phenomenon resulting in a membrane resistance increase. It should be mentioned that the pectin content of the initial date juice (0.08%) was completely...
removed by the membrane used in this study and, as a result, obtained permeates had no pectic materials. Therefore, the removed pectin is one of the major constituents responsible for pore blocking and fouling of the membrane. Hamachi et al. (2003) stated that reaching the permeate flux steady state is faster in smaller pore size membranes because of the higher fouling intensity. The permeate flux decreases with an increase in feed brix and a decrease in TMP. Several researchers have reported such a relation (Cassano et al. 2007a, b; Bhattacharya et al. 2001; Wei et al. 2008). The highest permeate flux (30-35 L/m²h) was obtained in the experiment with feed brix of 30 and TMP of 150 psi. When TMP was decreased to 70 psi, the rate of permeate flux reached 25-20 L/m²h. These findings show that TMP is the driving force for the mass transfer across the membrane. Thus, the increase of TMP will result in the permeate flux increase. The results of the experiments undertaken in this study showed that a higher concentration of date juice introduced to the filtration system increased the transfer rate of materials to the cake layer formed at the membrane surface. And this, therefore, increased the membrane resistance against the mass transfer. In addition, increasing brix led to the higher viscosity and decreased the permeability of the material in membrane.

**Effect of operating parameters on colour and turbidity reduction**

The colour values of permeates were significantly lower than the initial date juice as most of the colouring materials in date juice could not pass through the membrane pores. The average colour reduction obtained in this study was 77.3% while Hamachi et al. (2001) reported that the maximum colour removal from the cane sugar solution with the membrane of MWCO of 1 kDa was 58.67%. Similarly, the turbidity values of permeates were significantly lower than the initial date juice as almost all turbidity agents were removed by the membrane. The average turbidity reduction in this study was 96%, which is consistent with the results of other studies. For instance, Cassano et al. (2007a) reported that during ultra-filtration of kiwi fruit juice with membrane of MWCO of 15 kDa, suspended solids were completely removed and the resulting juice had a negligible turbidity. The small variation in these results might be explained due to differences between date juice and kiwifruit characteristics and also other operating conditions. The results showed that increasing feed brix and TMP led to higher removal of colour and turbidity agents. The highest colour reduction (83%) was obtained in the experiment carried out with feed brix of 50 and TMP of 150 psi (Fig. 1).

![Fig 1: Effect of feed brix and TMP on colour reduction of date juice](image-url)
Furthermore, in the filtration of date juice with feed brix of higher than 42, more than 97% turbidity reduction was obtained even at low TMPs (70 psi). This was due to the increased thickness and compactness of the cake layer and severe polarization concentration during ultra-filtration of date juice, leading to higher membrane resistance against permeation of colouring and turbidity materials. Similar results were reported in other studies (Jacob et al. 2007; Seres et al. 2004). According to our experimental results, the colour reduction percentage increased as the ultra-filtration process continued toward 30 min and reached even more than 80% in the case of treating date juice with feed brix of 35–50 for the duration of 25–30 min. Similarly, the highest turbidity reduction (more than 97.35%) was obtained by ultra-filtration of date juice with TMP of 140–150 psi for the duration of 25–30 min. As the ultra-filtration treatment continues, the thickness and compactness of the cake layer formed at the membrane surface increase, leading to higher removal of colouring and turbidity materials from date juice.

Effect of operating parameters on sugar reduction
Table 1 represents the variations of reducing sugar in each ultra-filtration experiment. Part of the reducing sugar in date juice was removed by the membrane where permeates had a lower sugar content than feed. In this regard, other studies show similar results. Carvalho et al. (1998) reported that by ultra-filtration of pineapple juice with the membrane of MWCO of 50 kDa, 10–11% reduction in glucose and 12–16% reduction in sucrose were observed. Given that the membrane used in this study had a lower pore size (i.e., MWCO of 5kDa) compared to the ones used in other studies, the higher sugar reduction can therefore be justified. The results showed that the reduction of sugar depended on the variation of feed brix so that the highest and lowest sugar reduction was in feed brix of 30 and 50, respectively. Similar trends were observed in other studies. For instance, Bhattacharya et al. (2001) reported that sugar loss during ultra-filtration decreased as the brix of sugar cane juice increased from 10 to 13. Wei et al. (2008) stated that during ultra-filtration of apple juice, the sugar recovery in permeate went up with an increase of feed brix from 10 to 15. These results suggest that there may be special points at the membrane surface that play an important role in the sugar removal during ultra-filtration of date juice. Thus, at low feed concentrations these points have more capacity to absorb and remove sugar due to the low rate of mass transfer to the membrane surface. In addition, polarization concentration and membrane resistance against the mass transfer goes up with an increase of TMP and operating time, implying sugar reduction of date juice during ultra-filtration increases.

Effect of different operating conditions on CIE colour parameters
The experimental results shown in Fig. 2 suggest that feed brix and TMP has a positive effect on the increase of $L^*$ value in date Juice. The same trend is observed with an increase of operation time. Therefore, the highest $L^*$ value (80%) is obtained by ultra-filtration of date juice with brix of 50 and TMP of 150 psi for 30 min. Reducing colour and turbidity of date juice during ultra-filtration with the described mechanisms normally result in increased date juice lightness. The $a^*$ value parameter refers to the red colour intensity of date juice, mostly provided by melanoidins which are not able to pass through the membrane because of their polymeric structure and high molecular weight (Belitz et al. 2009).
Therefore, the $a^*$ value of date juice reduces during the ultra-filtration treatment. Fig 3 shows that the $a^*$ value reduction increases with an increase of feed brix and TMP. The highest $a^*$ value reduction (more than 90%) was obtained by ultra-filtration of date juice with brix of 45–50 and TMP of 110–150 psi. The continuing process toward 30 min resulted in a higher reduction of melanoidins and other compounds responsible for the red colour of date juice, implying a higher $a^*$ reduction.

Table 1: Sugar reduction values during ultra-filtration. Results were reported in $\bar{x} \pm$ SD (n = 3)

<table>
<thead>
<tr>
<th>Run</th>
<th>Feed brix</th>
<th>TMP (psi)</th>
<th>Time (min)</th>
<th>Glucose reduction (%)</th>
<th>Fructose reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>70</td>
<td>10</td>
<td>20.48 ± 0.11</td>
<td>20.77 ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>70</td>
<td>10</td>
<td>0.36 ± 0.16</td>
<td>9.53 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>150</td>
<td>10</td>
<td>22.28 ± 0.11</td>
<td>22.87 ± 0.18</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>150</td>
<td>10</td>
<td>10.60 ± 0.06</td>
<td>15.35 ± 0.11</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>70</td>
<td>30</td>
<td>28.55 ± 0.09</td>
<td>28.61 ± 0.15</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>70</td>
<td>30</td>
<td>3.35 ± 0.13</td>
<td>9.51 ± 0.07</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>150</td>
<td>30</td>
<td>51.27 ± 0.06</td>
<td>58.57 ± 0.09</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>150</td>
<td>30</td>
<td>15.46 ± 0.06</td>
<td>20.11 ± 0.09</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>110</td>
<td>20</td>
<td>55.29 ± 0.04</td>
<td>54.28 ± 0.11</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>110</td>
<td>20</td>
<td>11.09 ± 0.13</td>
<td>17.56 ± 0.15</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>70</td>
<td>20</td>
<td>14.26 ± 0.06</td>
<td>17.16 ± 0.11</td>
</tr>
<tr>
<td>12</td>
<td>40</td>
<td>150</td>
<td>20</td>
<td>26.28 ± 0.13</td>
<td>26.50 ± 0.20</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td>110</td>
<td>10</td>
<td>15.40 ± 0.06</td>
<td>18.79 ± 0.11</td>
</tr>
<tr>
<td>14</td>
<td>40</td>
<td>110</td>
<td>30</td>
<td>18.42 ± 0.16</td>
<td>24.39 ± 0.07</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>110</td>
<td>20</td>
<td>17.13 ± 0.03</td>
<td>20.85 ± 0.09</td>
</tr>
<tr>
<td>16</td>
<td>40</td>
<td>110</td>
<td>20</td>
<td>17.26 ± 0.06</td>
<td>21.33 ± 0.08</td>
</tr>
<tr>
<td>17</td>
<td>40</td>
<td>110</td>
<td>20</td>
<td>17.38 ± 0.16</td>
<td>21.63 ± 0.09</td>
</tr>
</tbody>
</table>

Fig 2: Effect of feed brix and TMP on $L^*$ increase of date juice

Fig 3: Effect of feed brix and TMP on $a^*$ reduction of date juice
The $b^*$ value parameter refers to the yellow colour intensity of date juice, mostly provided by low molecular weight pigments such as carotenoids and flavonoids (MacDougall 2002; Socaciu 2008). Since the red-brown colour of date juice is related to the presence of high molecular weight pigments removed by the membrane, yellow becomes the predominant colour of the filtered date juice. This, therefore, means that the $b^*$ value goes up. The results show that feed brix and TMP had a positive effect on the $b^*$ value increase of date juice, and the highest $b^*$ value increase (more than 90%) was obtained by ultra-filtration of date juice with brix of 4–50 and TMP of 100–150 psi. On the other hand, the variation of the $b^*$ value was independent of operation time and no significant effects on the $b^*$ value increase of date juice were observed by continuing the process (P > 0.05).

**Effect of operating parameters on ash and minerals reduction**

The analysis of permeate showed that the amount of ash reduction went up with an increase of feed brix and TMP due to the higher concentration polarization and membrane resistance. The ash reduction reached more than 57% in the case of treating date juice with feed brix of 40–50 and TMP of 100–150 psi whereas operating time had a negative effect on the ash reduction. The measured ash content showed that its reduction was more than 60% in the first 15 minutes of ultra-filtration but it was reduced to less than 50% in the last 5 minutes of the process. This could be related to the presence of empty points on the clean membrane surface adsorbing minerals of date juice at the beginning of treatment, which would lose their adsorbing capacity as the process proceed. The presence of minerals can alter the sweetening attributes of date juice and other sweeteners. Schiffman et al. (2007), Schiffman et al. (2000) reported that 5 mM of Na, K and Ca had no effects on sweetness intensity of glucose and fructose solutions, but increased the time needed for reaching the maximum sweetness intensity. They also mentioned that K increased the bitter aftertaste in sweeteners solutions. As our results show, increasing feed brix and TMP led to a higher Na and K reduction, but had no significant effects on the Ca and Mg reduction. Increasing operating time decreased the K reduction but had no significant effects on the reduction of other minerals studied. The highest K reduction (more than 76%) occurred in the first 15 minutes of the date juice treatment using brix of 47–50 and TMP of 130–150 psi. The highest Na reduction (more than 65%) was obtained by ultra-filtration of date juice with brix of 45–50 and TMP of 110–150 psi for duration of 20–25 min. The highest Ca reduction (more than 80%) was obtained when date juice was treated with brix of 40–50 and TMP of 120–150 psi for duration of 15–28 min. The highest Mg reduction (more than 50%) was obtained in the first 12 minutes and the last 3 minutes of the date juice ultra-filtration.

**Effect of operating parameters on polyphenol and antioxidant activity reduction**

The membrane pore size is one of the most important factors affecting the polyphenols and antioxidant activity reduction. In this study, the average polyphenol and antioxidant reduction by the membrane of MWCO of 5kDa was 53 % and 61%, respectively. Cassano et al. (2007a) reported that ultra-filtration of kiwifruit juice with the membrane of MWCO of 30 kDa resulted in a 13.5% reduction of polyphenols while 65% of the antioxidant activity was recovered in the
retentate. The effect of twice processing parameter; feed brix and TMP on the polyphenol and antioxidant reduction showed that an increase of feed brix to 40 – 45 led to an increased polyphenol and antioxidant reduction, but these parameters decreased as feed brix further increased. Despite this, an increase of TMP led to the polyphenol and antioxidant reduction being increased. Ultrafiltration of date juice with brix of 40–45 and TMP of 130 – 150 psi resulted in the polyphenol and antioxidant reduction being more than 70% and 80%, respectively. The higher concentration polarization and membrane resistance against the mass transfer at increased levels of pressure led to higher removal of polyphenols and other compounds having antioxidant activities. It should be mentioned that operating time had no significant effect on the polyphenol and antioxidant reduction. Considering similar trends in the variation of CIE colour parameters and polyphenols, it can be concluded that polyphenols are one of the constituents responsible for the turbidity and dark red-brown colour of date juice. Thus, the reduction of these compounds leads to higher clarity and lightness (L*) as well as the higher reduction of red colour (a*) and an increase of yellow colour (b*) intensity in the filtered date juice.

Modelling the effects of operating parameters on the permeate flux and quality

By estimating the regression coefficients for all studied factors and omitting the insignificant variables (P > 0.05), the final models, as presented in Table 2, were obtained.

Optimization of the process

The optimization of operating parameters was carried out in order to maximize the decolourisation and clarification of date juice and, minimize sugar loss. Optimized levels determined for feed brix, TMP and operation

<table>
<thead>
<tr>
<th>Table 2: Regression equations of the fitted models for the permeate flux and quality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equation</strong></td>
</tr>
<tr>
<td>$PF = 11.38 - 5.89 x_1 + 2.88 x_2 - 0.90 x_3 + 1.88 x_1^2 + 0.87 x_3^2 - 0.45 x_1 x_2$</td>
</tr>
<tr>
<td>$CR = 78.07 + 1.22 x_1 + 1.25 x_2 + 3.30 x_3 - 1.26 x_1^2$</td>
</tr>
<tr>
<td>$TR = 96.99 + 2.26 x_1 + 0.35 x_2 - 1.56 x_1 x_2 - 0.33 x_2$</td>
</tr>
<tr>
<td>$GR = 20.36 - 13.70 x_1 + 5.89 x_2 + 4.79 x_3 + 8.07 x_1 x_2 - 8.21 x_3^2 - 3.65 x_1 x_3$</td>
</tr>
<tr>
<td>$FR = 24.34 - 11.30 x_1 + 5.78 x_2 + 5.39 x_3 + 9.27 x_1 x_2 - 4.82 x_2^2 - 5.06 x_3^2$</td>
</tr>
<tr>
<td>$LI = 70.84 + 6.75 x_1 + 6.11 x_2 + 1.81 x_3 - 2.15 x_1 x_2 - 1.48 x_2^2 - 1.29 x_1 x_3$</td>
</tr>
<tr>
<td>$aR = 73.84 + 12.78 x_1 + 4.32 x_2 + 0.89 x_3 - 1.85 x_2^2 + 0.78 x_3^2 + 1.61 x_1 x_2$</td>
</tr>
<tr>
<td>$bl = 78.96 + 16.58 x_1 + 4.90 x_2 - 2.54 x_1 x_2 - 4.05 x_2^2 + 1.17 x_1 x_2$</td>
</tr>
<tr>
<td>$AR = 55.13 + 4.57 x_1 + 2.88 x_2 - 2.65 x_2$</td>
</tr>
<tr>
<td>$NAR = 65.03 + 0.84 x_1 + 0.91 x_2 - 2.02 x_3 + 1.72 x_1 x_3$</td>
</tr>
<tr>
<td>$KR = 70.73 + 3.41 x_1 + 1.40 x_2 - 2.33 x_3 - 1.68 x_1 x_3$</td>
</tr>
<tr>
<td>$CAR = 79.27 + 1.80 x_1 - 3.27 x_2^2 + 1.58 x_1 x_3$</td>
</tr>
<tr>
<td>$MGR = 49.08 + 6.21 x_3^2$</td>
</tr>
<tr>
<td>$PR = 63.17 + 7.79 x_1 + 12.86 x_2 - 17.44 x_1 x_2 + 3.88 x_1 x_2$</td>
</tr>
<tr>
<td>$AAR = 70.90 + 6.49 x_1 + 12.92 x_2 - 16.67 x_1 x_2$</td>
</tr>
</tbody>
</table>

PF, permeate flux (L/m²h); CR, colour reduction (%); TR, turbidity reduction (%); GR, glucose reduction (%); FR, fructose reduction (%); LI, L’ increase (%); aR, a’ reduction (%); bl, b’ increase (%); AR, ash reduction (%); NAR, Na reduction (%); KR, K reduction (%); CAR, Ca reduction (%); MGR, Mg reduction (%); PR, polyphenol reduction (%); AAR, antioxidant activity reduction (%); $x_1$, feed brix; $x_2$, TMP (psi); $x_3$, time (min).
time were 47.13 °Brix, 82.11 psi and 30 min, respectively. Under such conditions, 80.94 % decolourisation and 97.78 % clarification were obtained while loss of glucose and fructose were only 3.49 % and 9.62 %, respectively.

Production and sensory evaluation of soft drinks
The decolorized date juice produced by ultra-filtration under optimized conditions and the stevioside concentration sweetness intensity were used to replace sugar at different ratios in the carbonated soft drink formulation. The sweetener specifications of the produced carbonated soft drink samples were presented in Table 3. The results of soft drinks sensory evaluation were reported in Table 3. Considering sweetness, the sample prepared by 100% sugar and 100 % stevioside had the highest and lowest desirability, respectively while samples prepared by 100% decolorized date juice, 50 % sugar, + 50 % stevioside, 50 % decolorized date juice and + 50 % stevioside did not have significant differences with the sample prepared by 100% sugar. Regarding the bitter aftertaste, the sample prepared by 100% sugar and 100% stevioside had the highest and lowest desirability, respectively while other samples did not have a significant difference with one another. In addition, there was no significant difference between the colour and clarity of the samples. In other words, replacing sugar with the decolorized date juice and stevioside was not limited by the colour and clarity of the final product.

Table 3: sweetener specifications and results of sensory evaluation of carbonated soft drinks (lowest=1, best=25)

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Sweetener specifications</th>
<th>Sweetness</th>
<th>Bitter aftertaste</th>
<th>Colour</th>
<th>Clarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 % sugar (100 g/L)</td>
<td>18.80 ± 3.38</td>
<td>20.73 ± 3.86</td>
<td>20.80 ± 2.91</td>
<td>20.73 ± 2.99</td>
</tr>
<tr>
<td>2</td>
<td>100 % decolourised date juice (150 g/L)</td>
<td>16.27 ± 3.92</td>
<td>17.40 ± 4.76</td>
<td>19.93 ± 2.79</td>
<td>19.67 ± 3.27</td>
</tr>
<tr>
<td>3</td>
<td>50 % sugar (50 g/L) + 50 % decolourised date juice (75 g/L)</td>
<td>14.07 ± 4.67</td>
<td>13.47 ± 5.58</td>
<td>18.47 ± 3.78</td>
<td>18.53 ± 3.91</td>
</tr>
<tr>
<td>4</td>
<td>50 % sugar (50 g/L) + 50 % stevioside (200 mg/L)</td>
<td>15.67 ± 5.21</td>
<td>15.67 ± 5.56</td>
<td>19.40 ± 2.95</td>
<td>19.13 ± 3.87</td>
</tr>
<tr>
<td>5</td>
<td>50 % decolourised date juice (75 g/L) + 50 % stevioside (200 mg/L)</td>
<td>16.00 ± 4.55</td>
<td>15.27 ± 5.23</td>
<td>19.40 ± 3.48</td>
<td>19.60 ± 3.74</td>
</tr>
<tr>
<td>6</td>
<td>100 % stevioside (400 mg/L)</td>
<td>10.53 ± 4.27</td>
<td>10.40 ± 5.89</td>
<td>19.00 ± 3.78</td>
<td>19.13 ± 3.87</td>
</tr>
</tbody>
</table>

Conclusions
The results obtained from this research study indicate that ultra-filtration using a membrane with MWCO of 5 kDa permitted a desirable level of the date juice decolourisation and clarification, while the loss of glucose and fructose were only 3.49 % and 9.62 %, respectively. The variation results in the permeate flux and quality attributes indicate that with an increase of feed brix, reduction of colour, turbidity, ash, polyphenols and antioxidant activity of date juice increases but the permeate flux and sugar reduction decreases. Increasing TMP leads to higher levels of all studied factors, while the permeate flux and ash reduction decreases as the process proceeds. In addition, no significant changes are observed in the reduction rate of polyphenols and antioxidant activity of date juice. More importantly,
treating date juice under optimized conditions produces a natural sweetener that can be used as a sugar substitute in the formulation of carbonated soft drinks without any considerable reduction in the quality of the final product. However, using stevioside as the only sweetener reduces the desirability of carbonated soft drinks due to a higher bitter aftertaste and less pure sweetness compared to the samples prepared by sugar and decolorized date juice.

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Nomenclature:
- \( P_{in} \) pressure at the inlet of the membrane
- \( P_{out} \) pressure at the outlet of the membrane
- \( P_{permeate} \) permeate pressure, is so close to 0 and often is ignored
- \( V_p \) permeate volume (L)
- \( A_m \) membrane area (m\(^2\))
- \( t \) time (h)
- \( J_p \) permeate flux (m/s)
- \( \Delta P \) transmembrane pressure difference(kPa)
- \( Y \) dependent variable (response)
- \( C_0, C_i, C_{ii}, C_{ij} \) constant coefficients
- \( x_i, x_j \) independent variables

References
Cassano A, Marchio M, Drioli E (2007b) Clarification of blood orange juice by ultrafiltration: Analyses of operating parameters, membrane fouling and juice quality. Desalination 212:15-27


