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Concentration of Hot Water Extracts of Anthocyanins obtained from Muscadine Grape Pomace using Membrane-Osmotic Distillation

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Abstract

Anthocyanins are well known for their health-promoting benefits. The goal of this study was to evaluate a distillation-based membrane technology to concentrate aqueous anthocyanins extracted from muscadine grape pomace using polypropylene (PP) or ethylene chlorotrifluoroethylene (ECTFE) membranes. A hot water extraction method was utilized to extract the anthocyanins from the pomace. A pre-experimental run using DI water as feed was conducted to optimize the NaCl brine concentration for osmotic distillation and it was determined that 4M was the optimal concentration. The aqueous anthocyanins extraction was filtrated through a series of filter of different pore sizes before the distillation process. Membrane distillation (MD), osmotic distillation (OD), and membrane osmotic distillation (OMD) were all carried out over a 6-hour period to determine the optimal distillation process. Among the three processes, OMD demonstrated the highest permeate flux value, therefore, it was utilized to conduct the concentration using either PP or ECTFE membranes. The anthocyanins contents before and after the concentration process were measured by High Performance Liquid Chromatography. The result shows that OMD paired with ECTFE membrane concentrated the aqueous anthocyanins up to 196%, but OMD with PP membrane exhibited a better performance, concentrating the aqueous anthocyanin extract 280% over a 12-hour period. In addition, the quality of the concentrate was also investigated in terms of NaCl transferring across the membrane to the feed solution. Only 0.02% - 0.03% of NaCl was transferred across the membrane to the feed side, indicating it is feasible to obtain concentrated anthocyanin extracts with low salt concentration

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1. Introduction

Muscadine grape is a species native to the Southeastern United States, and has been shown to be a rich source of phenolic compounds such as anthocyanins (Pastrana-Bonilla et al., 2003). It's been suggested that anthocyanins possess many health-benefiting properties such as anti-cancer and anti-inflammatory properties and they also play an important role in the prevention of cardiovascular illnesses and diabetes (Greenspan et al., 2005; Konczak & Zhang, 2004; Ju & Howard, 2005). Anthocyanins are also considered as natural pigments to replace the artificial food colorants (Anderson, 2006).

Commercially, muscadine grapes are pressed into juice product and the remaining solid residue is known as pomace. It's either recycled as animal feed or simply discarded as waste. However, grape pomace usually retains most of the polyphenolics after juicing, with 20-30% found in skins of the grapes and 60-70% found in the seeds (García-Marino et al., 2006). An opportunity exists to recover the remaining anthocyanins from muscadine pomace for application such as encapsulated supplements, natural colorants, or added nutraceuticals. Conventional anthocyanin extraction from pomace is usually achieved with organic solvent and harsh conditions such as high heat which could cause anthocyanin degradation and requires large quantity of organic solvents that are toxic to human health and environmentally hazardous. Hot water extraction, which uses water as the extracting solvent at elevated temperature, is non-toxic, non-flammable, cheap, easily available, and also "greener" than the conventional extraction method (Plaza & Turner, 2017; Francezon, Meda & Stevanovic, 2017). It has shown to be effective in recovering bioactive compounds such as polyphenols from blueberry pomace, grape pomace, and red onion (Avram et al., 2017; Monrad et al., 2012; Liu et al., 2013). In this study,

the extraction of anthocyanins from muscadine grape pomace was achieved by hot water extraction.

In order to reduce transport, storage, and packaging cost, aqueous anthocyanin extract from muscadine grapes needs to be concentrated. In the past decades, membrane technology has become a leading technology in food processing industry especially in the field of clarification and concentration of fruit juice and plant extract (Yilmaz & Bagci, 2018). It offers several advantages over the conventional thermal methods used in concentrating bioactive compounds since the membrane technology process does not require high heat which could degrade the structure and alter the bioactive properties of these compounds (Martín, Díaz-Montaña & Asuero, 2018). Membrane technology has shown to be effective in concentrating anthocyanins in many published studies with examples of anthocyanins extracted from blueberry, pomegranate, cranberry, and red cabbage (Avram et al., 2017; Conidi et al, 2017; Husson et al., 2013; Jampani & Raghavarao, 2015). However, membrane technique, membrane type, and operating parameters are important factors that can influence the flux rate, efficiency of the concentration process, and the quality of the concentrated anthocyanin extract (Johnson & Nguyen, 2015). The goal of this study was to evaluate a distillation-based membrane technology to concentrate anthocyanins from an aqueous muscadine grape pomace extract using polypropylene (PP) or ethylene chlorotrifluoroethylene (ECTFE) membranes.

2. Materials

The muscadine pomace used in the extraction process was obtained from the Food Science Department Pilot Plant, University of Arkansas. The sodium chloride used in brine solution was provided by Sigma Aldrich (St. Louis, MO, USA). The 0.2 μm pore size polypropylene

membrane (PP) and ethylene chlorotrifluoroethylene membrane (ECTFE) membrane were purchased from 3M (Maplewood, MN, USA).

3. Method

3.1 Extraction of Anthocyanins from Muscadine Grape Pomace

The muscadine grape pomace was collected after a juicing process at the Food Science Pilot Plant and stored at -20°C in a freezer. The muscadine pomace was allowed to slightly defrost before the extraction process. 15 g of pomace was measured and blended with 125 mL of water (98°C) with a commercial blender. The blended mixture was then filtered through Miracloth with a Buchner vacuum funnel set. The residue on the Miracloth was collected and blending and filtration process was repeated one more time. The filtrate was then collected and vacuum-filtered through a series of filter papers (Whatman) of different pore size with Buchner funnel. The filtrate first went through a 20-25 μm filter, and then was sequentially filtered through 11μm, 2.5μm, 1.6μm, and 0.7 μm filters. The extract collected from the 0.7μm filter was further filtered with a 0.45μm nylon membrane filter using a glass vacuum filtration set, and the extract was lastly filtered with a 0.22 μm nylon membrane filter. The final extract was stored at 2°C before the distillation process

3.2 Distillation

Three distillation techniques – osmotic distillation, membrane distillation, and combination of osmotic and membrane distillation – were investigated for the concentration of anthocyanins in the aqueous muscadine grape pomace extract. This work was performed at the Chemical Engineering Department (University of Arkansas) by Dr. Wickramasinghe and his students. For osmotic distillation, optimization of the methodology was performed before the experimental run with the focus on brine concentration and membrane material type. During the

distillation process, the anthocyanin extract (feed) was held at 40°C because anthocyanins are heat-sensitive and subject to degradation under continuous heat (Jiang et al., 2019).

3.3 Instrumentation

3.3.1 High Performance Liquid Chromatography (HPLC) Analysis of Anthocyanins

The aqueous muscadine extract (feed) and the concentrated membrane samples were analyzed for individual and total anthocyanins content using the HPLC method described by Cho et al [19]. The High Performance Liquid Chromatography (Waters Corp, Milford, MA) system was equipped with a 250 × 4.6 mm symmetry C_{18} column (Waters Corp, Milford, MA). The two mobile phases forming the mobile phase gradient consisted of (A) 5% formic acid and (B) 2-60% methanol ran over a period of 60 minutes at a flow rate of 1 mL/min. Anthocyanin peaks were monitored at 510 nm. Individual anthocyanins in the chromatograms were quantified as delphinidin, cyanidin, petunidin, peonidin and malvidin glucoside equivalents using external calibration curves of authentic standards obtained from Polyphenols (Sandnes, Norway). Total anthocyanins were determined as the sum of delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside equivalents. Results are expressed as mg of total anthocyanins per 100g of muscadine pomace.

3.3.2 HPLC-Electrospray Ionization Mass Spectrometry (ESI-MS) Identification of Anthocyanins

The ESI-MS was performed using the HPLC system described above interfaced to a Bruker Esquire LC/MS ion trap mass spectrometer. The solvents and column used in this analysis were the same as the HPLC analysis described in the previous section. The conditions for performing the ESI-MS were: (A) positive ion electrospray mode; (B) nebulizing pressure at 32 psi; (C)

capillary voltage of 4000V; (D) temperature of 300°C; (E) drying gas flow 12 ml/min; (F) skim voltage 53.7V (Cho et al., 2004)

4. Results and Discussion

4.1 HPLC and HPLC ESI-MS Identification of Anthocyanins in Muscadine Grape Pomace Extract

The HPLC detected five peaks from the hot water extract of anthocyanins from muscadine grape pomace (**Fig.1**). The identification of the individual peaks was performed using HPLC ESI-MS. The HPLC ESI-MS result of the aqueous extract of muscadine pomace is shown in **Figure 2**. Figure 2a illustrates a molecular ion *m/z* of 627 which fragmented into two ions with *m/z* of 465.2 and 303.1, respectively. The difference between the molecular ion and the first ion is 162, indicating the loss of one glucose while the difference between the first and second ion is also 162, which again indicates the loss of another glucose. The molecular weight of the second ion (*m/z* 303) indicates that it is the aglycone of delphinidin. Therefore, the compound was identified as delphinidin 3,5-O-diglucoside. Figure 2b shows a molecular ion with *m/z* 611 which yielded two fragmented ions with *m/z* of 449 (Δ= -162) and 287.1 (Δ= -162), respectively. The latter fragmented ion is the aglycone of cyanidin. The difference means that two glucose were fragmented, therefore, this compound was identified as cyanidin 3,5-O-diglucoside. Figure 2c shows a molecular ion with *m/z* 641 which yielded two fragmented ions with *m/z* 479 (Δ= -162) and 317.2 (Δ= -162), respectively. The second fragmented ion is the aglycone of petunidin. The difference in MW means that two glucose were fragmented, therefore, the compound was identified as petunidin 3,5-O-diglucoside. Figure 2d shows a molecular ion with *m/z* 625 which yielded two fragmented ions with *m/z* 463 (Δ= -162) and 301.2 (Δ= -162), respectively. The latter fragmented ion is the aglycone of peonidin. The difference in MW indicates two glucose

were fragmented, so this compound was identified as peonidin 3,5-O-diglucoside. Figure 2e shows a molecular ion with *m/z* 655 which yielded two fragmented ions with *m/z* 493 (Δ= -162) and 331.3 (Δ= -162), respectively. The latter fragmented ion is the aglycone of malvidin. The difference in MW indicates two glucose were fragmented, therefore, this compound was identified as malvidine 3,5-O-diglucoside.

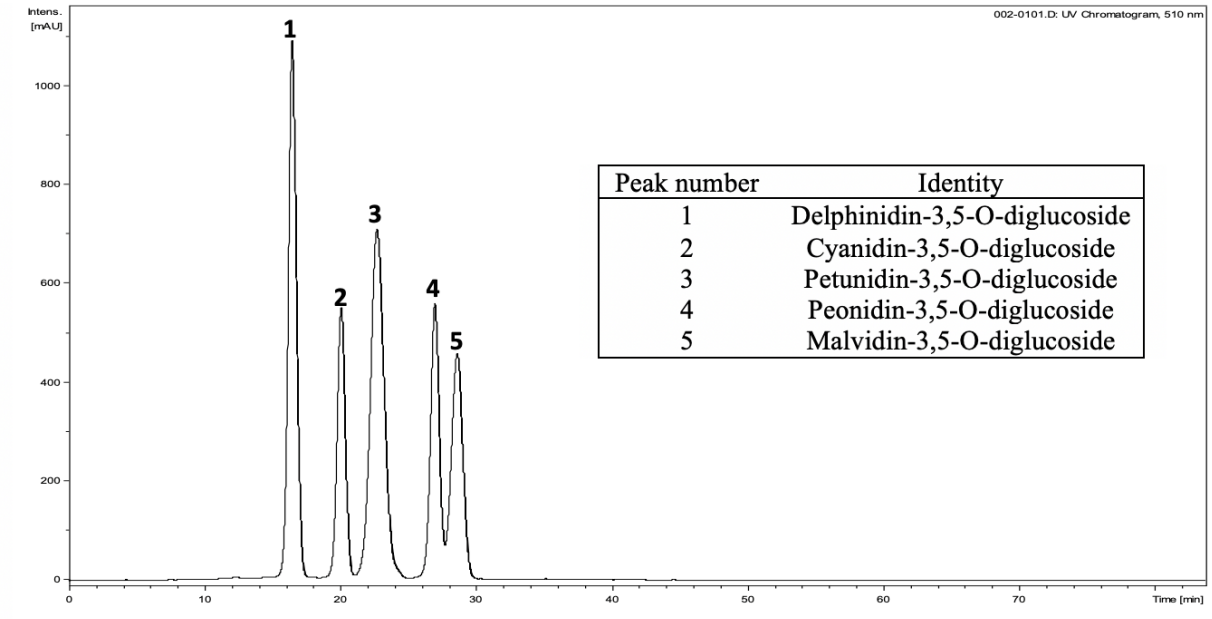
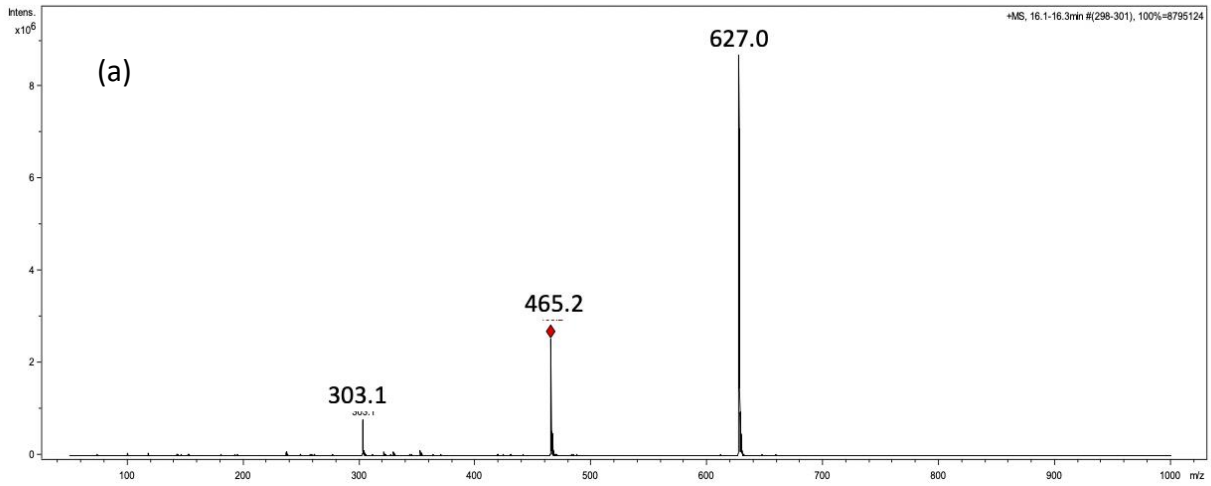
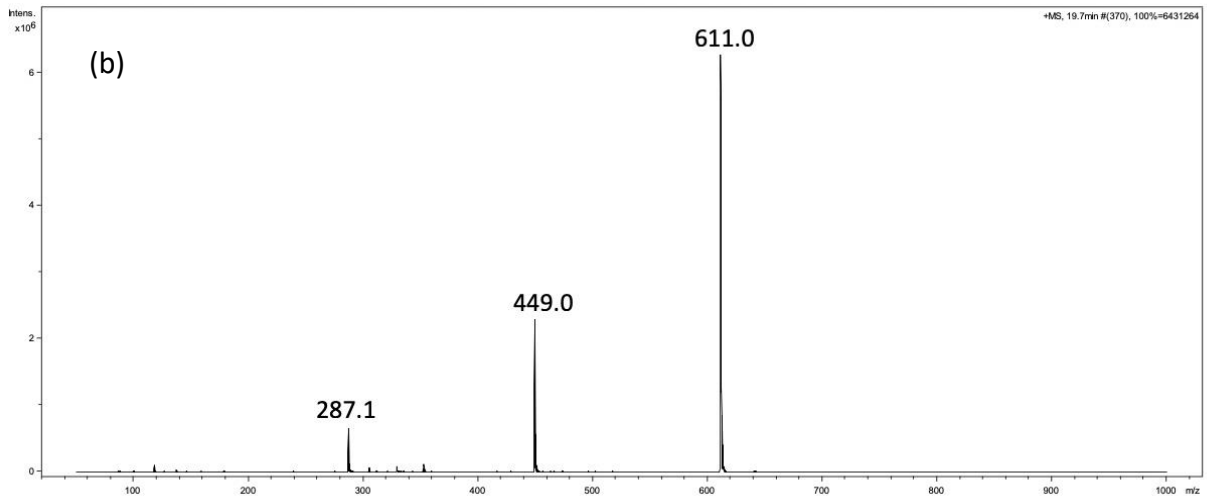
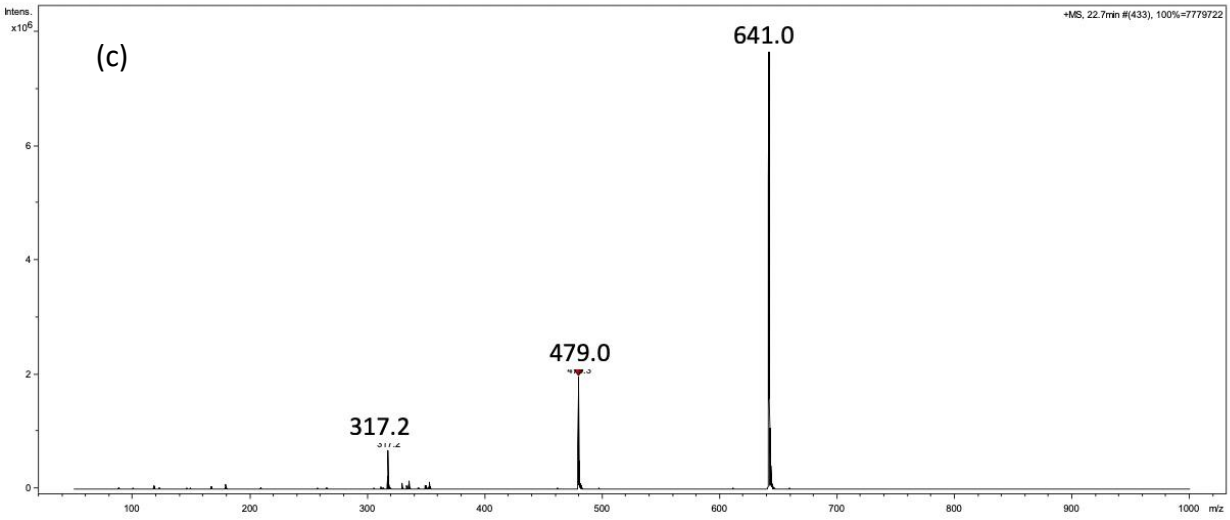
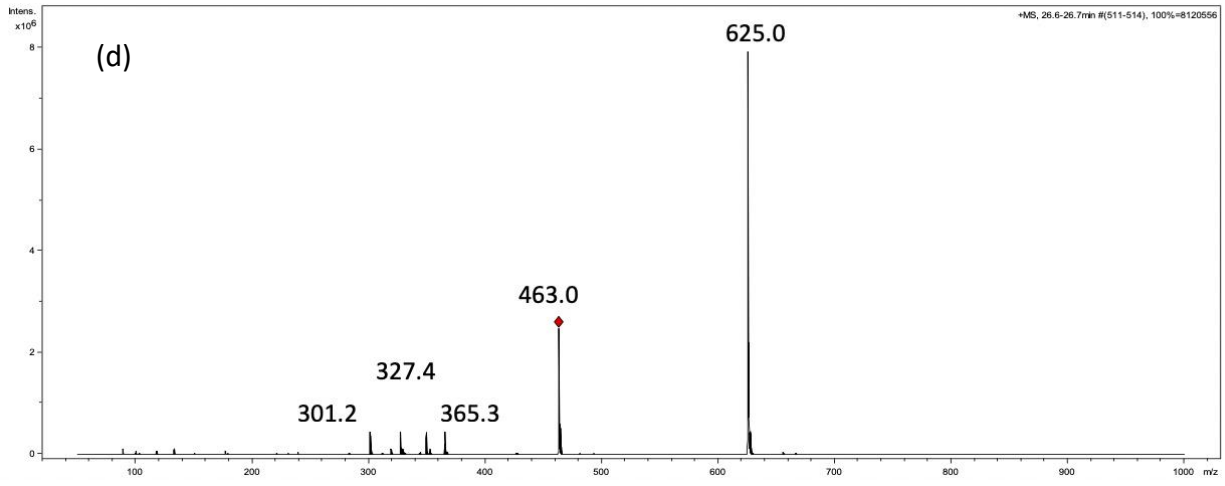


Figure 1. HPLC chromatogram (Abs 510 nm) of anthocyanins in aqueous anthocyanins extract of muscadine grape pomace




(b)
611.0
449.0
287.1
+MS, 19.7min #(370), 100%=6431264
Intens.
x10^6
m/z


(c)
641.0
479.0
317.2
+MS, 22.7min #(433), 100%=7779722
Intens.
x10^6
m/z


(d)
625.0
463.0
327.4
301.2
365.3
+MS, 26.6-26.7min #(511-514), 100%=8120556
Intens.
x10^6
m/z

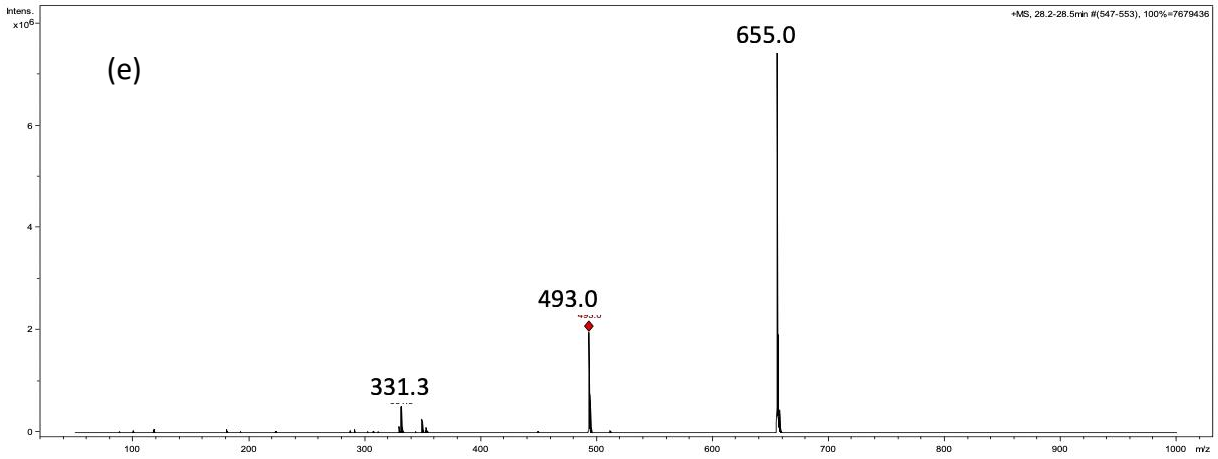


Figure 2. HPLC ESI-MS identification of anthocyanins in aqueous extract of muscadine grape pomace

4.2 Optimization of Osmotic Distillation Methodology

The optimization of osmotic distillation methodology was performed at the Chemical Engineering Department (University of Arkansas) by Dr.Wickramasinghe and his students. A preliminary study was conducted to optimize the experimental parameters for osmotic distillation. In this experiment, NaCl brine of different concentrations (2M, 4M, and 5M) were used at the permeate side with ECTFE membrane, and DI water was used as the feed. Figure 3 shows the flux of three different brine concentrations over a six-hour period and Table 1 presents the average flux. It is shown that 2M brine had the lowest flux value over the membrane among the brines. This could be due to the lack of driving force, which was produced by lowering the water vapor pressure with salt (Johnson & Nguyen, 2015). 4M and 5M brines had very similar flux result with 5M brine having slightly higher flux. Concentrations above 5M were not tested because the brine solution had reached saturation at this point. Therefore, it was determined that 4M was the optimal concentration for brine solution used in osmotic distillation.

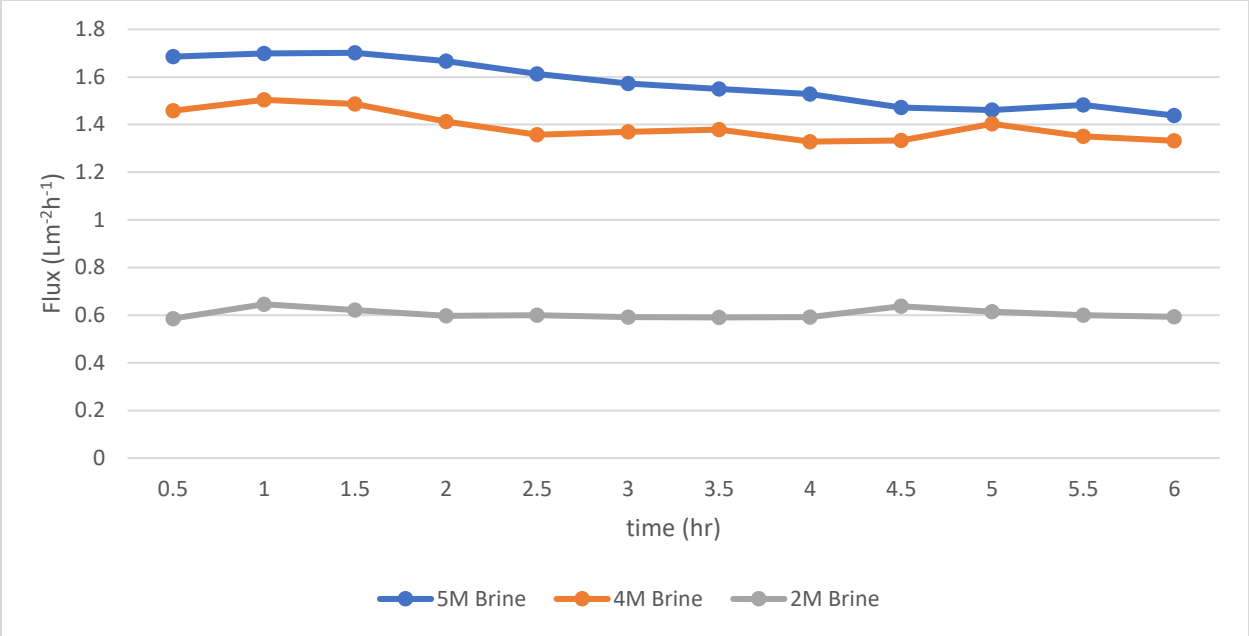


Figure 3. Flux value of different brine solutions over time.

Brine Concentration	5M	4M	2M
average flux ($Lm^{-2}h^{-1}$)	1.5724	1.3930	0.6056

Table 1. Average flux value of different brines over six hours

4.3 Comparison of Distillation Methods

The comparison of distillation methods was performed at the Chemical Engineering Department (University of Arkansas) by Dr.Wickramasinghe and his students. Figure 4 presents the flux of different combination of membranes and distillation methods over a six-hour period. The methods using only osmotic distillation were shown to have a very low flux value while the methods using the membrane distillation had much higher flux result, with PP-MD slightly higher than ECTFE-MD. The methods that combined both MD and OD were also evaluated. The result demonstrates that the combination of MD and OD gave larger flux value than the individual distillation method. In addition, it was found that the flux for PP membrane was marginally higher than ECTFE membrane which could be due to the different adsorptive

properties of the membrane types and their interaction with the anthocyanins (lower adsorption tendency leads to higher flux) (Kujawa et al., 2015; Cassano et al. 2017; Terki et al., 2018).

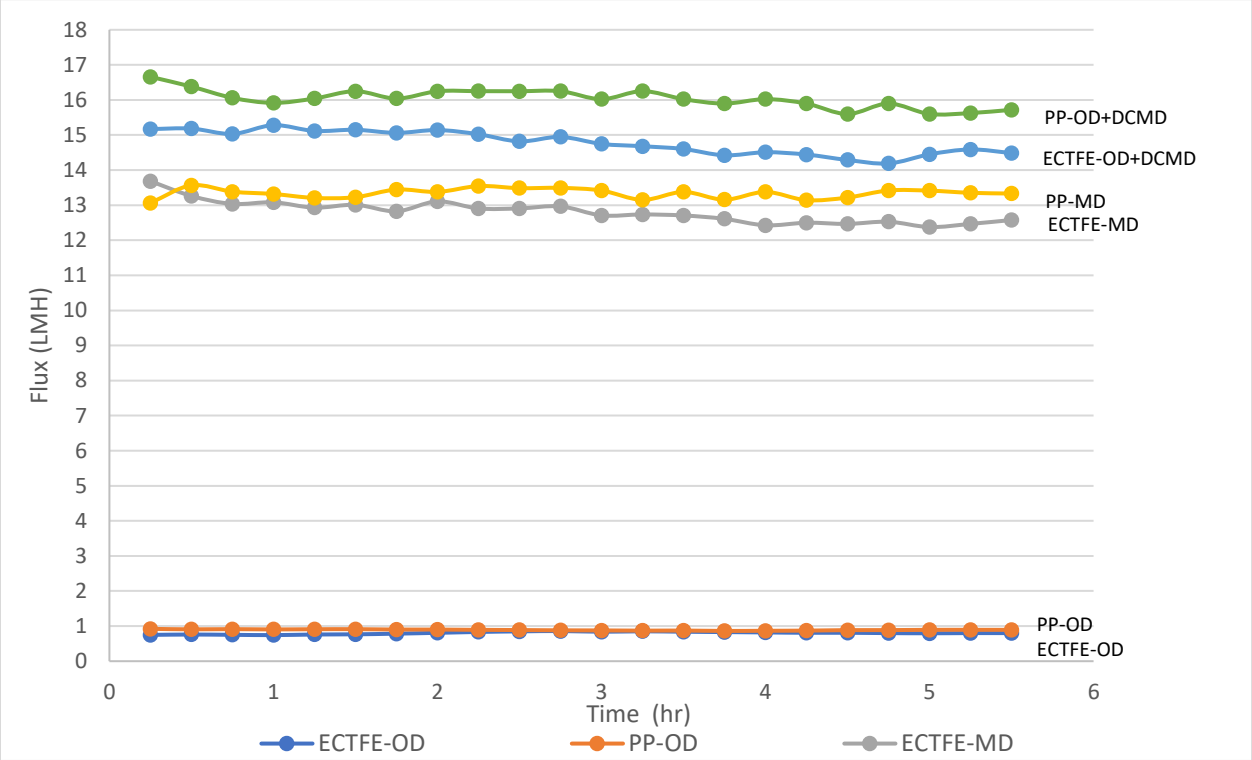


Figure 4. The permeate flux of different combinations of membranes and distillation methods

4.4 Concentration of Aqueous Anthocyanin Extract Before and After Distillation

Table 2 displays the individual and total anthocyanins content before and after each membrane process. The total anthocyanins content was calculated by the summation of the individual anthocyanins. The result shows that membrane osmotic distillation paired with ECTFE membrane can concentrate the aqueous anthocyanins up to 196% while the combination with PP membrane can concentrate up to 280%.

It was also essential to investigate the quality of the concentrated anthocyanins regarding the amount of sodium chloride transferred to the feed. The NaCl content of the aqueous anthocyanin extract before the concentration process was 6.82 mg/L, after the first run of concentration was 48.85 mg/L, and after the second run of the concentration was 73.88 mg/L. There was a certain

degree transferring but only 0.02% (48.85 mg from 233.76 g per liter) and 0.03% (73.88 mg from 233.76 g per liter) of the NaCl was transferred from the 4M brine to the samples during 1st and 2nd run of concentration, respectively.

Process / Anthocyanins (mg /100g)	ECTFE before	ECTFE after	PP before	PP after
Cyanidin-3,5-*O*-diglucoside	56.9	90.7	57.6	130.1
Delphinidin-3,5-*O*-diglucoside	22.6	46.9	22.6	66.4
Petunidin-3,5-*O*-diglucoside	45.3	94.2	46.0	137.6
Peonidin-3,5-*O*-diglucoside	22.9	49.6	22.9	71.9
Malvidin-3,5-*O*-diglucoside	23.5	51.1	23.5	74.6
Total anthocyanins	170.0	333.5	171.7	480.7

Table 2. Individual and total anthocyanin content before and after membrane osmotic distillation with ECTFE and PP membranes.

5. Conclusion

The combined process of osmotic distillation and membrane distillation was found to be the more effective compared to the individual processes for concentrating anthocyanins from the aqueous muscadine grape pomace extract. The combined process also demonstrated a constant flux during the concentration. In addition, it was discovered that membrane osmotic distillation paired with PP can concentrate aqueous anthocyanins up to 280% and with ETCFE by 196% over a 12-hour period. Therefore, membrane osmotic distillation with PP membrane exhibited a better concentration performance compared to ETCFE membrane. The amount of NaCl transferred from the permeate side to the feed was also studied to investigate the quality of the concentrated anthocyanin extract. The result was promising since only 0.02% - 0.03% of NaCl

was transferred to the feed across the membrane. This indicates that a concentrated anthocyanin extract can be obtained using membrane osmotic distillation with low salt content.

References

Pastrana-Bonill, E., Akoh, C. C, Sellappan, S., & Krewer, G. (2003). Phenolic content and antioxidant capacity of muscadine grapes. *Journal of Agricultural and Food Chemistry* 51:5497–5503.

Andersen, O. M., & Jordheim, M. (2006). The anthocyanins. In O. M. Anderson, K. R. Markham, & Flavonoids. (Eds.), *Chemistry, biochemistry and applications* (pp. 471–

Greenspan, P., Bauer, J. D., Pollock, S. H., Gangemi, J. D., Mayer, E. P., Ghaffar, A., … Hartle, D. K. (2005). Antiinflammatory properties of the muscadine grape (*Vitis rotundifolia*). *Journal of Agricultural and Food Chemistry*, 53:8481–8484.

Konczak, I., & Zhang, W. (2004). Anthocyanins-more than nature's colours. *Journal of Biomedicine and Biotechnology*, 5:923-933

Ju, Z., & Howard, L. R. (2005). Subcritical water and sulfured water extraction of anthocyanins and other phenolics from dried red grape skin. *Journal of Food Science*, 70:S270-S276.

García-Marino, M., Rivas-Gonzalo, J. C., Ibáñez, E., & García-Moreno, C. (2006). Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction. *Analytica Chimica Acta*, 563:44–50.

Plaza, M., & Turner, C. (2017). Pressurized hot water extraction of bioactives. *Comprehensive Analytical Chemistry Green Extraction Techniques - Principles, Advances and Applications*,53-82. doi:10.1016/bs.coac.2016.12.005

Francezon, N., Meda, N. R., & Stevanovic, T. (2017). Optimization of bioactive polyphenols extraction from Picea Mariana bark. *Molecules,22*(12), 2118. doi:10.3390/molecules22122118

Avram, A. M., Morin, P., Brownmiller, C., Howard, L. R., Sengupta, A., & Wickramasinghe, S. R. (2017). Concentrations of polyphenols from blueberry pomace extract using nanofiltration. *Food and Bioproducts Processing,106*, 91-101. doi:10.1016/j.fbp.2017.07.006

Monrad, J. K., Srinivas, K., Howard, L. R., & King, J. W. (2012). Design and optimization of a semicontinuous hot–cold extraction of polyphenols from grape pomace. *Journal of Agricultural and Food Chemistry,60*(22), 5571-5582. doi:10.1021/jf300569w

Liu, J., Sandahl, M., Sjöberg, P. J., & Turner, C. (2013). Pressurised hot water extraction in continuous flow mode for thermolabile compounds: Extraction of polyphenols in red onions. *Analytical and Bioanalytical Chemistry,406*(2), 441-445. doi:10.1007/s00216-013-7370-7

Yilmaz, E., & Bagci, P. O. (2018). Production of phytotherapeutics from broccoli juice by integrated membrane processes. *Food Chemistry,242*, 264-271. doi:10.1016/j.foodchem.2017.09.056

Martín, J., Díaz-Montaña, E. J., & Asuero, A. G. (2018). Recovery of anthocyanins using membrane technologies: A review. *Critical Reviews in Analytical Chemistry,48*(3), 143-175. doi:10.1080/10408347.2017.1411249

Conidi, C., Cassano, A., Caiazzo, F., & Drioli, E. (2017). Separation and purification of phenolic compounds from pomegranate juice by ultrafiltration and nanofiltration membranes. *Journal of Food Engineering,195*, 1-13. doi:10.1016/j.jfoodeng.2016.09.017

Husson, E., Araya-Farias, M., Desjardins, Y., & Bazinet, L. (2013). Selective anthocyanins enrichment of cranberry juice by electrodialysis with ultrafiltration membranes

stacked. *Innovative Food Science & Emerging Technologies,17*, 153-162. doi:10.1016/j.ifset.2012.09.011

Jampani, C., & Raghavarao, K. (2015). Process integration for purification and concentration of red cabbage (Brassica oleracea L.) anthocyanins. *Separation and Purification Technology,141*, 10-16. doi:10.1016/j.seppur.2014.11.024

Johnson, R. A., & Nguyen, M. H. (2017). *Understanding membrane distillation and osmotic distillation*. Hoboken, NJ: John Wiley & Sons.

Jiang, T., Mao, Y., Sui, L., Yang, N., Li, S., Zhu, Z., . . . He, Y. (2019). Degradation of anthocyanins and polymeric color formation during heat treatment of purple sweet potato extract at different pH. *Food Chemistry,274*, 460-470. doi:10.1016/j.foodchem.2018.07.141

Cho, M. J., Howard, L. R., Prior, R. L., & Clark, J. R. (2004). Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture,84*(13), 1771-1782. doi:10.1002/jsfa.1885

Kujawa, J., Guillen-Burrieza, E., Arafat, H. A., Kurzawa, M., Wolan, A., & Kujawski, W. (2015). Raw juice concentration by osmotic membrane distillation process with hydrophobic polymeric membranes. *Food and Bioprocess Technology,8*(10), 2146-2158. doi:10.1007/s11947-015-1570-4

Cassano, A., Luca, G. D., Conidi, C., & Drioli, E. (2017). Effect of polyphenols-membrane interactions on the performance of membrane-based processes. A review. *Coordination Chemistry Reviews,351*, 45-75. doi:10.1016/j.ccr.2017.06.013

Terki, L., Kujawski, W., Kujawa, J., Kurzawa, M., Filipiak-Szok, A., Chrzanowska, E., . . . Madani, K. (2018). Implementation of osmotic membrane distillation with various hydrophobic porous membranes for concentration of sugars solutions and preservation of the quality of cactus pear juice. *Journal of Food Engineering,230,* 28-38. doi:10.1016/j.jfoodeng.2018.02.023