EXTRACTION OF TOKAJI ASZÚ MARC AND CONCENTRATION OF THE EXTRACT BY REVERSE OSMOSIS

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Abstract

Grapes and even wine-making wastes such as marc and stalks, are rich in phenols. The polyphenolic content has many favourable effects on human health. Our aim was to find the optimal conditions of the extraction of the antioxidant and phenolic compounds from Tokaji aszú marc. Absolute ethanol and deionized water were used to prepare the solvent, 4:1 solvent-to-sample ratio was choosen. The solvent contains different volumes of ethanol (0 – 25 – 50 – 75 – 100%). The temperature was 30 °C and 60 °C. The time of the extraction was half-, one-, two-, three-, four- and five-hours long. The extractions were more efficiency using ethanol solvent compared with the water solvent. In all cases the phenol concentration and antioxidant activity were two and three times higher at higher temperature (60 °C) than at lower temperature (30 °C). The maximum value of total phenol content (67830±509 μM GS/L) was reached at 60 °C temperature, 25% ethanol solvent after 3 hours. The maximum value of antioxidant activity (11126±145 μM AS/L) was reached at 60 °C temperature, 50% ethanol solvent after 5 hours. The retention of RO membrane for total phenol content was 99.7 and for antioxidant activity 98.8%.

Keywords: tokaji aszú marc, extraction, reverse osmosis, total phenol content, antioxidant activity

Introduction

Grapes are one of the world's largest fruit crops, and even wine-making wastes such as marc (the remains of grapes or other fruit that have been pressed for wine-making) and stalks, are rich in phenols (Amendola et al. 2010, Vatai et al. 2009). Grapes, wine, grape seeds and skins extracts have many favourable effects on human health due to their polyphenol content, such as the anti-carcinogenic effects and the inhibition of the oxidization of low-density lipoproteins, thereby decreasing the risk of cardiovascular diseases. Therefore, phenolic compounds can be considered to be added-value by products, corroborating their isolation

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from the industrial waste (Spigno et al. 2007, Bonilla et al. 1999). Furthermore, the activity of these compounds as food lipid antioxidants is well known. By adding antioxidants is a method which is increase the shelf life, especially of fats, oil and fat containing food products. Since synthetic antioxidants, such as BHA and BHT have restricted use in foods because their toxicological effects on different species and suspected carcinogenic potential, the search of natural and safe antioxidants, especially of plant origin, has increased latterly (Spigno et al. 2007). The goal of an extraction process is to provide the maximum yield of substances and of the highest quality (concentration of phenolic compounds and antioxidant power of the extracts) (Shi et al. 2003). Membrane technologies is one of the technological answer to the problem of the production of concentrates with high quality, natural fresh taste and additive-free. It can be achieved by mild conditions to avoid the damage of the valuable compounds (Torun et al. 2014).

Materials and methods

The marc of Tokaji aszú was provided by the Fitomark Ltd. (Tolcsva). The marc was stored in freezer till the experiments.

Extraction measurements

Our aim was to find the optimal conditions of the extraction from Tokaji aszú marc. Absolute ethanol and deionized water were used to prepare the solvent, 4:1 solvent-to-sample ratio was choosen. Continuous stirring was ensured during all experiments. The picture and flow sheet of the equipment can be seen at Figure 1.

In the experiments three parameters of the extraction were changed: the temperature, the solvent concentration and the time of the extraction. The temperature was 30 °C and 60 °C. To keep the temperature at constant value a Lauda Ecoline E100 Immersion Thermostat was used. The solvent contains different volumes of ethanol (0 – 25 – 50 – 75 – 100%). The time of the extraction was half-, one-, two-, three-, four- and five-hours long.

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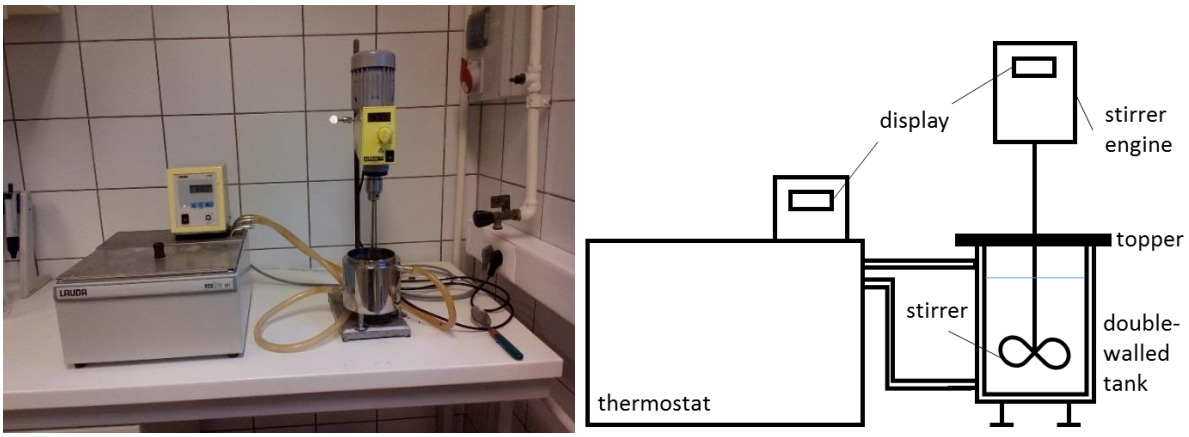

a) b)

Figure 1. (a) picture of the experimental equipment and (b) flow sheet of the experimental equipment

Analitical measurements

The TPC, FRAP assays were run with a Nicolet Evolution 300 BB type spectrophotometer (Thermo Electron Corporation, Cambridge, UK) at the respective wavelengths. Measurements were run triplicate.

Analysis of total phenol content (TPC)

Total phenol content was determined by the Folin-Ciocalteu assay (Singleton et al. 1999) applying gallic acid as the standard at 760 nm. Total phenol content was expressed in µmol equivalents of gallic acid (GS)/L.

Antioxidant activity measurements (FRAP)

The FRAP antioxidant activity assay was run as described by Benzie and Strain (1996) using ascorbic acid as standard. The absorbance was measured at 593 nm and results were determined in µmol equivalents of ascorbic acid (AS)/L.

Reverse osmosis measurement

Our aim was to concentrate the extract from tokaji aszú marc. For this purpose a TRISEP reverse osmosis membrane (ACM2, polyamide) was used (Torun et al. 2014). The membrane performance: active membrane area 0.18 m^2, maximum applied pressure 41 bar,

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recommended operating temperature 2 – 45 °C, feed pH range 4 – 11, minimum salt rejection 98.5 %. The concentration was carried out in cross-flow mode, at 25 °C temperature and at 40 bar TMP with the equipment can be seen in Figure 2.

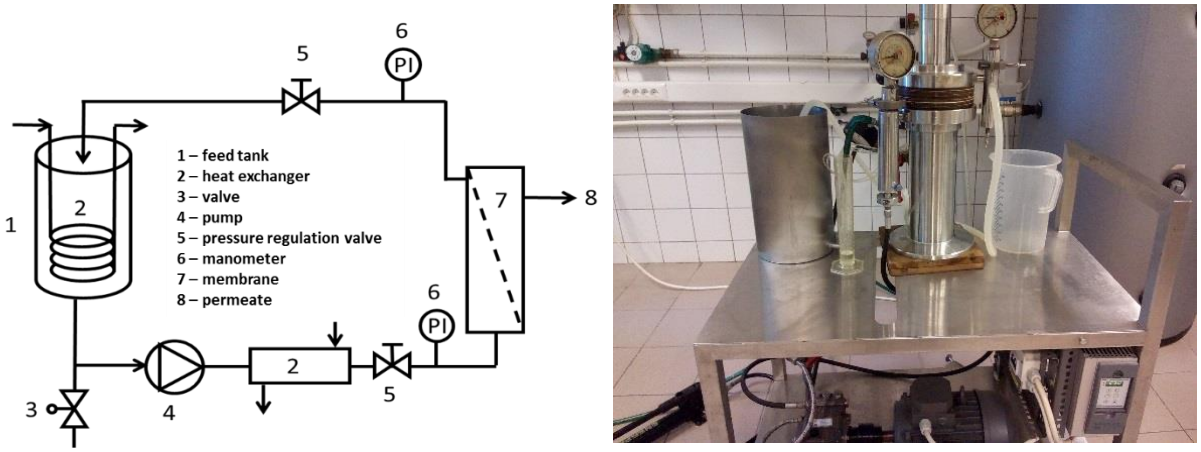

Figure 2. Flow sheet and picture of reverse osmosis equipment

For the calculation of membrane and fouling resistance the resistance-in-series model (Strahtmann et al. 2006) was used:

$$J = \frac{TMP}{\eta \cdot (R_M + R_F + R_C)} \qquad (1)$$

where J – permeate flux (m^3 m^{-2} s^{-1}), TMP – transmembrane pressure difference (Pa), η – dynamic viscosity of permeate (Pa·s), R_M – membrane resistance (m^{-1}), R_F – fouling resistance due to internal fouling inside the membrane pores (m^{-1}), R_C – cake layer resistance due to concentration polarization and desposition of solids on the membrane surface (m^{-1}).

The membrane resistance can be calculated by measuring the hydraulic permeability of the clean membrane from equation (1) where R_F and R_C is zero, they can be neglected. After the concentration R_C is removed by cleaning the membrane with water. Knowing the membrane resistance R_F can be calculated from the resistance-in-series model (1) by measuring the hydraulic permeability on the fouled membrane.

Results and discussion

In case of water solvent the phenol concentration and antioxidant activity at higher temperature (60 °C) were two and three times higher than at lower temperature (30 °C). The

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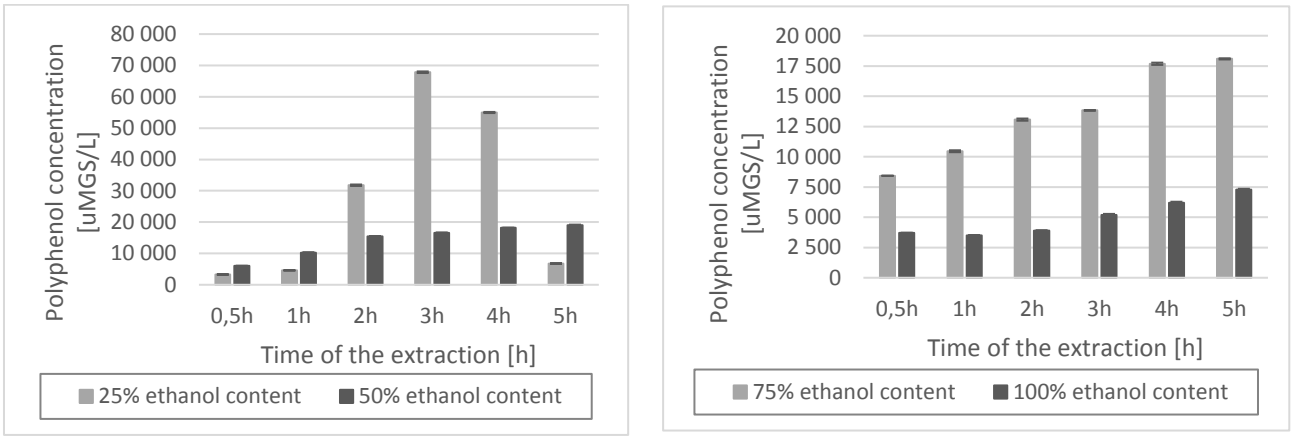
extraction time generally increased the total phenol content and the antioxidant activity. In case of water solvent the maximum values of polyphenol concentration was 3000±17 µM GS/L (after 5 hours), and the maximum of antioxidant activity was 1575±33 µM AS/L (after 5 hours).

In our experiments the phenol content and the antioxidant activity of the extracts were much higher at 60 °C temperature than at 30 °C temperature (the variance analysis showed significant difference), therefore we only represent the results at 60 °C temperature comparing the various solvent concentrations.



a) b)

Figure 3. (a) Polyphenol concentration in case of 25% and 50% ethanol solvent (b) Polyphenol concentration incase of 75% and 100% ethanol solvent at 60 °C temperature

The total phenol content of the extracts can be seen in Figure 3. in case of 25% – 50% (a) and 75% – 100% (b) ethanol solvent versus extraction time. The different volumes of ethanol in the solvent reached a more varied result than the water solvent. In case of 25% ethanol solvent the total phenol content was increased in the first 3 hours and after that it was decreased. In case of 50%, 75% and 100% ethanol solvent a continuously raise was observed in total phenol content during the five hours. The maximum value of total phenol content (67830±509 µM GS/L) was reached at 60 °C temperature, 25% ethanol solvent after 3 hours.

The antioxidant activity of the extracts can be seen in Figure 4. in case of 25% – 50% (a) and 75% – 100% (b) ethanol solvent versus extraction time. The same trend was observed in antioxidant activity with 75% ethanol solvent than the total phenol content with 25% ethanol:

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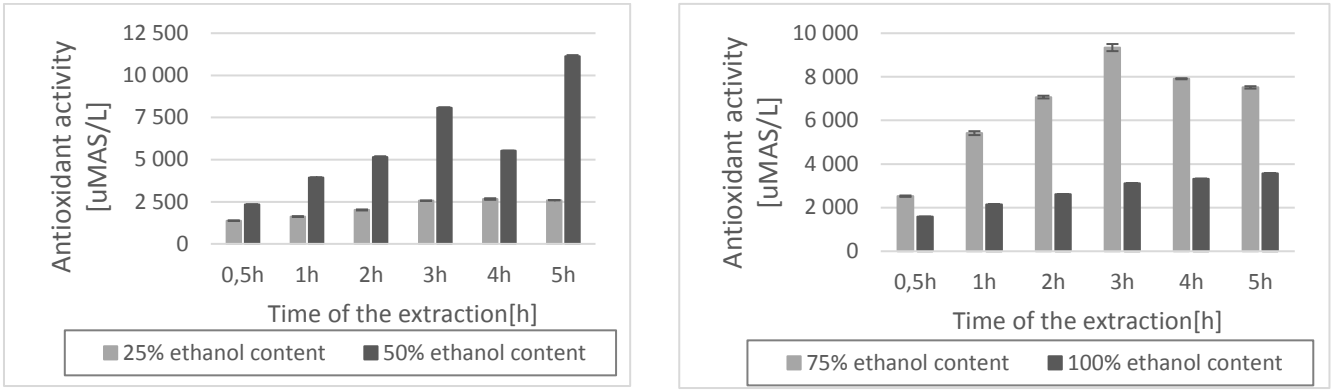
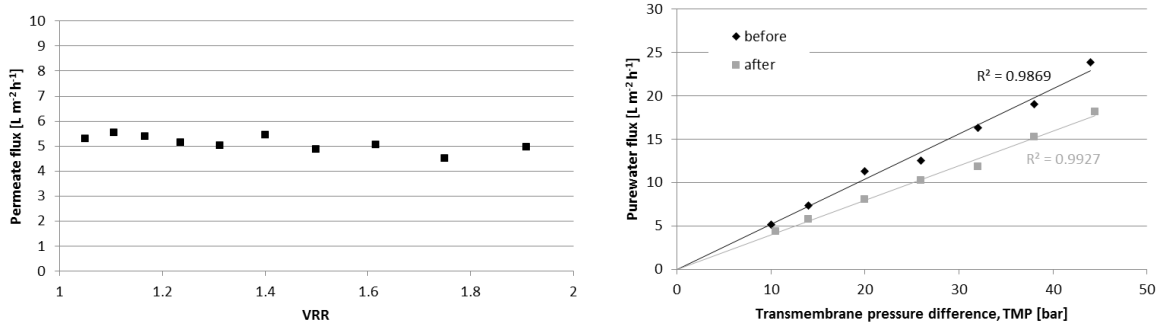
the antioxidant activity of the extracts was increased in the first 3 hours and after that it was decreased. In other cases the antioxidant activity was increased with the extraction time. The maximum value of antioxidant activity (11126±145µM AS/L) was reached at 60 °C temperature, 50% ethanol solvent after 5 hours.



a) b)

Figure 4. (a) Antioxidant activity in case of 25% and 50% ethanol solvent (b) Antioxidant activity incase of 75% and 100% ethanol solvent at 60 °C temperature



a) **b)**

Figure 6. a) Permeate flux vs. VRR during RO concentration of the extract

b) Pure water flux before and after the RO concentration

The permeate flux can be seen in Figure 6. (a) during the RO concentration of the extract. The permeate flux decreasing was not significantly. The reached volumetric reduction rate (VRR) was 1.9. The permate flux was changed between 5.53 and 4.96 L m^{-2} h^{-1}. The concentration kept 1 hour long.

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The polyphenol content and antioxidant activity were measeured in the samples (initial extract, permeate and retentate), these results can be seen in Table 1. In both cases the concentration of valuable compounds was increased during the concentration by reverse osmosis. The retention of polyphenol content was 99.7% and of antioxidant activity 98.8%. The applied RO membrane (TRISEP, ACM2) is appropriate for the concentration of tokaji aszú extract.

Table 1. Concentration of total phenol content and antioxidant activity during RO concentration

	Polyphenol concentration [μM GS L^{-1}]	Antioxidant activity [μM AS L^{-1}]
Extract (after circulated in the system)	4770.41	3804.74
Permeate	22.11	61.07
Retentate	7017.43	4991.02

For calculating the membrane- and fouling resistances the pure water flux was measured before and after the concentration (Figure 6. (b)). In both cases the pure water flux was linear, the correlation coefficient were 0.9869 and 0.9927. The decreasing of the pure water flux was 22%. This small value shows that the RO membrane did not fouled appreciably. This fact is very important in economic respect. By raising of the permeate flux the operating cost will be lower. The value of membrane- and fouling resistance were calculated from equation (1). The value of membrane resistance (R_M) was $6.65 \cdot 10^{14} \pm 3.67 \cdot 10^{13}$ m^{-1} and the value of fouling resistance (R_F) was $2.23 \cdot 10^{14} \pm 3.7 \cdot 10^{13}$ m^{-1}. The fouling resistance was thirds of the membrane resistance. The low fouling resistance is more economic, the concentration can be achieved longer time without stop for cleaning of the membrane.

Conclusion

The extraction experiments were achieved successfully. In all cases the concentration of total phenol content and antioxidant activity was higher at higher temperature. The extractions were more efficiency using ethanol solvent compared with the water solvent. Determine the optimal parameters of the extraction (temperature, solvent concentration and extraction time)

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is not easy because of the different optimum of the total phenol content and antioxidant activity. Our suggestion to choose the optimal operating parameters according to the more important component. The reverse osmosis is an appreprate method for the concentration the extract. The retentions of the RO membrane for the valuable compound were over 98% in all cases.

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