EXTRACTION OF ANTIOXIDANT AND POLYPHENOL COMPOUNDS FROM TOKAJI ASZÚ MARC WITH ISO-PROPANOL – WATER SOLVENT

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Abstract
The aim of this study was to determine the best parameter of extracting total phenol content (TPC) and antioxidant capacity (AC) from Tokaji Aszú using isopropanol – water solvent. The solid/liquid ratio was used 1:4. Different parameters such as solvent concentration (0, 25, 50, 75, 100%), time (0.5, 1, 2, 3, 4, 5) and temperature (30, 45 and 60 ºC) were investigated. The total phenol content (TPC) and antioxidant capacity (AC) were determined in the extracts using spectrophotometric analysis. SPSS statistical software was used to determine the significant difference (One-Way ANOVA, post hoc multiple comparisons, Scheffe test at significance level 0.05). The binary solvent (a mixture of water and isopropanol) was better than mono-solvent (pure water or 100% isopropanol). The optimal extracting condition for TPC was achieved at 60 ºC (temperature), 50% (isopropanol) and 4 hrs (time) 7419±36 µM GAE/L while maximized value of AC was 5228±11µM ASE/L reached at 60 ºC (temperature), 25% (isopropanol) and 4 hrs (time).

Introduction

Wine production generates a huge amount of waste which is considered as unbeneficial and potentially causes environment problems. However, due to the advance technology, minimization of waste production in several wine industries has drawn attention to many. The necessary development of innovation and effective valorization procedures has been implemented to reduce winery waste (1). Winery wastes are regarded as a low-cost source of antioxidant and phenolic compounds (2).
Wine making residues includes organic wastes (grape pomace, seeds, pulp and skins, grape stems and leaves); wastewater; emission of greenhouse gases and inorganic wastes (1). In the present paper, organic wastes (grape pomace) were studied. This waste is produced during the production of must (grape juice) after pressing the whole fruit (1). Phenolic compounds are recovered through solvent- extraction procedure whereby isolation, identification, and quantification are performed (2).
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).

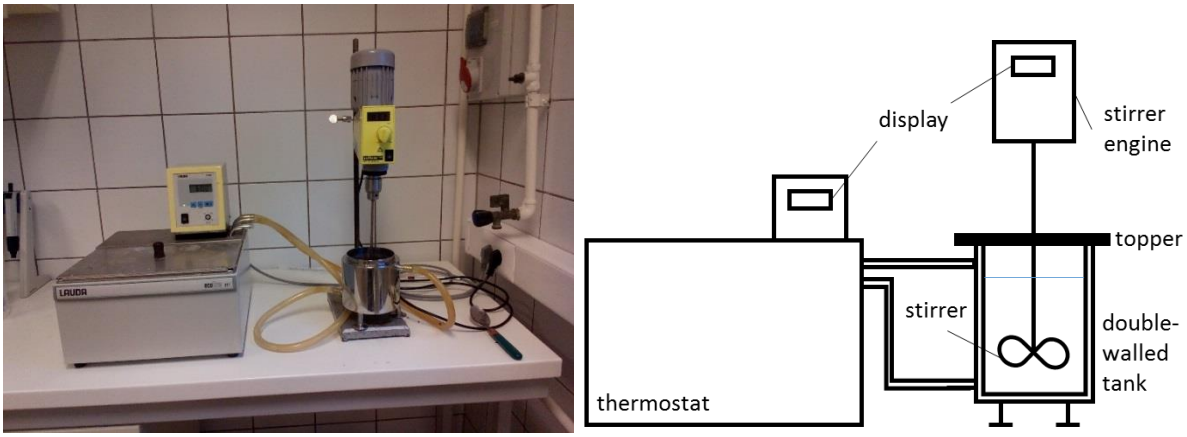
Experimental

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. Isopropanol 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, 45 °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 isopropanol (0 – 25 – 50 – 75 – 100%). The time of the extraction was half-, one-, two-, three-, four- and five-hours long.



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 [3] applying gallic acid as the standard at 760 nm. Total phenol content was expressed in µmol equivalents of gallic acid (GS)/L.

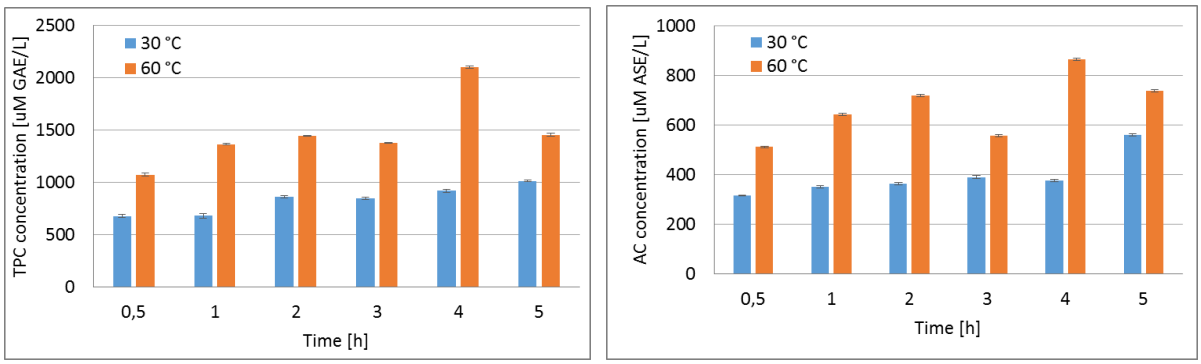
Antioxidant capacity measurements (AC)

The FRAP antioxidant capacity assay was run as described by Benzie and Strain [4] using ascorbic acid as standard. The absorbance was measured at 593 nm and results were determined in µmol equivalents of ascorbic acid (AS)/L.

SPSS Statistics 22 software was used to determine the significant difference between the samples (one-way ANOVA, post hoc multiple comparisons).

Results and discussion

Figure 2. shows the phenol concentration (a) and antioxidant capacity (b) of the extracts in case of water solvent at different temperatures versus extraction time. At higher temperature (60 °C) the phenol concentration and antioxidant capacity were two and three times higher than at lower temperature (30 °C). The extraction time generally increased the total phenol content and the antioxidant capacity. 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 capacity was 1575±33 µM AS/L (after 5 hours).



a) b)

Figure 2. (a) Polyphenol concentration incase of water solvent and b) Antioxidant capacity incase of water solvent

In our experiments the phenol content and the antioxidant capacity of the extracts were much higher at 60 °C temperature than at 30 °C temperature, therefore we only represent the results at 60 °C temperature comparing the various solvent concentrations.

The total phenol content of the extracts can be seen in Figure 3. in case of 25% – 50% (a) and 75% – 100% (b) isopropanol solvent versus extraction time. The different volumes of isopropanol in the solvent reached a more varied result than the water solvent. In case of 25% isopropanol solvent the total phenol content was increased in the first 3 hours and after that it was almost constant. The Scheffe test did not show significant difference between the TPC values at 3-4-5 hrs. Using 50% isopropanol the TPC concentration in the extract was the same after one hour. Next to 75% isopropanol solvent a continuously raise was observed in TPC during the five hours. Using 100% isopropanol the TPC concetration was low like using pure water. The maximum value of total phenol content (7419±36 µM GAE/L) was reached at 60 °C temperature, 50% ethanol solvent after 4 hours.

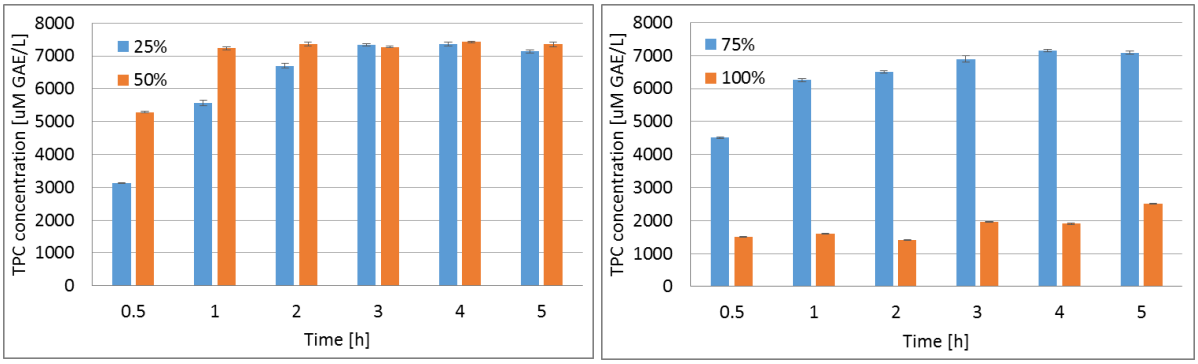


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Figure 3. (a) Polyphenol concentration in case of 25% and 50% isopropanol solvent (b) Polyphenol concentration incase of 75% and 100% isopropanol solvent at 60 °C temperature

The antioxidant capacity of the extracts can be seen in Figure 4. in case of 25% – 50% (a) and 75% – 100% (b) isopropanol solvent versus extraction time at 60 °C temperature.
The interaction of water and isopropanol have shown a positive effect on antioxidant capacity in the extracts. Using 25% isopropanol solvent the AC increases directly proportional to time until 4 hours and then decrease during 5 hours of extraction.
The maximum value of antioxidant capacity (5228±11µM ASE/L) was reached at 60 °C temperature, 25% ethanol solvent after 4 hours. Applying 50-75% isopropanol solvent an almost continuously raise was observed in AC during the five hours. In all cases there are significantly difference between AC concentrations. Using 100% isopropanol for the extraction resulted low AC concentrations. The AC values were almost the same values like in case of pure water.

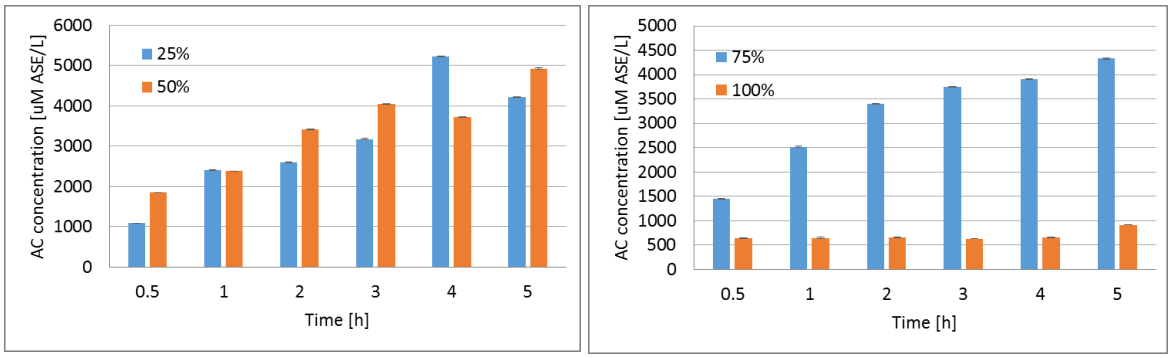

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Figure 4. (a) Antioxidant capacity in case of 25% and 50% isopropanol solvent (b) Antioxidant capacity incase of 75% and 100% isopropanol solvent at 60 °C temperature

Figure 5. shows the influence of temperature on TPC and AC in case of different concentration of solvent after 3 hrs extraction. The temperature has a great impact on phenolic compounds and antioxidant recovery. TPC increased with temperature and Chew et al. [5], Wang et al. [6] reported similar results.

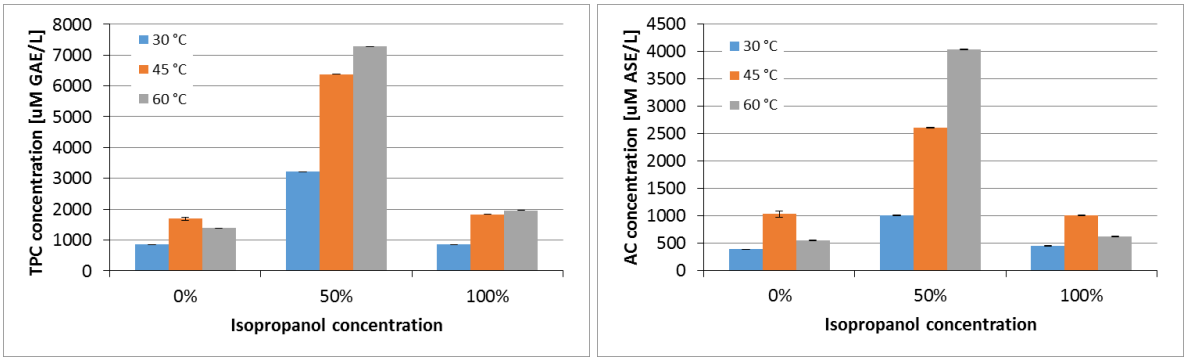

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Figure 5. Effect of temperature and solvent concentration on TPC (a) and AC (b) content after 3 hours of extraction

Thoo et al. [7] mentioned that to improve phenolic compounds adequate heating is applied to promote solvent extraction by enhancing both diffusion coefficients and the solubility of polyphenol content. Wang et al. [6] findings demonstrated that high temperature has a positive impact on the extraction of phenolic compounds excluding antioxidant since it increases solubility and diffusion coefficient of any compounds.

Our results show that the extraction at 60 °C has the highest yield of total phenolic content to compare with 30 °C and 45 °C while for antioxidant capacity extracted at 45 °C was high and decreases at 60 °C except for using 50% of isopropanol. Antioxidant capacity was sensitive to high temperatures. High-temperature degrade and promote molecular collisions, favouring polymerization which reduces antioxidant compounds [8].

From the economic point of view, working at 45 °C is much better than 60 °C to maximised the yield. This need to be verified by evaluation of the energy cost of the extraction step on the overall production cost [2].

The high temperature is in favoured of the released bound of polyphenol in a sample with the breakdown of cellular constituents of plant cells which lead to increased cell membrane permeability. These bound polyphenols could further reduce the chances of polyphenol coagulating with lipoprotein, thereby enhancing the solubility of the polyphenol and diffusion increasing polyphenol yield [7].

There is no significant difference between AC extracted using 0% and 100% isopropanol concentration at all temperature (30, 45 and 60 ºC), respectively. There is also no significant difference between AC values at 30 °C, 50% isopropanol and 45 °C, 0% isopropanol and 45 °C, 100% isopropanol concentration. But in all other cases, there are significantly difference. There is a significant difference between all TPC concentration except at 30 °C temperature using 0% and 100% isopropanol.

Conclusion

Winery waste is rich in phenolic compounds. The extraction process can be used to separate these compounds from waste. The extraction experiments were achieved successfully. In all cases the concentration of total phenol content and antioxidant capacity was higher at higher temperature. The maximum recovery TPC from the grape marc using traditional solid-liquid extraction was 7419±36 µM GAE/L at the optimum conditions of 50% isopropanol concentration, 60 ºC temperature and 4 hours, respectively. In contrast, the maximum AC was 5228±11µM ASE/L under optimum conditions of 25 % isopropanol concentration, 60 ºC temperature and 4 hours, respectively. These results indicate that an increase in temperature

and solvent concentration has a positive impact on both TPC and AC. There was a gradual increase in TPC and AC as the temperature rises from 30 ºC to 60 ºC. The TPC and AC rise during binary solvent (25-75% isopropanol concentration) and decrease in the mono-solvent system (pure water and 100 % isopropanol concentration). Determine the optimal parameters of the extraction (temperature, solvent concentration and extraction time) is not easy because of the different optimum of the total phenol content and antioxidant capacity. Our suggestion to choose the optimal operating parameters according to the more important component.

Acknowledgements

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