EXTRACTION OF PHENOLIC COMPOUNDS FROM TOKAJI ASZU MARC

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Abstract

Phenolic compounds are main antioxidant compounds in grapes and grape by-products. Wine production generates a huge amount of waste which is considered as unbeneficial and potentially causes environment problems.

The aim of this study was to determine the optimal parameters (temperature, solvent concentration, time) of extracting total polyphenol content (TPC) from Tokaji Aszu marc using two different extraction solvent: ethanol-water and isopropanol-water (1:4 solid/liquid ratio). The extractions were achieved based on 2^p full factorial design (CCRD-RSM). The TPC content was measured by Folin method.

The optimal extraction parameters in case of ethanol-water solvent: 60 °C temperature, 60% ethanol concentration in solvent, 5 hours. At this parameters the probable TPC concentration is 23270.5 uM GA/L. The optimal extraction parameters in case of isopropanol-water solvent: 60 °C temperature, 52% ethanol concentration in solvent, 4.63 hours. At this parameters the probable TPC concentration is 6441.5 uM GA/L. In both cases the binary solvent was better than mono-solvent. Ethanol-water solvent was more efficient than the isopropanol-water solvent.

Keywords: extraction, grape marc, phenolics, ethanol, iso-propanol

1. Introduction

The grapes are one of the world's largest fruit crops, which are rich in phenols. After the wine-making process many by-products remain. These by-products are the marc, stalks, skin and seeds which are also rich this is valuable component. The polyphenol content has many favourable effect on the human health, such as the inhibition of the oxidization of low-density lipoproteins and the anti-carcinogenic effects (SPIGNO et al., 2007; BONILLA et al., 1999).

The grapes are also rich in antioxidants. Antioxidants are beneficial to health because they have protective role against oxidative stress. Antioxidants have an important role in

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preventing the development of many diseases such as cancer and coronary heart disease (ALÍA et al., 2003).

In Hungary the most famous grape species is the Tokaji aszú. The natural or induced development of noble rot caused by the fungus Botrytis cinerea. There must be three basic conditions for the noble rot: (1) the grape should be in full maturity when the wet weather indicate the growing of the fungi, (2) at the same time the grape should be intact and free from injury, (3) a few days of rainy weather followed by a long and dry period (EPERJESI et al., 1998). There is just a few research which was done to optimise the yield of bioactive compounds extracted from Tokaji Aszu (winery waste) using solvent concentration. The wine marc has many valuable components which is not utilized. The industry does not use this material in notable volume. They made some spirit of it but the is a lot of it whih are will not be used. The marc is hazardous waste that needs to be transported by the producer.

The objective of the present study is to find the optimal extraction parameters to maximize the phenolics from tokaji aszu marc.

2. Material and Methods

2.1. Material

The Fitomark Ltd. (Tolcsva) provided the Tokaji aszu marc. The marc was stored in freeze until the experiments.

2.2. Extraction procedure

The carried-out extraction is based on a central composite design. Three parameters were changed during the extractions: the time of the extraction, the temperature and the solvent concentration. Every parameter has a minimum, a central and a maximum point (Table 1). The solvent-to-sample ratio was 4:1. Lauda Ecoline E100 Immersion Thermostat was used to keep the temperature on constant level. Continuous stirring was used during extractions. A cover was used to avoid evaporation of the solvent. The extraction solvent contained water and ethanol or iso-propanol, in different ratio.

RSM technique was used to optimize the extraction conditions aimed at maximum recovery of polyphenol. The experiments were made in randomized order starting and finishing with a center point run the experiment series (Table 2).

Table 1. The levels of extraction parameters

	Temperature	Time	Solvent conc.	
			Water	Alcohol
Minimum	30 °C	1 hr	100%	0%
Central	45 °C	3 hrs	50%	50%
Maximum	60 °C	5 hrs	0%	100%

Table 2. The design of the experiments

Order	**The coded level of parameters**			**Temperature (°C)**	**Solvent conc. (%)**	**Time (hrs)**
1.	0	0	0	45	50	3
4.	-1	-1	-1	30	0	1
3.	-1	-1	1	30	0	5
10.	-1	1	-1	30	100	1
5.	0	0	0	45	50	3
8.	-1	1	1	30	100	5
11	1	-1	-1	60	0	1
6.	1	-1	1	60	0	5
9.	0	0	0	45	50	3
7.	1	1	-1	60	100	1
2.	1	1	1	60	100	5
15.	1	0	0	60	50	3
13.	0	0	0	45	50	3
16.	-1	0	0	30	50	3
18.	0	1	0	45	100	3
12.	0	-1	0	45	0	3
17.	0	0	0	45	50	3
14.	0	0	1	45	50	5
19.	0	0	-1	45	50	1
20.	0	0	0	45	50	3

The center point measurements were dispersed as evenly as possible throughout the design matrix. The center point measurements were repeated 6 times. Design Expert 11.0 software was used for optimization of extractions parameters and statistical analysis.

2.3. Analysis of total phenol content (TPC)

Total phenol content was determined by the Folin-Ciocalteu assay (SINGLETON & ROSSI, 1965) applying gallic acid as the standard at 760 nm. Total phenol content was expressed in μmol equivalents of gallic acid (GS)/L.

2.4. Antioxidant capacity measurements (FRAP)

The FRAP antioxidant capacity 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.

3. Results and discussion

RSM technique was used to optimize the extraction conditions aimed at maximum recovery of polyphenol. The regression equation with linear and square coefficients as follows:

for ethanol: $Y(\mathrm{Sqrt}(\mathrm{TPC})) = 125{,}25 + 17{,}67 \cdot A + 15{,}31 \cdot B + 10{,}53 \cdot C + 0{,}44 \cdot A^2 - 38{,}79 \cdot B^2 - 3{,}44 \cdot C^2$

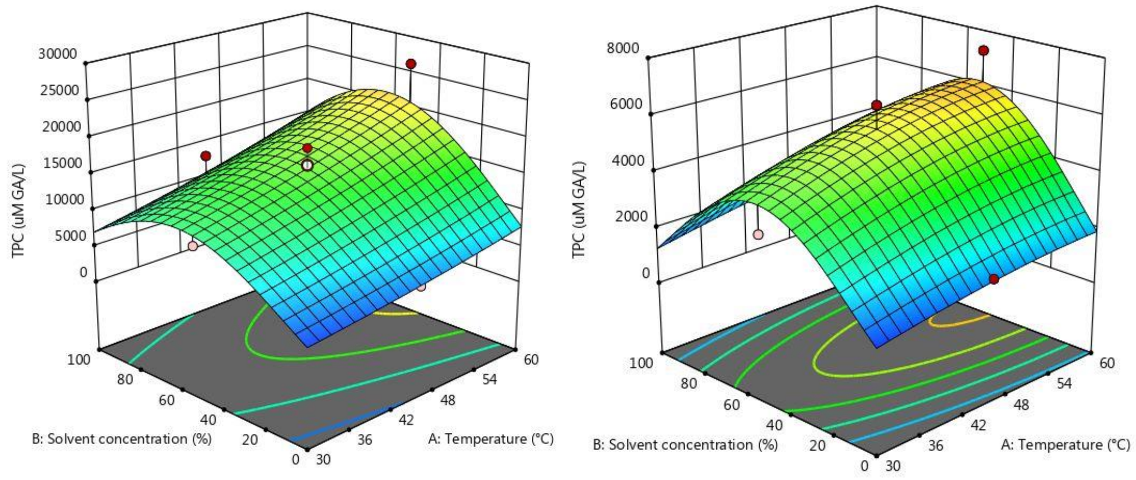
for iso-propanol:

$$Y(\mathrm{Sqrt}(\mathrm{TPC})) = 74{,}07 + 6{,}75 \cdot A + 2{,}72 \cdot B + 4{,}85 \cdot C - 2{,}82 \cdot A^2 - 31{,}87 \cdot B^2 - 3 \cdot C^2$$

Predicted values of dependent variables were obtained from the regression model. Statistical analysis showed that some linear and quadratic coefficients of regression model were significant ($p < 0.05$) whereas the lack of fit was non–significant ($p \geq 0.05$) which validates the model. In both cases (using ethanol-water or iso-propanol-water solvent) the interaction coefficients were not significant, so the model was reduced so it contains only the linear and quadratic coefficients finally (Table 3.).

Table 3. ANOVA table for reduced quadratic models for ethanol – water and iso-propanol – water solvent

	Using ethanol – water solvent					Using iso-propanol – water solvent				
Source	Sum of squares	df	Mean Square	F-value	p-value	Sum of squares	df	Mean Square	F-value	p-value
Model	7092,01	6	1182,00	29,62	<0,0001	14849,59	6	2474,93	13,78	<0,0001
A-Temp	455,12	1	455,12	11,41	0,0050	3123,67	1	3123,67	17,39	0,0011
B-Solv conc	73,76	1	73,76	1,85	0,1971	2343,98	1	2343,98	13,05	0,0032
C-Time	234,93	1	234,93	5,89	0,0305	1108,86	1	1108,86	6,17	0,0274
A^2	21,92	1	21,92	0,5494	0,4171	0,5265	1	0,5265	0,0029	0,9576
B^2	2793,72	1	2793,72	70,02	<0,0001	4137,15	1	4137,15	23,04	0,0003
C^2	24,71	1	24,71	0,6192	0,4454	32,63	1	32,63	0,1817	0,6769
Residual	518,70	13	39,90			2334,65	13	179,59		
Lack of Fit	422,56	8	52,82	2,75	0,1403	1606,43	8	200,80	1,38	0,3765
Pure Error	96,14	5	19,23			728,22	5	145,64		
Cor Total	7610,71	19				17184,24	19			

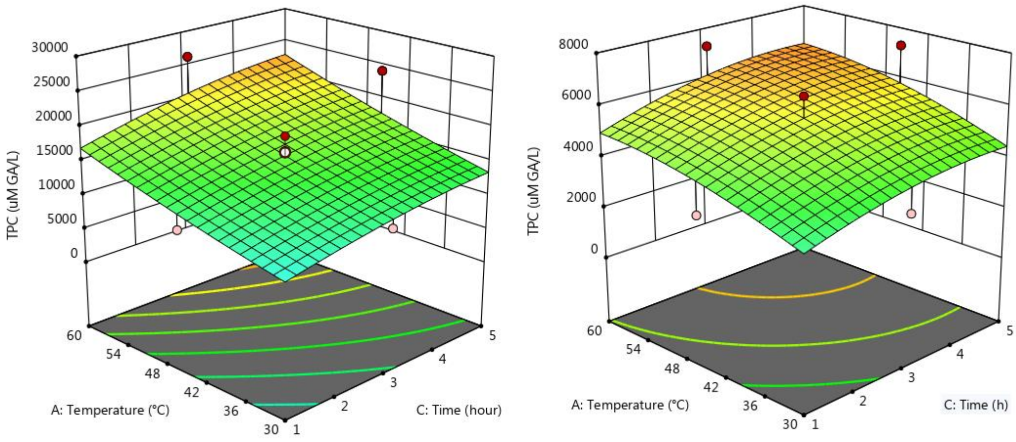


(a) (b)

Figure 1. Response surface plots showing the effect of solvent concentration and temperature on the phenolics yield (uM GA/L) from tokaji aszu marc waste while the time kept at coded zero level.: (a) Using ethanol – water solvent, (b) Using iso-propanol – water solvent

In order to obtain the optimum values, surface plots were plotted selecting two independent values while remaining one at zero level. The increase in temperature showed increase in the phenolics yield (Fig. 1). In both cases the binary solvent (1:1) was better than mono-solvent.

CHEW et al. (2011) reported similar results. Ethanol-water solvent was more efficient than the isopropanol-water solvent.



(a) (b)

Figure 2. Response surface plots showing the effect of temperature and time on the phenolics yield (uM GA/L) from tokaji aszu marc waste while the solvent concentration kept at coded zero level.: (a) Using ethanol – water solvent, (b) Using iso-propanol – water solvent

Increasing in temperature has greater effect than the increase in extraction time on phenolics concentration in tokaji aszu marc extracts. A slightly increase in TPC concentration can be observed in 5 hours, but increase in temperature the TPC concentration increased with 2 – 2.5 times (Fig. 2a). In case of iso-propanol – water solvent the same trend was found.

The optimal extraction parameters in case of ethanol-water solvent: 60 °C temperature, 60% ethanol concentration in solvent, 5 hours. At this parameters the probable TPC concentration is 23270.5 uM GA/L. The optimal extraction parameters in case of isopropanol-water solvent: 60 °C temperature, 52% ethanol concentration in solvent, 4.63 hours. At this parameters the probable TPC concentration is 6441.5 uM GA/L.

4. Conclusion

The phenolics were extracted from tokaji aszu marc waste following twenty selected combination of temperature, solvent concentration, and extraction time. A second order model was developed for polyphenol content. Ethanol-water solvent was more effective than iso-propanol-water solvent. The present study helps for the utilization of tokaji aszu marc waste and for optimization of extraction parameters in maximizing of the recovery of polyphenols. This optimization process provides valuable data which can be utilized in process design and industrial scale-up operations.

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