EVALUATION OF EXTRACTION METHODS OF GALICJANKA CHOKEBERRY POMACE ON THE ANTHOCYANINS AND ANTIOXIDANT CAPACITY

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Abstract
Chokeberry pomace is an abundant source of anthocyanins. Anthocyanins are important phenols, which give berries the red, purple, and dark colour. The anthocyanins found in the chokeberry pomace have the potential to be used in the food industry as a natural food additive, however, the effectiveness will depend on the level of anthocyanins in the extract. This study evaluated the level of anthocyanins and antioxidant capacity found in samples extracted from Galicjanka Polish variety chokeberry pomace, extracted with 50% ethanol + 1% citric acid and 50% glycerol +1% citric acid, at 50 and 60 °C for 60 and 120 minutes. The results indicated a significantly higher ($P< 0.05$) level of anthocyanins with 50% ethanol + 1% citric acid (ranged from 1546 ±5 4 mg/100g to 1680 ± 36 mg/100g DW) than 50% glycerol 1% citric acid (ranged from 1355 ±73 mg/100g DW to 1456 ± 48 mg/100g DW) at all extraction conditions, with the highest level yielded at 50 °C at 120 minutes. The 50% ethanol +1 citric acid has also yielded the highest antioxidant capacity with both DPPH and TEAC, at 60 °C for 120 minutes. This study reveals that 50% ethanol +1% citric acid, 50-60 °C, and 120 minutes serve as a potent solvent, incubation temperature, and duration respectively, for obtaining extracts containing a high level of anthocyanins, antioxidant capacity, and good colour intensity.

Introduction
In connection with saving the planet and human health, the interest in utilizing by-product extracts as natural food additives and pharmaceutical active agents has increased globally [1]. Chokeberry pomace is a by-product produced after juice extraction from the chokeberry fruits. Chokeberries have been identified as rich in phytochemicals including anthocyanins [2]. Anthocyanins are polyphenols that contribute to the dark purple colour of chokeberry. This distinctive colour can be utilized in the food industry as a natural colourant. Anthocyanins are attributed to the predominant percentage of total phenolic compounds found in fruits, ranging between 25-50% [3]. Several studies have identified cyanidin-3-galactoside, cyanidin-3-arabinoside, and cyanidin-3-xyloside as predominant anthocyanins in chokeberry extracts accounting for proximately 90% of the total anthocyanins [4–6]. Anthocyanins are also identified with antioxidant effects, which play a significant role in inhibiting oxidation reactions and delaying ripening in fruits [7,8]. However, the effective utilization of anthocyanins is determined by their concentration in the extracts. Anthocyanins are not so stable and can easily be affected by many factors including temperature, solvents used, and the extraction duration. Therefore, this study aimed to study the effectiveness of 50% ethanol +1 citric acid and 50% glycerol +1 citric acid in extracting anthocyanin and the antioxidant capacity under different extraction conditions.

Material and methods
Garijanca (Polish) variety chokeberry fruits collected from a farm located near Lajosmizse (47◦02′44.4″ N 19◦35′14.8″ E) in Hungary in 2023, were pressed under controlled conditions

in the laboratory to produce juice and pomace. The produced pomace was lyophilized using a Leybold Heraeus Lyovac CT2 freeze dryer (Labexchanger, Burladingen, Germany), and ground into 2.2 mm powder, then used to extract bioactive compounds. The extraction was carried out with 50% ethanol and 50% glycerol all acidified with 1% citric acid (to stabilize anthocyanins), at 50 and 60 °C incubation temperature each for 60 and 120 minutes. This was then assisted by sonication for 15 minutes. These extraction conditions were selected based on our previous studies' results [5]. Thereafter, samples were centrifuged at 4500 rpm for 5 minutes, then the supernatant was collected and measured for total anthocyanins, antioxidants capacity (FRAP and TEAC), and colour parameters. Extraction and all tests were done in triplicate.
The total anthocyanin concentration was spectrophotometrically measured following the pH differential method [9]. The antioxidant capacity was evaluated following the Ferric Reducing Ability of plasma (FRAP) assay [10], and Trolox equivalent antioxidant capacity (TEAC) assay as indicated by Müller et al. [11] with some modifications. The colours intensity of samples was measured with a digital colourimeter (Konika Minolta, Chroma-400). The differences in colour (ΔE) between ethanol and glycerol samples were evaluated and reported by looking at the noticeability level (0 - 0.5 = not noticeable, 0.5 - 1.5 = slightly noticeable, 1.5 - 3.0 = noticeable, 3.0 - 6.0 = clearly visible, and 6.0 - 12.0 = great visibility) described by Lukács [12]

Statistical Analysis

The IBM SPSS statistics software, version 27 (IBM Corp., New York, NY 10022, USA, 2020) was used to analyse the mean difference between samples, using a one-way analysis of variances (ANOVA) post-hoc test (Turkey's, and Games' Howell). The normality of error for different analyses was proved by Skewness, Kurtosis, and Kolmogorov-Smirnova ($P > 0.05$). Levene's test was used to test the homogeneity of variances between the mean values of the samples. The significant difference between factors was determined at the interval level of $P < 0.05$.

Results and Discussions

Total anthocyanins

The mean values of total anthocyanins between samples were compared between the solvents (50% ethanol and 50% glycerol samples all acidified with 1%) and the extraction conditions (50 °C and 60 °C each at 60 minutes and 120 minutes) to determine the solvent and extraction conditions giving the highest level of anthocyanin from Galicjanka variety. The results are indicated in Figure 1. Based on the results 50% ethanol has given significantly ($P < 0.05$) higher levels of anthocyanins at all extraction conditions (ranging from 1546 ±5 4 mg/100g to 1680 ± 36 mg/100g DW) compared to 50% glycerol (ranging from 1355 ±73 mg/100g DW to 1456 ± 48 mg/100g DW). The highest levels of anthocyanins from ethanol (1680 ± 36 mg/100g DW) and glycerol (1456 ± 48 mg/100g) extracts were obtained at 50 °C incubation temperature for 120 minutes. These results are in line with our previous findings [5], in which 50% ethanol + 1% citric acid yielded a higher level of anthocyanins compared to other solvents including 50% glycerol + 1% citric. Our previous study has also found 50 °C at 120 optimal for anthocyanins and TPC extraction with ethanol and glycerol respectively. However, both solvents are green solvents that can extract a significant yield of anthocyanins that can used as a natural food additive.

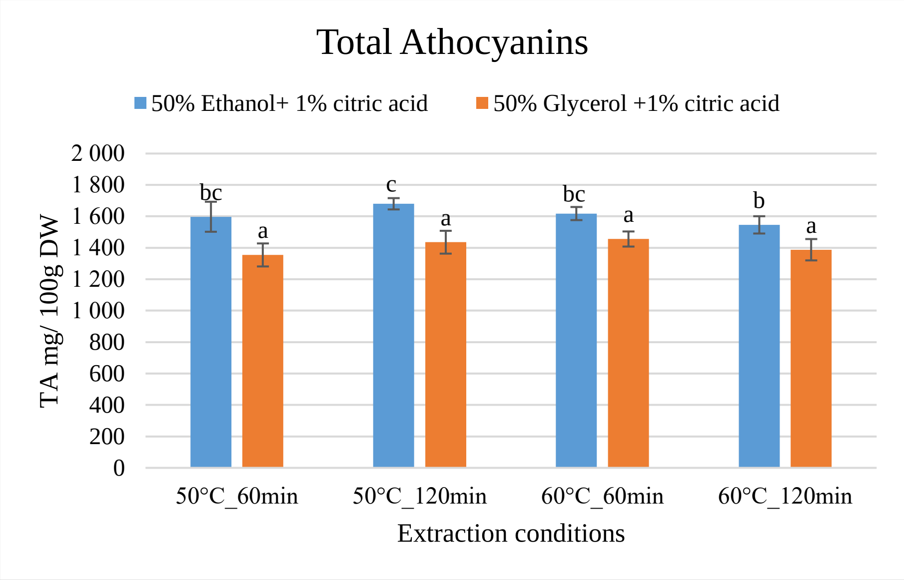

Figure 1. Anthocyanin mean values obtained with 50% ethanol +1% citric acid and 50% glycerol +1% citric acid at different extraction conditions. Different letters indicate significant differences (0.95 confidence interval) between treatments. Error bars the standard deviation.

In a study done by Kowalska et al. [13] 65% glycerol at 80 °C was found optimal in extracting anthocyanins. This could mean that 50% glycerol + 1% citric acid concentration might be too low to dissolve a high quantity of anthocyanins. The plant properties matrices, anthocyanin solubility, and polarity can influence anthocyanin yield.

Antioxidant capacity

The level of antioxidant capacity of polyphenols obtained from the chokeberry pomace using 50% ethanol and 50% glycerol acidified with 1% citric acid is indicated in Figures 2 and 3. Regarding FRAP (Figure 2), 50% ethanol has yielded a significantly higher ($P < 0.05$) level of antioxidant activity (4476 ± 488 mg AA/100g DW) compared to glycerol at 60 °C for 120 minutes. However, glycerol yielded a significantly higher ($P < 0.05$) level of antioxidant activity (3662 ± 439 mg AA/100g DW) at 50 °C and 120 minutes.

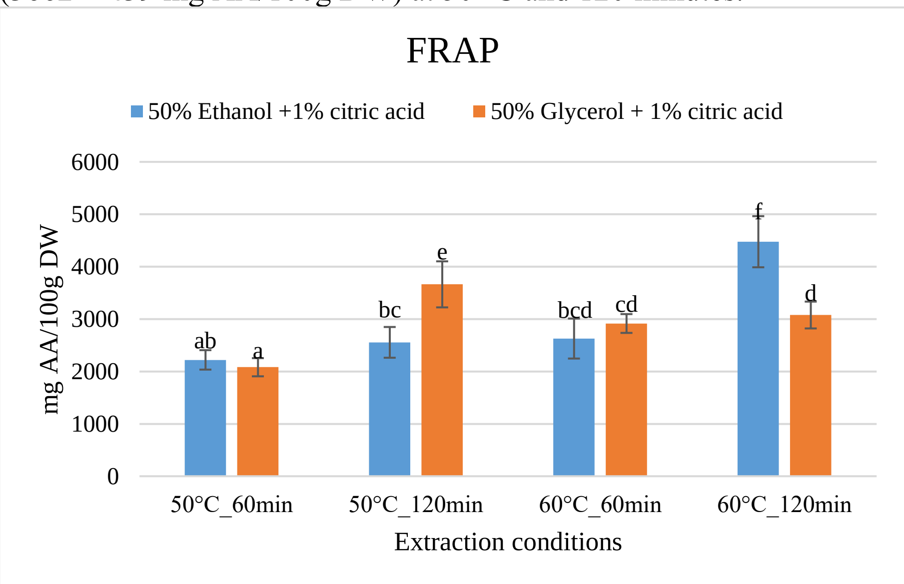

Figure 2. FRAP mean values obtained with 50% ethanol +1% citric acid and 50% glycerol +1% citric acid at different extraction conditions. Different letters indicate significant differences (0.95 confidence interval) between treatments. Error bars the standard deviation.

TEAC results (Figure 3) have also indicated ethanol extracts to yield a higher level of antioxidant capacity compared to glycerol, and the highest was obtained at 60 °C for 120 minutes similar to FRAP. the efficiency of ethanol in yielding extracts with high antioxidants over other solvents has been reported by [11]. Extracts with a higher antioxidant capacity are needed for utilization in the food industry as anti-senescence, antibacterial, and to prevent oxidation.

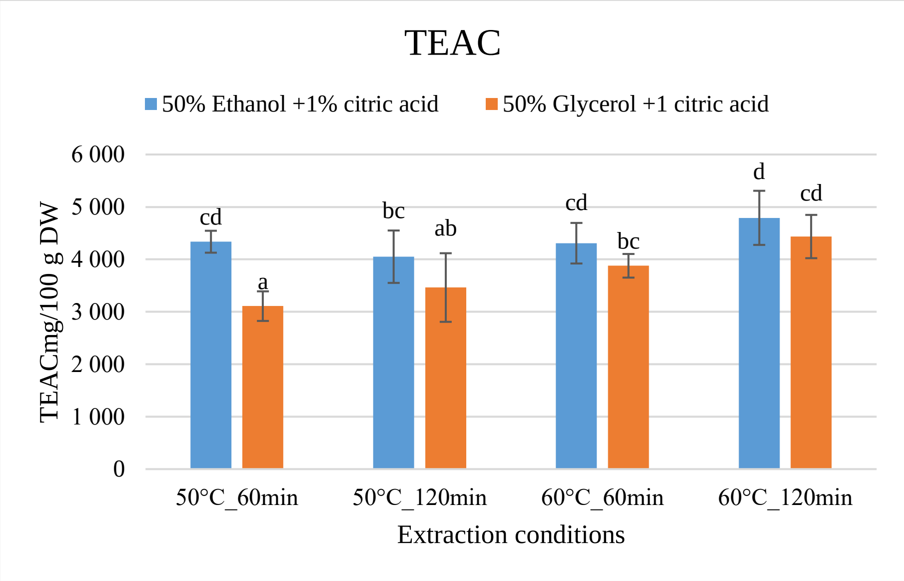

Figure 3. TEAC mean values obtained with 50% ethanol +1% citric acid and 50% glycerol +1% citric acid at different extraction conditions. Different letters indicate significant differences (0.95 confidence interval) between treatments. Error bars the standard deviation.

Colour Differences

The colour differences between 50% ethanol + 1% citric acid and glycerol +1% citric acid at each extraction condition were evaluated, and the results are given in Table 1. The difference in colour between the two solvents was noticeable. These results align with the anthocyanins results, in which 50% ethanol + 1% citric acid extracts have yielded the highest level of anthocyanins.

Table 1. The colour differences between 50% ethanol +1% citric acid and 50% glycerol + 1% citric acid

	Ethanol			Glycerol				
Extraction conditions	**L***	**a***	**b***	**L***	**a***	**b***	**ΔE***	**Evaluation**
50°C_60min	9,07	1,59	-0,40	10,49	1,93	-0,31	1,46	Noticeable
50°C_120min	9,05	1,56	-0,44	10,90	1,69	-0,50	1,85	Noticeable
60°C_60min	9,06	1,38	-0,43	10,67	1,49	-0,51	1,619	Noticeable
60°C_120min	9,03	1,37	-0,46	10,44	1,45	-0,49	1,41	Noticeable

Conclusion

This study assessed the level of anthocyanins and antioxidant capacity in samples extracted with 50% ethanol and 50% glycerol all acidified with 1% citric acid at different extraction conditions previously known to yield a high level of polyphenols. In overall, 50% ethanol +1% citric acid has given the maximum yield for anthocyanins, antioxidant capacity and the colour. For the extraction conditions, the highest anthocyanins were obtained at 50 °C for 120 minutes, whereas at 60°C for 120 minutes extracts yielded the highest antioxidant capacity. Therefore, this study suggests these two extraction settings can be suitable for extracting a high level of anthocyanins and antioxidant compounds.

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