EXTRACTION OF POLYPHENOL COMPOUNDS FROM CHOKEBERRY (*ARONIA MELANOCARPA* (MICHX)) POMACE USING METHANOL AND ACETONE AT DIFFERENT CONDITIONS

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Abstract
Fruits are appreciated in the food industry, due their high of bioactive compounds and sensory characteristics. Chokeberry is one of the fruits that are bioactive compounds abundant. It is used in juice production and other products, and large amount of chokeberry pomace believed to be rich in bioactive compounds is produced. Therefore, this pomace can be reused by extracting the bioactive compounds such as anthocyanins, which can be potentially used as natural food additives in the food industry. However, some compounds can be sensitive to different extraction conditions. Therefore, in this study, methanol (50%) and acetone (50%) were used as solvents for extracting antioxidants, total anthocyanins, and total phenolic compounds at room temperature and 60 °C at the duration of 30, 60 and 120 minutes. The shaker, ultrasonic sound and at room stand were used to hold the sample for 15 minutes. Room temperature extraction has been found not optimal for TPC and antioxidant at all extraction length in time, however, it is suitable for anthocyanins. Alternatively, acetone at room temperature (A20) for 120 minutes at room temperature can also be ideal. A combination of methanol at 60 °C for 60 min and holding the sample on shaker has been found appropriate for anthocyanins. The combination of extraction with acetone at 60ºC for 120 minutes and holding for 15 minutes on a shaker has been identified to be optimal TPC and antioxidant capacity extraction.

Introduction
Chokeberry (*Aronia melanocarpa* (Michx)) fruits have grown popularity in the food industry due to its abundance of phenolic compounds [1]. Aronia fruits are used in juice processing, which produces a large amount of Aronia pomace [2]. Aronia pomace has been reported rich in anthocyanins which is responsible for colours in fruits, making Aronia pomace a potential food colourant source [3]. Aronia pomace has also been reported rich in other polyphenols which can be used to preserve food [3]. However, some compounds are sensitive to different environmental conditions, and may degrade [4]. Therefore, for a significant quantity of bioactive compounds to be extracted from Aronia pomace, potential extraction methods and conditions must be considered. Solvents like methanol, acetone, ethanol, and glycerol are commonly used in polyphenol extraction from fruits [5–7]. However, there is little information on the optimal conditions and solvents for extracting polyphenols from Aronia pomace. Therefore, the aim of this study was to evaluate the efficiency of two commonly used solvents under different conditions (20°C and 60ºC) at a three different length of time (30, 60, 120 min) combined with different extraction methods (ultrasound, shaking).

Experimental

Materials and Methods

Aronia pomace were provided. Solvents were used acetone/distilled-water and methanol/distilled in 1:1 v/v and acidified with 1% formic acid.

Extraction was carried out by adding 30 mL of the solvent to ±1 g of the sample and thoroughly mixed. Some samples were extracted at room temperature (20ºC) and some at 60°C (in the water bath set at 60°C) in both methanol (50%) and acetone (50%). Both room temperature (20ºC) and 60°C samples were left to react for 20 min, 60 min and 120 minutes. After reaction time, samples were held for 15 minutes on the shaker, ultrasonic sound and on the bench (on stand) for 15 mins. This was then followed by centrifugation at 4000 rpm for 5 minutes, and the supernatant was collected and used for total anthocyanins (TA), total phenolic contents (TPC), total antioxidant capacity (FRAP) and colour parameters (L*, a*,b*).

The total anthocyanin concentration was assessed using the pH differential method[8]. The total phenolic contents were analyzed using the Folin-Ciocalteu method[9]. The antioxidant capacity was evaluated following the Ferric Reducing Ability of plasma (FRAP) assay [10].

Data analysis

The results obtained were statistically analyzed with IBM SPSS statistics software, version 27. The mean differences between factors were analyzed using the one-way analysis of variance (ANOVA) Post-hoc test (Turkey's). The significant difference between factors were determined at the interval level of $P<0.05$.

Results and discussion

The effect of time on the level of bioactive compounds

To determine the optimal extraction conditions of total anthocyanins, TPC, and antioxidants from aronia pomace, the effect of 50% methanol and 50% acetone at room temperature (20ºC) and 60ºC was evaluated at different extraction length in time (at 30, 60, and 120 minutes).

Total phenolic content: Figure 1A, indicates that the length of extraction time on samples extraction with both acetone (A20) and methanol (M20) at room temperature have no significant effects ($P<0.05$) on TPC content. However, a significant ($P>0,05$) increase of 3644 mg GA/mL (from 30 to 120 minutes) and 2945 mg GA/mL (from 30 to 120 minutes) on TPC content has been observed on samples extracted with Acetone (A60) and methanol (M60) at 60ºC, respectively. Figure 1A also indicates that acetone is more effective comparing to methanol.

Antioxidant capacity: Different extraction length in time have also been observed to have no significant effects ($P>0.05$) on the antioxidant capacity on samples extracted with both acetone (A20) and methanol (M20) at room temperature (Figure 1B). Similar to Figure 1A, a gradual increase on antioxidant capacity on samples extracted with acetone at 60ºC at 30 min, 60 min and 120 min has been observed with a significant increase ($P<0.05$) of 6013 µg AA/mL at 120 minutes from 30 minutes. Methanol extraction at 60°C has indicated no significant effects on antioxidant capacity on samples extracted at 30, 60 and 120 minutes, however the extraction of methanol at 60ºC is more effective comparing to methanol at room temperature. Studies that have looked at different durations of extraction[7] have also found similar results, where better yield of FRAP was obtained as the extraction duration increases.

Anthocyanins: In Figure 1C, 60 minutes of extraction has indicated better recovery of anthocyanins comparing to 30 and 120 minutes, on samples extracted with both acetone and ethanol. In general, anthocyanins has poor stability, factors like heat, pH and other environmental factors can affect the content of anthocyanin [11]. A significant decrease ($P<0.05$) of 243 mg/mL in anthocyanin content has been observed on sample extracted with acetone at

60ºC from 60 minutes (1032±199) to 120 minutes (789±135). This could be a degradation due to lengthy exposure of anthocyanins to high temperatures. Unlike in Figure 1A&B for TPC and FRAP; the results obtained in Figure 1C indicate extraction at room temperature for both acetone (A20) and methanol (M20) to have better recovery of anthocyanins comparing to extraction at 60º

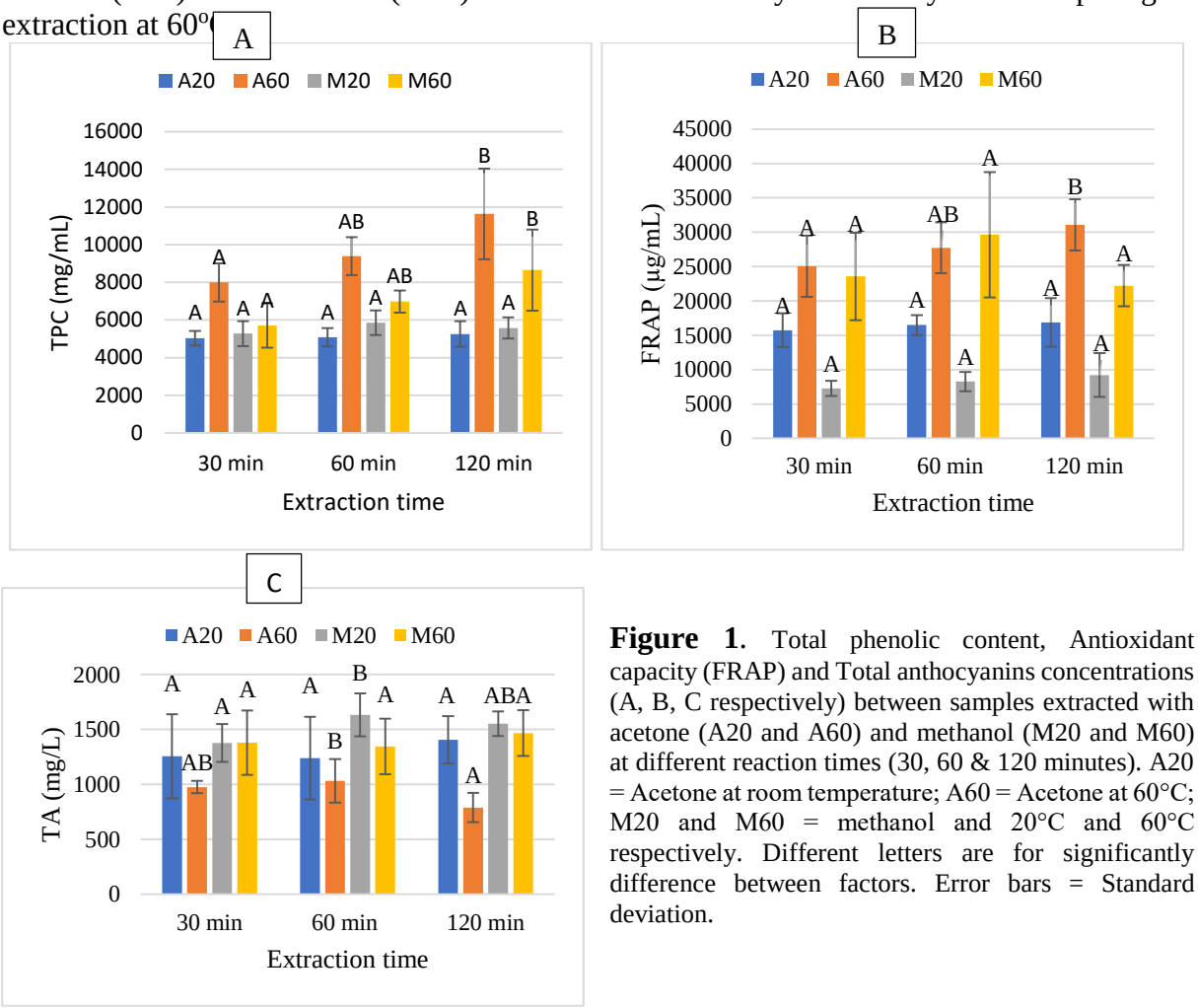

Figure 1. Total phenolic content, Antioxidant capacity (FRAP) and Total anthocyanins concentrations (A, B, C respectively) between samples extracted with acetone (A20 and A60) and methanol (M20 and M60) at different reaction times (30, 60 & 120 minutes). A20 = Acetone at room temperature; A60 = Acetone at 60°C; M20 and M60 = methanol and 20°C and 60°C respectively. Different letters are for significantly difference between factors. Error bars = Standard deviation.

The effect of a Shaker and Ultrasonic bath on the level of Anthocyanins, TPC and antioxidant

Total phenolic content: Figure 2A indicates the extraction with both acetone and methanol at 60ºC to be more effective comparing to extraction at room temperature. According to the results (Fig. 2A), the shaker has higher effects on TPC compared to room stands and ultrasonic sound for both acetone and methanol samples at 60ºC, with significantly higher recovery of 11012±263 mg/mL compared to 8113±896 mg/mL of room stands on acetone samples.

Antioxidant capacity: The results also indicate the extraction at 60ºC for both acetone (A60) and methanol (M60) to be more effective on antioxidant recovery compared to extraction at room temperature (A20 and M20) (Fig. 2B). The shaker has indicated better recovery of anthocyanins (31380±3701 µg/mL) on samples extracted with acetone, highly significant ($p<0.005$) compared to room stands (25292±2484 µg/mL). However, antioxidant capacity on samples extracted with methanol was recovered with the ultrasonic bath although not as high as antioxidants recovered with acetone on a shaker.

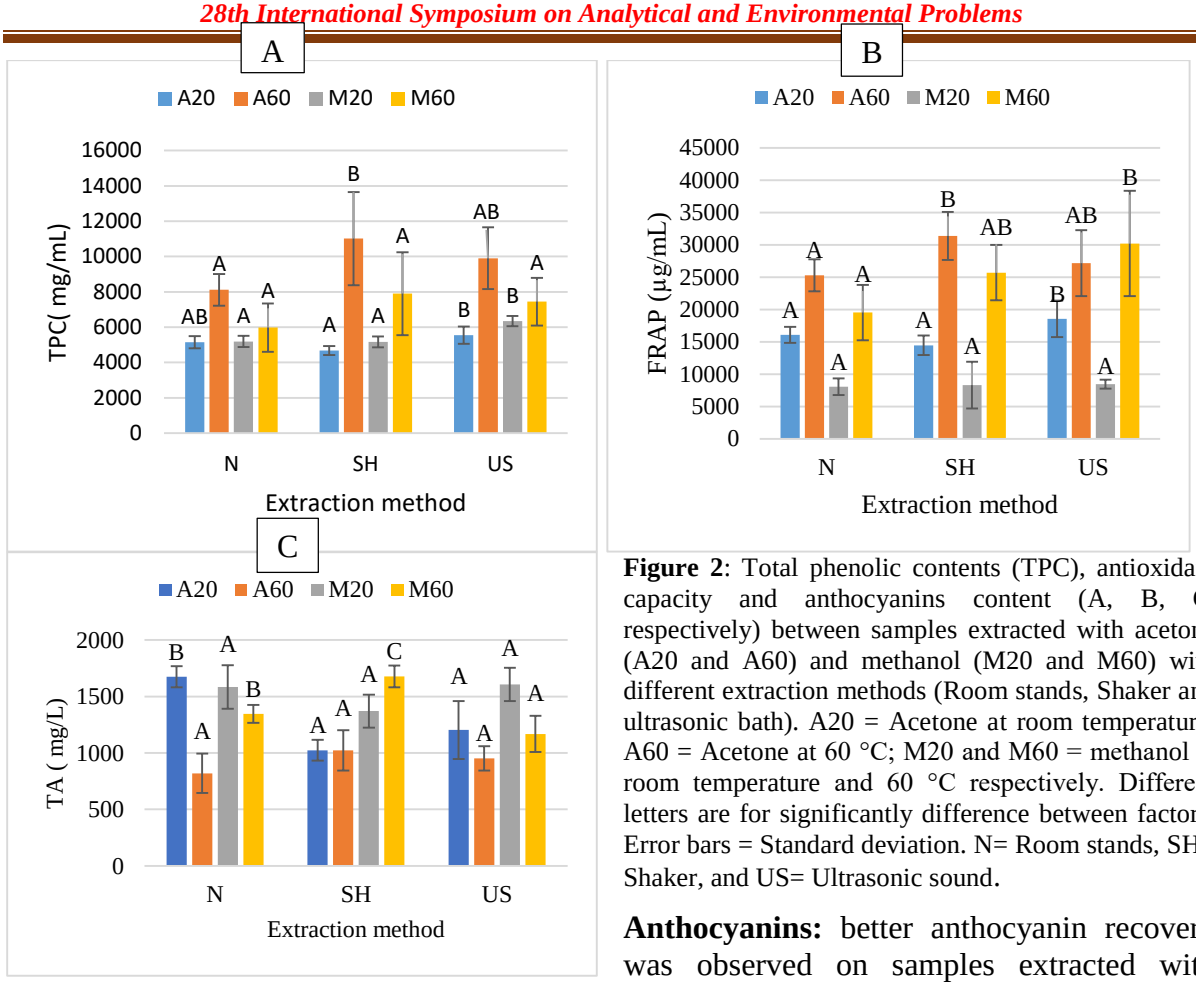

Figure 2: Total phenolic contents (TPC), antioxidant capacity and anthocyanins content (A, B, C, respectively) between samples extracted with acetone (A20 and A60) and methanol (M20 and M60) with different extraction methods (Room stands, Shaker and ultrasonic bath). A20 = Acetone at room temperature; A60 = Acetone at 60 °C; M20 and M60 = methanol at room temperature and 60 °C respectively. Different letters are for significantly difference between factors. Error bars = Standard deviation. N= Room stands, SH= Shaker, and US= Ultrasonic sound.

Anthocyanins: better anthocyanin recovery was observed on samples extracted with acetone (1674±93) and methanol (1584±193) at room temperature (A20 and M20) at room stands (N), as well samples extracted with methanol (M60) on a shaker (1678±96) and with methanol (M20) with ultrasonic bath (1607±148). Although in this case methanol at 60ºC (M60) on a shaker has yielded the highest anthocyanins, Acetone (A60) at room stand could be advisable since anthocyanin is not stable, and at room stands, there are no many factors such as heat.

Conclusion

Extraction of food components can sometimes be challenging due to the sensitivity if the compounds to be extracted. Therefore, selection of solvents and extraction conditions is very crucial for maximum concentration of polyphenols. In this study, two solvents were used at room temperature (20°C) and 60ºC at different extraction length in time and with different extraction methods. It has been concluded that room temperature extraction does not yield good results for TPC and antioxidant at all extraction length in time, however, it is suitable for anthocyanins. The optimal combination to yield high TPC and antioxidant capacity extraction would be extraction with acetone at 60ºC for 120 minutes and holding for 15 minutes on a shaker. Anthocyanins optimal conditions would be extraction with methanol at 60ºC for 60 minutes on a shaker. Alternatively, acetone at room temperature (A20) for 120 minutes at room temperature can also be ideal.

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