

Purification of the apple pomace polyphenol extract using adsorbent resin

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ABSTRACT

Polyphenols from agro-industrial waste particularly of fruit origin are a reliable source of antioxidants and antimicrobials that can be used as natural food additives. Organic solvents play an important role in extracting the polyphenols, however, inefficiency in exerting bioactivity and interference with the organoleptic properties are among the reasons that hinder their use as food additives. These problems can be alleviated by purification. In this study, the effect of resin types and elution solvent for purification of the apple pomace extracts on total phenolic content (TPC) and antioxidants were investigated. Crude ethanolic extracts were purified using amberlite resins (XAD7HP and FPX66) in a glass column (25 × 310 mm). The sorption flow rate was 2 Bed volume (BV) per hour, rinse 2 BV per hour, and desorption was 2 BV per hour. Final wash and regeneration were each done by 2 BV per hour. Polyphenol content and antioxidant capacity were quantified spectrophotometrically using Folin-Ciocalteu and Ferric reducing ability of plasma (FRAP) assays respectively. Polyphenol recovery was 50% in XAD7HP (Lowest) using ethanol and 69% in FPX66 (Highest) using acetone. For the case of FRAP recovery, 76% (Lowest) was observed in FPX66 using ethanol while 93% (Highest) was observed in XAD7HP using acetone. Conclusively, FPX66 is the ideal resin for the purification of apple pomace extracts for enhancing antioxidant activity compared to XAD7HP. Further, acetone seems to be a good desorption solvent compared to ethanol.

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KEYWORDS

plant extracts, amberlite resin, apple pomace, antioxidant, TPC and food additives

INTRODUCTION

In recent years, a great deal of interest has been observed in exploring the use of natural products such as plant extracts in developing food additives, cosmetics and pharmaceutically active agents. This is due to the presence of a high amount of bioactive compounds, including that of polyphenol origin. Polyphenols have a vital role as antioxidants, antimicrobial, antiviral, anticancer and anti-inflammatory activities among others (Chojnacka et al., 2021; Landis-Piowar et al., 2007; Raederstorff, 2009; Sun et al., 2021) hence becoming a potential prospect at solving food and medicine challenges. Exploration of cheap, reliable and sustainable sources of polyphenols harboring bioactive compounds has been on the rise (Romani et al., 2020). Among the promising polyphenol sources are pomaces. Pomaces are the waste products from the agroindustrial processing of fruits such as apples.

Apple pomace extracts have gained exciting interest from the scientific communities due to their wide range of bioactivities including antioxidant and antimicrobial properties. These extracts are showing promising use in food products as well as pharmaceutical compounds (Barreira et al., 2019; Carpes et al., 2021). Although various solvents, methods and techniques for extracting polyphenols from the apple pomace have been explored and widely documented, there is little information on the purification of the polyphenols from the extracts (Chemat et al., 2019; Iqbal et al., 2021). Provided that purification helps in getting rid of unwanted compounds that might otherwise negatively affect the organoleptic properties of food as well as affect visual appearance through color changes, purifying plant extracts could be an important means of advancement towards their use in food products (Alsobh et al., 2022).

The use of resins in purifying crude plant extracts is an important technique that can facilitate the exploitation of their biological properties in food and pharmaceuticals. In industrial settings, resins are used to purify and concentrate protein and pharmaceutically active compounds (Oliveira et al., 2015; Pérez-Larrán et al., 2018). There is ongoing research on the use of resins in purifying crude extracts from plant sources. For example, great results have been observed in purifying and preserving biological activities of grapes pomace and soybean extracts (Mariotti-Celis et al., 2018; Tran et al., 2022). In apple pomaces, a study by Kammerer et al. (2010), provides an important clue about the possibility of using resins to purify the extracts for industrial-scale use in food and pharmaceuticals.

In this study, the effect of resin type (Amberlite resins XAD7 HP and FPX66) and desorption solvent (Acetone and ethanol) were evaluated for their potential use in purifying crude extracts of the apple pomace. Their effect on the recovery of total phenolic content (TPC) and antioxidant capacity in terms of ferric reducing ability of plasma (FRAP) were evaluated.

MATERIALS AND METHODS

Extraction

Ultrasound-assisted extraction was used to obtain extracts from dried apple pomace as described by Murphy et al. (2020) with some modifications. Briefly, 20 g of the dried apple pomace was



mixed with 60 mL of 80% ethanol followed by sonication 20 kHz, 25 °C for 30 min. Thereafter, the extracts were centrifuged at 4,500 rpm for 5 min. The obtained supernatant was filtered using Whatman filter paper No.1 followed by rotary evaporation to remove the extraction solvent. The concentrated extracts were further dried at 60 °C to obtain complete dried extracts. The weight of the extracts was determined and the extracts were redissolved in the distilled water making a final concentration 200 mg mL⁻¹. The obtained extracts were stored at -20 °C until further analysis.

Resin purification

Purification of the crude extracts using two types of Amberlite resins (XAD7 HP and FPX66, from DuPont Company) was performed according to [Seif Zadeh and Zeppa \(2022\)](#) with some modifications. 20 g of each resin was activated by mixing with 40 mL of absolute ethanol under a shaker (20 rpm) for three hours. Thereafter, ethanol was removed through filtration and the resins were packed into a glass column (25 × 310 mm). Diluted crude extracts of apple pomace (5 mg mL⁻¹) were then loaded into the column and the sorption flow rate was set to 2 Bed volume per hour. Rinse and desorption flow rates were also set to be 2 Bed volume per hour. Distilled water was used as the rinse solvent while 96% ethanol or acetone was used as the desorption solvent. The amount of polyphenols (TPC) and ferric reducing ability of plasma (FRAP) in the desorption solvent were quantified.

Total phenolic content assay

Total phenolic content (TPC) was determined spectrophotometrically by the method of [Singleton and Rossi \(1965\)](#) with few modifications. Briefly, a small volume of sample (50–250 µL) was mixed with 1,250 µL of Folin reagent solution. If needed, the volume of the sample plus Folin reagent was brought to 1,500 µL using 80% methanol. The mixture was then allowed to stand for 1 min followed by adding 1,000 µL of sodium carbonate solution. Thereafter, the mixture was well shaken and incubated for 5 min at 50 °C in a water bath. The absorbance was read at 760 nm using a spectrophotometer (HITACH 2900). The obtained readings were converted into concentration; Garlic acid equivalent (GAE) using a standard calibration curve. TPC recovery was deduced using the equation below

$$\text{TPC recovery(\%)} = (\text{TPC in eluent} \div \text{TPC before purification}) \times 100$$

where TPC is total phenolic content, TPC in eluent is the amount of TPC (GAE) determined in the eluant solvent after passing the extracts in resin and TPC before purification is the amount of TPC (GAE) in extracts prior to subjecting the extracts to resin.

The ferric reducing ability of plasma assay

The antioxidant activity of the apple pomace was measured by the means of ferric reducing ability of plasma (FRAP) according to [Benzie and Strain \(1996\)](#) with some modifications. A small volume of sample (10–50 µL) was mixed with 1,500 µL of FRAP reagent. Distilled water (0–50 µL) was used to adjust the total volume to 1,550 µL if needed and the mixture was allowed to stand for 5 min in a room temperature. After that, absorbance was read at 593 nm in a spectrophotometer (HITACHI2900). The concentration of the extracts was given as Ascorbic



acid equivalent (AAE) using a standard calibration curve. The antioxidant recovery was given using the equation below

$$\text{FRAP recovery}(\%) = (\text{FRAP in eluent} \div \text{FRAP prior purification}) \times 100$$

where FRAP is the Ferric reducing ability of plasma, FRAP in eluent is the concentration of extracts (AAE) in the eluent solvent after passing through the resin. FRAP prior to purification is the concentration of extracts (AAE) in the crude extracts before subjecting the extracts to the resin.

RESULTS AND DISCUSSION

The effect of Resin type (Amberlite XAD7HP and Amberlite FPX66) as well as desorption solvent (Ethanol and Acetone) were investigated on the ability to recover the polyphenols from the extracts during purification. Results show that the lowest recovery was 50% observed on amberlite XAD7HP resin when ethanol was being used as a desorption solvent. The highest recovery was 69% observed on amberlite FPX66 resin using acetone (Fig. 1). Statistical analysis however indicated that there was no significant effect of both resin type $F(1, 8) = 1.50$, $P = 0.27$, and desorption solvent $F(1, 8) = 1.18$, $P = 0.31$ on TPC recovery. The interaction of the resin type and desorption solvent did not have a significant effect on the TPC recovery

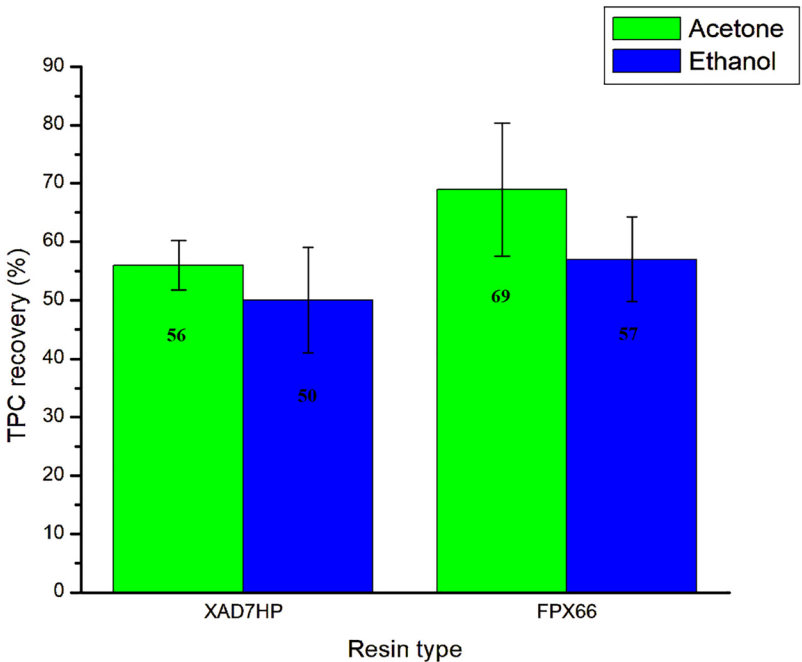


Fig. 1. The effect of resin type and desorption solvent on TPC recovery of the apple pomace extracts. Bars represent the mean value of triplicate reading. Error bars represent standard error



either, $F(1, 8) = 0.13$, $P = 0.73$. In a study by [Monsanto et al. \(2015\)](#), on the effectiveness of resins amberlite XAD7 HP and FPX66 on the recovery of useful polyphenols from black tea, they concluded by recommending FPX66 to be the optimal resin for recovery of catechin with the ability to recover 59% of the aflavins. [Ostrihoňová et al. \(2023\)](#) studied the ability of 17 different types of industrial resin including the amberlite XAD 7HP and FPX66. Their study indicated that all of them had good performance however FPX68 which is closely related to FPX66 had the best performance among all. They concluded that particle porosity, pore size distribution, surface morphology and functionalized components such as sulphonyl groups are among the most important parameters for resin functioning.

[Figure 2](#) displays the results of the effect of resin type and desorption solvent on the recovery of the antioxidant capacity of the purified extracts. The highest antioxidant recovery 93% was observed on the resin Amberlite XAD7 HP using acetone as a desorption solvent. The lowest recovery 76% was observed on FPX66 when ethanol was used as a desorption solvent. Similar to TPC recovery, statistical analysis indicated that there was no significant effect of the resin type $F(1, 8) = 0.10$, $P = 0.77$ and solvent type $F(1, 8) = 4.16$, $P = 0.08$. Moreover, the interaction between resin type and desorption solvent did not have a significant effect on the recovery of the polyphenols $F(1, 8) = 0.09$, $P = 0.77$. A study by [Green et al. \(2022\)](#) investigating four types of resin on the recovery of antioxidants from the fruit non-palatable fruit juice of *Aronia mitschurinii*, showed that FPX66 resin which displayed the best results managed to recover 40% of the anthocyanins responsible for antioxidant activity. Similarly, for the case of total flavonoids,

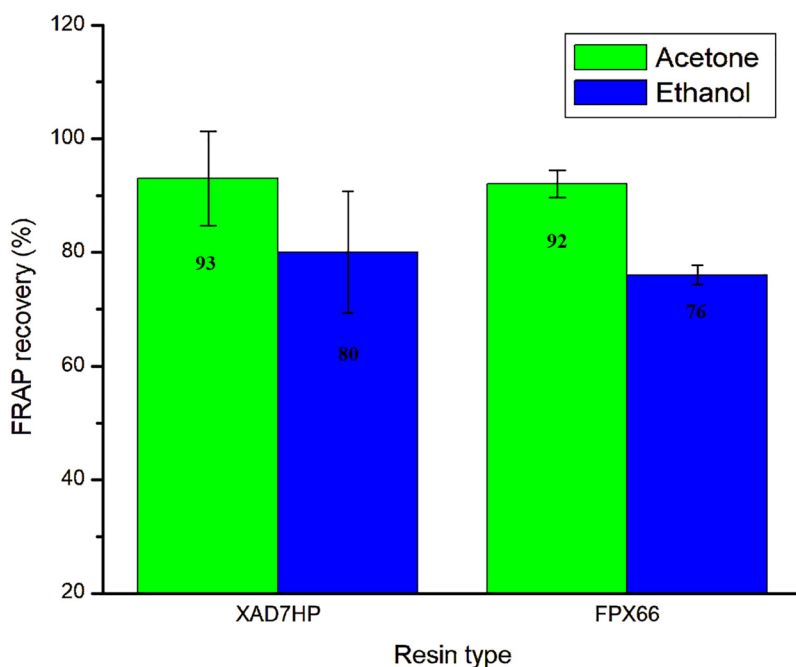


Fig. 2. The effect of resin type and desorption solvent on antioxidant recovery of the apple pomace extracts. Bars represent the mean value of triplicate reading. Error bars represent standard error



FPX66 recorded the highest with a recovery of 45.5%. For the case of polyphenols, FPX66 was also the best resin with a recovery percentage of 33%. In their study, acidified ethanol was used as a desorption solvent. Results from this study support a wide range of literature where it has been shown that resin FPX66 is a good choice for the recovery of polyphenols from various sources (Green et al., 2022; Monsanto et al., 2015; Yangui et al., 2017).

Several studies suggest that ethanol is widely used as a desorption solvent during resin purification of polyphenols and other high-value bioactive compounds (Green et al., 2022; Mariotti-Celis et al., 2018; Monsanto et al., 2015; Pérez-Larrán et al., 2018; Tran et al., 2022; Yangui et al., 2017). Little is known about the use of acetone as an effective solvent for the purification of extracts from plant sources. This study, however, demonstrates that, although there was no significant difference attributed to solvent in the recovery of the polyphenols as well as antioxidant ability, in all cases, acetone had slightly higher performance than ethanol (Figs 1 and 2). This suggests that acetone could be a good desorption solvent for purifying apple pomace extracts similar to or more than ethanol.

CONCLUSION

The search and subsequent use of natural food additives have gained much interest in the food science communities due to interests of consumers' concern about the safety and well-being of the commonly used synthetic food additives. Despite being a reliable source of natural food additives, the use of polyphenols including that of apple pomace is limited by the purification and concentration of the useful bioactive compounds. In the present study, among the investigated resins and desorption solvents, it was found that resin FPX66 exerts good recovery of total phenolic content as well as antioxidant activity. Furthermore, acetone showed great potential in the recovery of both total phenolic content and antioxidant activity when used as a desorption solvent. These results demonstrate that the use of resins particularly FPX66 and acetone can be reliable in the recovery and concentration of polyphenols from crude extracts of the apple pomace for the development of natural food additives.

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