Extraction of anthocyanins from *Aronia melanocarpa* skin waste as a sustainable source of natural colorants

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ABSTRACT
Black chokeberry (*Aronia melanocarpa*) contains anthocyanins in high concentrations and has a simpler anthocyanin profile compared to other anthocyanin-containing berries.[1] Anthocyanin content in *A. melanocarpa* skin is found to be higher than in the juice, which is the plant's strategy to protect the seeds from the UV radiation and harmful insects.[2] Anthocyanins display different colours in various ranges of pH, however, their instability towards light, heat, oxygen, pH, and hydrolysis limits their application as natural colorants. The raw material used in this study is waste *A. melanocarpa* skins generated as a by-product following pressing of the berries for the production of *Aronia* fruit juice, hence, this raw material has significant potential as a sustainability and renewable resource.

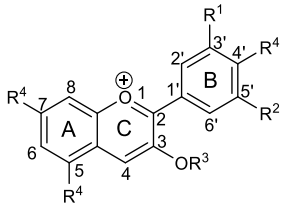

Figure 1. The general structure of anthocyanins, R^1, R^2 = H, OH, or OCH_3; R^3 = glycosyl or H; R^4 = OH or O-glycosyl.[3]

In this study, *A. melanocarpa* skins were extracted, isolated, characterised, and also chemically modified to improve stability. The batch extraction method was compared with a new proposed method, namely an integrated extraction-adsorption method. This new method was developed to extract and selectively recover anthocyanins from *A. melanocarpa* skin in a single run.[4] Anthocyanins extracted from *A. melanocarpa* skin were then characterised by UV-Vis, HPLC, HRMS and ^{1}H-NMR. Chemical modification of anthocyanins extracted from *A. melanocarpa* skins was a chemical esterification by reacting with an acylating reagent to make them more lipophilic.

To obtain the better recovery of anthocyanins, the process was initially optimised using a batch method. The best conditions used for the batch method are as follows: extraction temperature of 60 °C, extraction time of 3 h, acid additive (0.1% v/v HCl), biomass-solvent ratio of 1:16 and biomass-SPE resin ratio of 1:1. A new proposed method was investigated to obtain higher anthocyanin yields. Higher anthocyanin yields are obtained when the process was performed for 3 h without cooling and the flow rate was 1.3 mL s^{-1}. Overall, a new proposed method showed a better anthocyanin yield and purity

[1] L. Jakobek, M. Šeruga, M. Medvidović-Kosanović and I. Novak, *Dtsch. Leb.*, 2007, **103**, 58–64.
[2] J. Oszmianski and A. Wojdylo, *Eur. Food Res. Technol.*, 2005, **221**, 809–813.
[3] J. M. Kong, L. S. Chia, N. K. Goh, T. F. Chia and R. Brouillard, *Phytochemistry*, 2003, **64**, 923–933.
[4] L. Galván D'Alessandro, P. Vauchel, R. Przybylski, G. Chataigné, I. Nikov and K. Dimitrov, *Sep. Purif. Technol.*, 2013, **120**, 92–101.

(0.91% dry weight) compared to a batch method (0.52% dry weight). This method also simplified the process as three steps were eliminated during the process which eventually save more time and energy. Furthermore, an integrated extraction-adsorption method is an industrial scalable which potentially will be applied to produce a large quantity of anthocyanins. Anthocyanins present in *A. melanocarpa* skins were identified as cyanidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-xyloside and the cyanidin aglycon; cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinoside were the major components. This is a particularly interesting observation as only one anthocyanin parent structure (cyanidin) and only monosaccharide glycosides were identified in the fruit, which is not typical compared to other berries which have a wider range of anthocyanins and/or more diverse glycosylation.

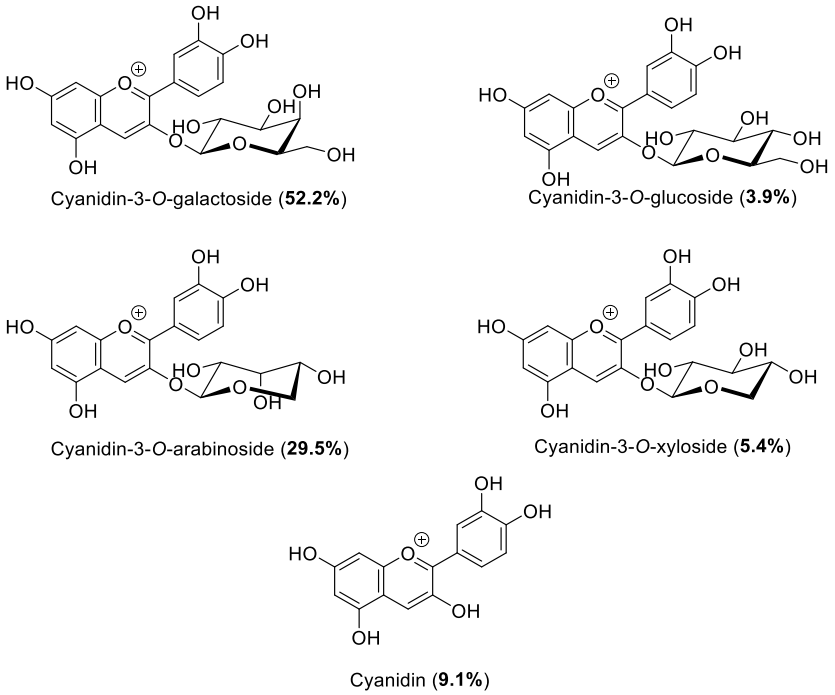

Figure 2. Anthocyanin glycosides and aglycon identified in *A. melanocarpa* skin.

Esterification of anthocyanins lead to the formation of the heptaacetate derivative of cyanidin-3-*O*-galactoside and the hexaacetate derivative of cyanidin-3-*O*-arabinoside. This is the first time the chemical modification of anthocyanins extracted from *A. melanocarpa* waste skins through a simple esterification is presented. These structural transformations of anthocyanins to be more lipophilic derivatives have the main advantage of improving the technological application potential in lipophilic systems such as polyester-based textile and cosmetic formulations.

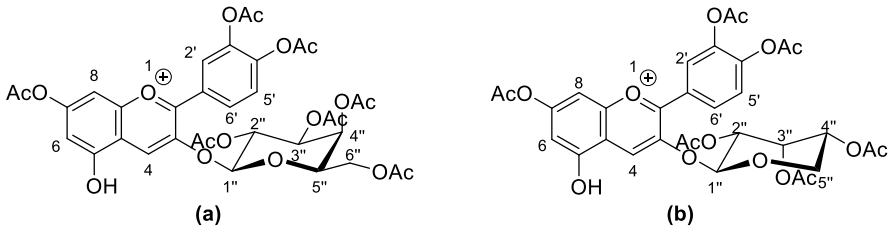

Figure 3. Proposed structure of ester derivatives of (a) cyanidin-3-*O*-galactoside and (b) cyanidin-3-*O*-arabinoside.