POLYPHENOL RICH EXTRACTS FROM BERRY PRESS RESIDUES OF *VACCINIUM* SPECIES, CHARACTERIZATION OF CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY

Author: Linards Klavins
University of Latvia, Faculty of Biology, Jelgavas street 1, LV-1004, Riga, Latvia

Abstract:
Berries of Northern bogs and forests (*Vaccinium* spp.) contain significant quantities of various phenolic compounds. Most of these compounds are recovered when berry juice is produced. However, a considerable part of polyphenols remain in berry press residues and are discarded as food industry waste. The aim of the study was to compare the methods of extraction of polyphenols from press residues of American cranberry and optimize the extraction conditions. The impact of main extraction parameters (e.g., extraction time, solid/solvent ratio, solvent type) on the yield of extracted polyphenols was examined. Ultrasound-assisted extraction showed the highest potential from all studied methods, given its fast, convenient use and low cost. Response Surface Methodology (RSM) was used to identify the optimal solvent composition. Antimicrobial activity of extracts was evauated by the Agar Difussion method, which showed a potential use of extracts to inhibit growth of human pathogens. Proliferation of dermal fibrobalsts was inhibited, and cell cytometry showed high antiradical activity *in vitro.*

Materials and Methods:

- Berry sample material – Lyophilized American cranberry (*Vaccinium macrocarpon* L.) for the optimization of extraction. Highbush blueberry (*Vaccinium corymbosum* L.), bilberry (*Vaccinium myrtillus* L.), lingonberry (*Vaccinium vitis-idaea* L.) ang bog cranberry (*Vaccinium oxycoccos* L.) press residues for the validation of RSM optimized extraction conditions (Figure 2.C.)
- Response surface methodology – to achieve optimum response from the used independent variables (solvent and acid concentration).
- Quantitative methods – Folin-Ciocaulteu (total polyphenols), total carbohydrates, pH differential method (total anthocyanins), UPLC-PDA, Orbitrap-MS
- Selection of solvent– identifying most efficient solvent for phenolic extraction.
- Choice of acid – considering the application and further processing of extracts.
- Extraction methods – Choice of the best extraction method (Soxhlet, microwave, ultrasound assisted extraction, extraction with critical CO_2.
- Microbiological methods – Agar difussion method, determination of MIC and MIB.
- Human cell cultures – Cell cytometry, cell proliferation tests of dermal fibroblasts.

Results and Discussion:

- Most efficient solvents identified – Methanol, ethanol and acetonitrile (Table 1) give the highest polyphenol/anthocyanin yields, the first two were chosen for further optimisation considering their use in food industry.
- Acid for assisting extraction – TFA gives higher yields of anthocyanins; HCl gives higher yields of total polyphenols, however HCl is less preferable as it can contribute to the hydrolysis of anthocyanins.
- Extraction method – 15-25 minutes of ultrasound assisted extraction at various powers with solid:solvent ratio 1:90 for methanol and 1:30 for ethanol. Soxhlet and microwave assisted extraction are least favourable methods.
- Content of polyphenols– 15 anthocyanins and 150 other polyphenols identified in 5 studied berries allowing to perform chemotaxonomic analysis (Figure 1 ;3B).
- Analysis of Response Surfaces – Ethanol and methanol together with formic acid or TFA was tested and the response, anthocyanins and polyphenols, was observed. For the extraction of anthocyanins and polyphenols different optimal conditions were found (Figure 2A and B). Optimal conditions summarized in Figure 2C.
- Validation of optimal conditions – to test the robustness of the identified optimal conditions, extractions using the optimal conditions and press residues/whole, dried berries of 5 *Vaccinium* spp. were performed (Figure 4). Press residues were identified as a material rich in compounds with biological activity (Figure 4).
- Antimicrobial activity – Activity against various human pathogens at concentrations ranging from 0.07 mg/mL to 1.9 mg/mL (Figure 3A).
- Antiproliferative activity –Prepared extracts show antiproliferative acitivity in dermal fibroblasts as well as the ability to effectively scavenge free radicals (Table 2)

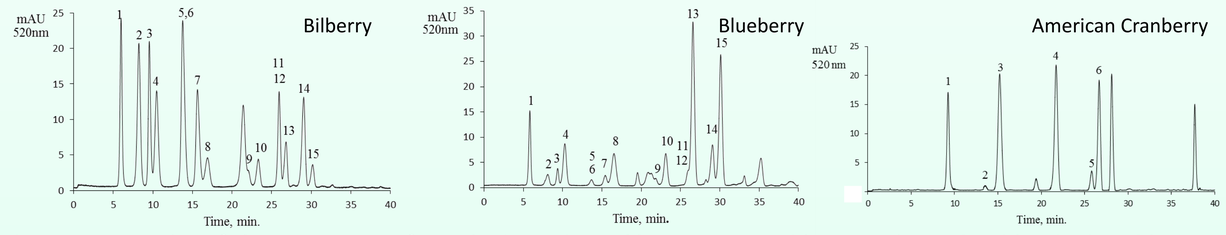


Figure 1. Examples of chromatograms from the investigated berry press residues. Peak number identity in Table 3.

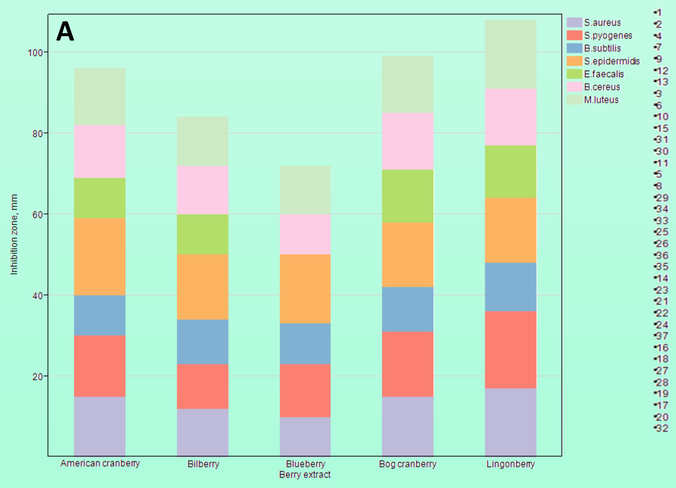
Solvent	Dry residue	Carbohydrates	Anthocyanins	Polyphenols
Acetonitrile 49.5%, TFA 0.5%	37.24 ± 1.53	7.82 ± 0.27*	0.228 ± 0.006	3.84 ± 0.12*
Acetone 50%	34.29 ± 1.41	12.17 ± 0.43	0.151 ± 0.004	2.70 ± 0.08
Acetone 75%	36.01 ± 1.48	18.52 ± 0.65*	0.156 ± 0.004	2.69 ± 0.08
Methanol 60%, acetone 30%	37.94 ± 1.56	16.86 ± 0.59	0.184 ± 0.005	2.34 ± 0.07
Methanol, HCl 1%	48.38 ± 1.98*	17.93 ± 0.63	0.451 ± 0.011*	4.80 ± 0.14*
Water, HCl 1%	16.91 ± 0.69*	14.82 ± 0.52	0.098 ± 0.002*	0.89 ± 0.03*
Ethanol 70%, HCl 1%	39.62 ± 1.59	16.85 ± 0.51	0.204 ± 0.005	3.43 ± 0.09

Table 1. Comparison of various solvents used for phenolic extraction. All values as g/100g berry press residues

Sample	Events	Value (MFI)	Antiradical activity
Solvent	4542	590,87*	Low
Vitamin-C	7045	403,15	Antioxidant level
Blueberry	8384	207,21*	Very good
Bilberry	5466	203,51*	Very good
Bog Bilberry	3376	283,87*	Good
American Cranberry	3010	159,63*	Very good
Lingonberry	2871	296,93*	Good

Table 2.Measured antiradical activity in vitro for five berry polyphenol extracts using cell cytometry. Value (MFI) of vitamin-C compared to the observed values of berry extracts. Asterisk (*) represents signifficant difference when ompared with Vitamin C.

C	Solv., v/v. %	Acid, v/v %
Anthocyanins	MeOH, 97	TFA, 0,3
	EtOH, 40	TFA, 1,0
Polyphenols	MeOH, 70	TFA, 1,0
	EtOH, 57	TFA, 0,9



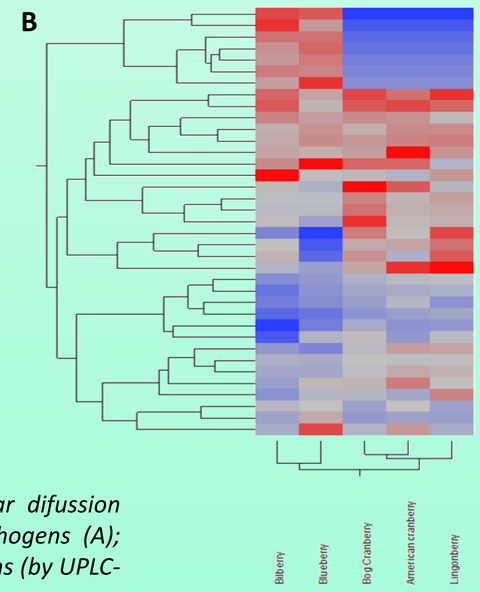


Figure 3. Observed antimicrobial inhibition zones using agar difussion assay, prepared extracts were testd on various human pathogens (A); Cluster analysis of the identified and quantified 15 anthocyanins (by UPLC-PDA) and 24 other polyphenols (by Orbitrap-MS) (B)

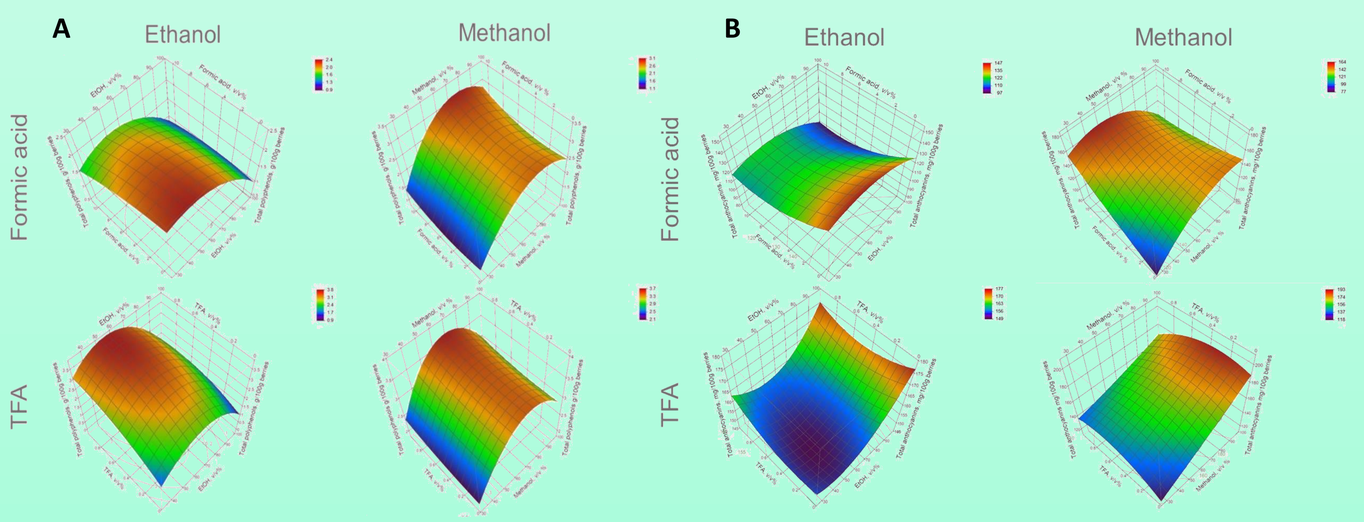


Figure 2. Response surfaces showing the effect on (A) total polyphenol and (B) total anthocyanin content depending on the solvent/acid concentrations. (C) Optimal extraction conditions for anthocyanins and polyphenols.

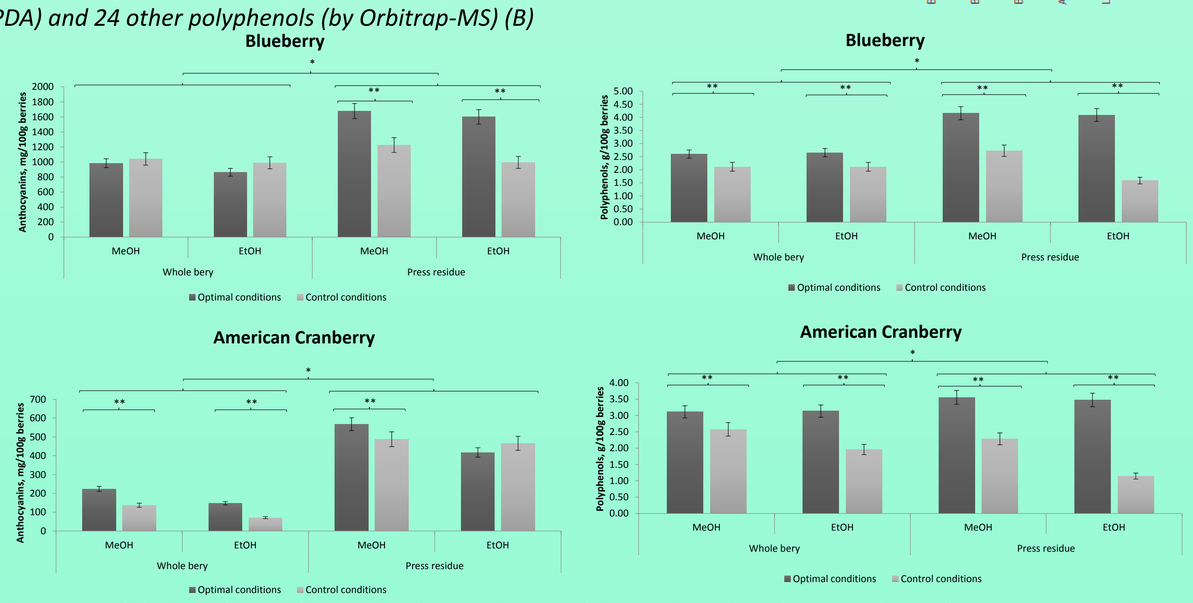


Figure 4. Validation of identified optimal extraction conditions with ethanol and methanol for various Vaccinium spp. berries (whole berries and press residues).

Conclusions: This study successfully identified the most efficient conditions for polyphenol and anthocyanin extractions, namely, 15-25 minutes of ultrasound assisted extraction using methanol and TFA as the extraction solvent with the solid/solvent ratio 1:90. It was also shown that the identified 15 anthocyanins don't differ in whole berries and dried press residues. The used RSM approach proved to be a useful tool in identifying and providing optimal extraction conditions that can be applied for various sample types. Performed validation experiments revealed the value of berry press residues containing high levels of polyphenols as a possible source of valuable polyphenols, thus pinpointing a possible use of this product in nutraceuticals. The purified polyphenol extracts contained up to 15 different anthocyanins and 150 other polyphenols of which 37 were quantified and used for chemotaxonomic analysis. Polyphenol extracts showed antimicrobial activity against various human pathogens. Proliferation of dermal fibroblasts is interrupted after applying the prepared extracts while the free radicals within the cell are effectively scavenged.

Acknowledgements: This work was supported by the European Regional Development Fund within the project No. 1.1.1.1/16/A/047 "Genus Vaccinium berry processing using 'green' technologies and innovative, pharmacologically characterized biopharmaceutical products".

BerriesPro