ANTIOXIDANT AND ANTIHYPERGLYCAEMIC ACTIVITY OF FIVE *VACCINIUM* SPP. BERRY POMACE EXTRACTS

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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. Berry pomace extracts showed strong antioxidant activity and efficacy to inhibit activities of enzymes involved in carbohydrate digestion and to protect hepatic cells from tert-Butyl hydroperoxide caused oxidative damage at non-toxic concentrations.

Results

Most efficient solvents identified

Methanol, ethanol and acetonitrile provide the highest polyphenol/anthocyanin yields from berry press residues, the first two were chosen for further optimisation.

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

Response surface

Ethanol and methanol acidifed with formic acid/TFA was tested and the response (anthocyanin and polyphenol yield) was observed (Figure 1). For the extraction of anthocyanins and polyphenols different optimal conditions were found. Optimal conditions for both observed responses are summarized in Figure 2B.

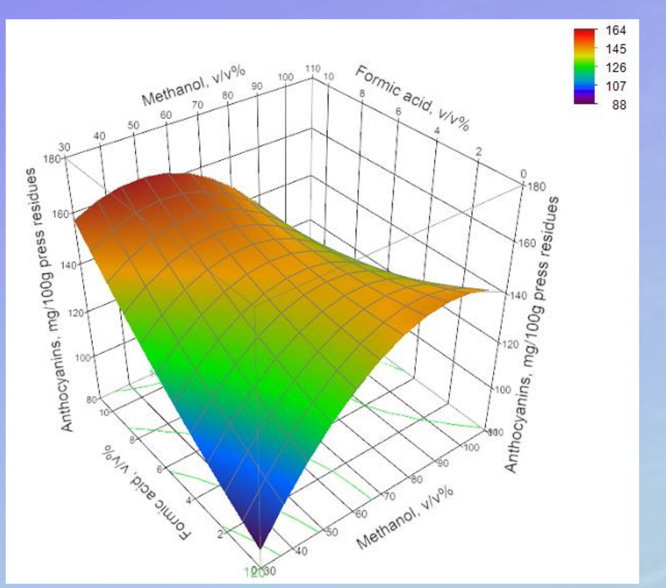


Figure 1. An example of the prepared Response Surface where the concentration of methanol and formic acid is plotted as a function of anthocyanin extraction yield. n=15, central composite design.

Optimal Conditions

Robustness of the identified optimal conditions was determined, extractions using the optimal conditions and press residues or whole, dried berries of 5 *Vaccinium* spp. were performed. Press residues contained more compounds with biological activity (Figure 2A).

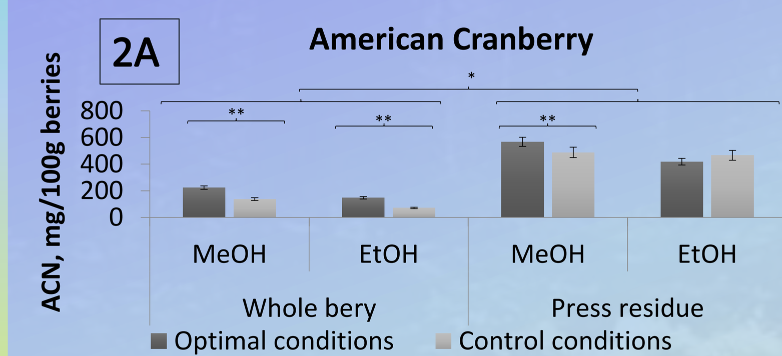


Figure 2. Comparison of extraction at optimal conditions vs. control conditions for the extraction of American cranberry press residues and whole, dried berries (2A). Optimal conditions for extraction of polyphenols and anthocyanins determined by RSM(2B).

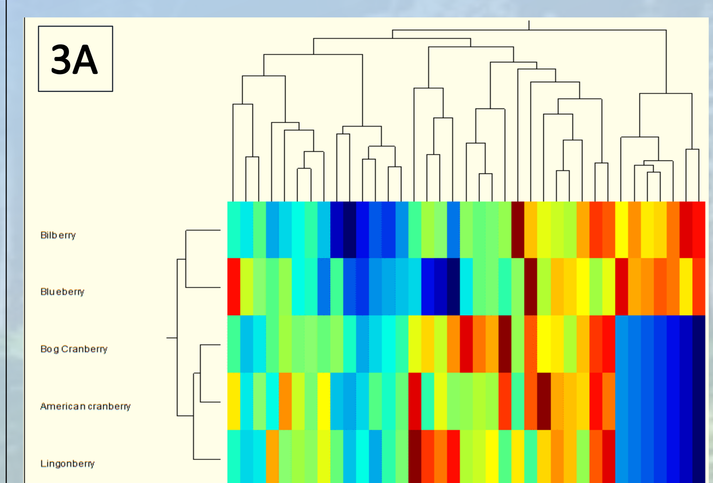
2B

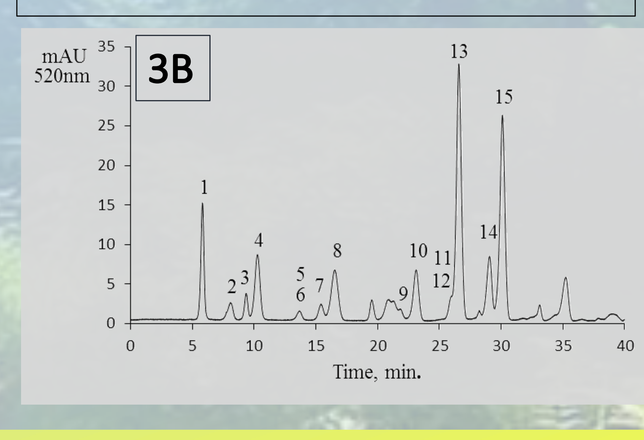
	Solv., v/v. %	Acid, v/v %
Anthocy.	MeOH, 97	TFA, 0,3
	EtOH, 40	TFA, 1,0
Polyph.	MeOH, 70	TFA, 1,0
	EtOH, 57	TFA, 0,9

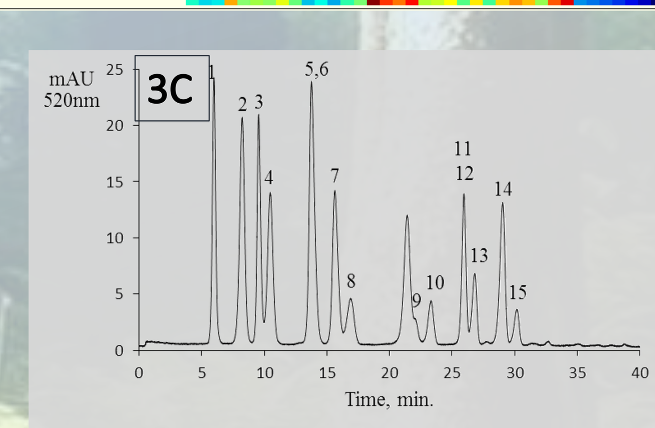
Content of polyphenols

15 anthocyanins and 150 other polyphenols identified in 5 studied berries allowing to perform chemotaxonomic analysis.

Figure 3. Chemotaxonomic analysis of *Vaccinium* spp. where the quantitative results of HPLC and ORBITRAP-MS analysis were used, in total 32 polyphenols were quantified (**3A**). Examples of Bilberry (**3B**) and Blueberry (**3C**) chromatograms at 520 nm, where the numbers 1-15 represent various anthocyanins found in these berries.







Antioxidative and enzyme activity

Berry extracts	TAC AAE µg/mg	DPPH IC50 µg/ml	SOD IC50 µg/ml
Bilberry	385 ± 14	230 ± 21$^{\#}$	16 ± 3*
Blueberry	221 ± 15	235 ± 24$^{\#}$	120 ± 14*
Bog cranberry	465 ± 16	120 ± 14	32 ± 8*
American cranberry	224 ± 22	125 ± 20	130 ± 23*
Lingonberry	317 ± 20	150 ± 19$^{\#}$	114 ± 10*
Ascorbic acid		94 ± 18	
SOD			1.15 ± 0.11

Table 1. Total antioxidant capacity (TAC), DPPH scavenging effects and SOD-like activity of *Vaccinium* spp. berry pomace extracts. *P ≤ 0.05 vs. SOD, $^{\#}$P≤ 0.05 vs. ascorbic acid.

Obtained data show that bog cranberry extract possesses the highest TAC, following with bilberry, lingonberry, American cranberry and blueberry extracts. TAC includes all antioxidant and the synergistic effects between extract components. Scavenging action was assessed as DPPH reducing effect compared ascorbic acid. Scavenging activity of cranberry and American cranberry extracts didn't differ from that of ascorbic acid. Cranberry extracts showed superior free radical scavenging activities over other extracts. The strongest SOD-like activity was found for the bilberry extract. Cranberry extract showed two times less activity (Tabel 1).

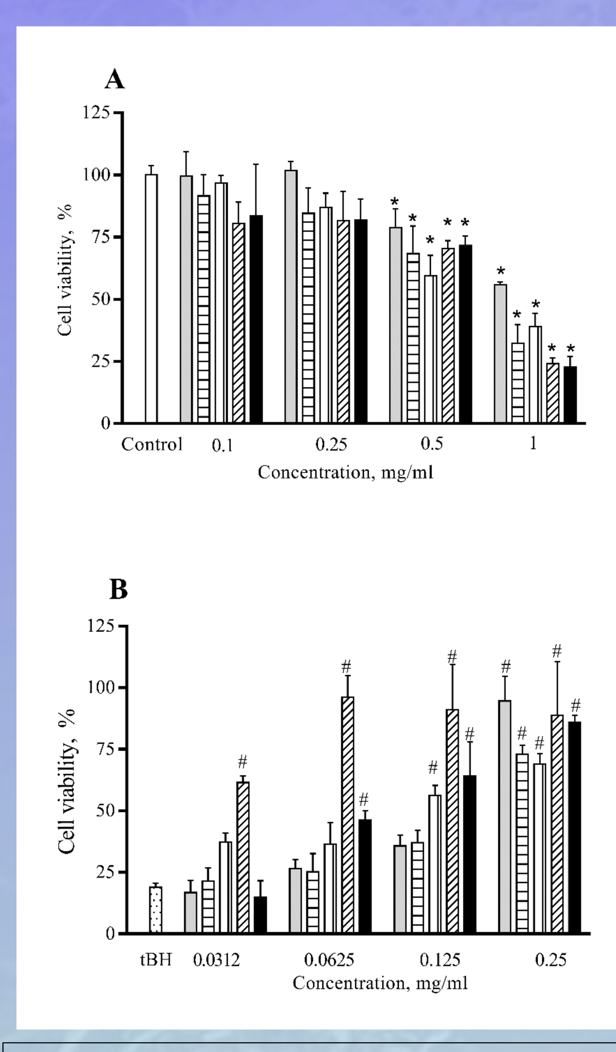


Figure 4. Effects of *Vaccinium* spp. berry extracts on HepG2 cell viability after 4 h treatment (A) and after 1 h pre-treatment before following 2.5 h exposure to 0.5 mM tBH (B). Data represent the mean ± S.D. of at least three independent experiments. * P ≤0.05 vs. control, $^{\#}$ P ≤0.05 vs. tBH, ANOVA followed by Dunnett's Multiple Comparison test.

Extract	α-amylase, IC50 µg/ml	α-glucosidase, IC50 µg/ml
Bilberry	430 ± 26*	7.7 ± 0.24*
Blueberry	550 ± 39*	35 ± 2.4*
Bog cranberry	340 ± 40*	7.0 ± 0.7*
American cranberry	510 ± 39*	10 ± 0.4*
Lingonberry	380 ± 22*	16 ± 1.1*
Acarbose	820 ± 52	125± 27

Table 3. Inhibition of α-amylase and α-glucosidase activity. Berry pomace extracts exerted inhibitory effect on α-amylase and α-glucosidase activity with preferable inhibition of α-glucosidase activity.

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 compared to the MFI vlaue of Vitamin C. The lower the MFI value, the more effective radical scavenging properties are observed. All the observations were done using the extracts at 0.025 mg/mL on 24h old cell culture.

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. 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. Our results support use of berry pomace extracts for design of standardized formulation which in the future could be used for the prevention of chronic diseases associated with oxidative stress. Hepatoprotective and antidiabetic activity of the extracts might be justified by the presence of active polyphenols or their potential synergistic effect.

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