# POLYPHENOL EXTRACTS FROM BERRY PRESS RESIDUES: CHARACTERIZATION OF CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY



Abstract

Authors: Linards Klavins\*, Jorens Kviesis, Vizma Nikolajeva, Martins Boroduskis, Maris Klavins Correspondence: linards.klavins@lu.lv

# **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	$0.151 \pm 0.004$	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%	<u>48.38 ± 1.98*</u>	<u>17.93 ± 0.63</u>	<u>0.451 ± 0.011*</u>	<u>4.80 ± 0.14*</u>
Water, HCI 1%	16.91 ± 0.69*	14.82 ± 0.52	0.098 ± 0.002*	0.89 ± 0.03*
Ethanol 70%, HCl 1%	<u>39.62 ± 1.59</u>	<u>16.85 ± 0.51</u>	<u>0.204 ± 0.005</u>	<u>3.43 ± 0.09</u>

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*.

# **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

## **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).



Solv., v/v.

MeOH, 97

# **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.







extraction yield. n=15, central composite design.



Acid, v/v

TFA, 0,3





## **Biological activity of the obtained extracts**

Antimicrobial effect was observed by using the Agar-Diffusion Method. Highest antimicrobial activity was observed for the red-coloured berries. Bactericidal activity against various human pathogens at concentrations ranging from 0.07 to 1.9 mg/mL was observed by measuring the bacterial growth (Figure 4).



# Figure 6.

Obtained optimal berry extracts were further processed to remove any unneccessary substances like sugars and organic acids, that could influence the growth of bacteria or human cells. Extracts were purified using Amberlite XAD7-HP sorbent, the purified extracts was the rotary-evaporated and

freeze dried to obtain a fine, water-soluble berry polyphenol powder.

#### Figure 4.

Observed antimicrobial inhibition zones using agar difussion assay, prepared extracts were tested on various human pathogens. Bilberry and blueberry, berries that contain high amounts of anthocyanins showed a lesser inhibitory effect than the redcoloured cranberries and lingonberries.

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. 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 (Figure 6). 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 (Table 2).

BerriesPro

#### Figure 5.

*Prepared* berry polyphenolic concentrate effect on the proliferation of dermal fibroblasts. S10 medium used as a control without added extract or solvent. Error bars represent standard deviation. All used extracts were prepared in the concentration of 20 mg/mL and diluted in S10 medium in the concentrations shown in the figure. All 5 of the tested berry polyphenol extracts show similar effect in cell culture- in concentrations as low as 0.0125 mg/mL cell proliferation is inhibited.



<u>Acknowledgements</u>: 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".



\* European Regional Development Fund

EUROPEAN UNION

INVESTING IN YOUR FUTURE