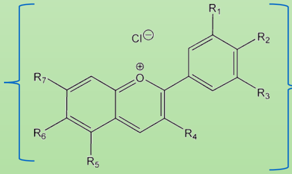
Berry press residues as a valuable source of polyphenolics: extraction optimisation and analysis

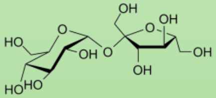
Maris Klavins, Agnese Kukela, Linards Klavins, Jorens Kviesis

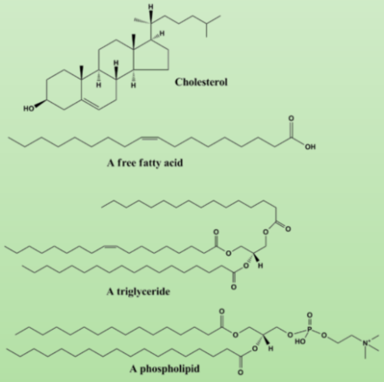
Application possibilities of fruits and berries

G.Arcimboldo Louvre

Berry pomace or press residues







Berry press residues as a source of valuable compounds

- Berry press residues (pomace) often is a waste material
- Berry press residues can serve as a source of many biologically active substances with diverse fields of applications
- One of most prospective groups of substances - polyphenolics

Study object – *Vaccinium* berries

Vaccinium myrtillus L	Blueberry	
Vaccinium uliginosum L	Bog bilberry	
Vaccinium corymbosum L	Highbush blueberry	
Vaccinium vitis-idaea L	Lingonberry	
Vaccinium oxycoccos L	Bog cranberry	
Vaccinium macrocarpon	Large cranberry	

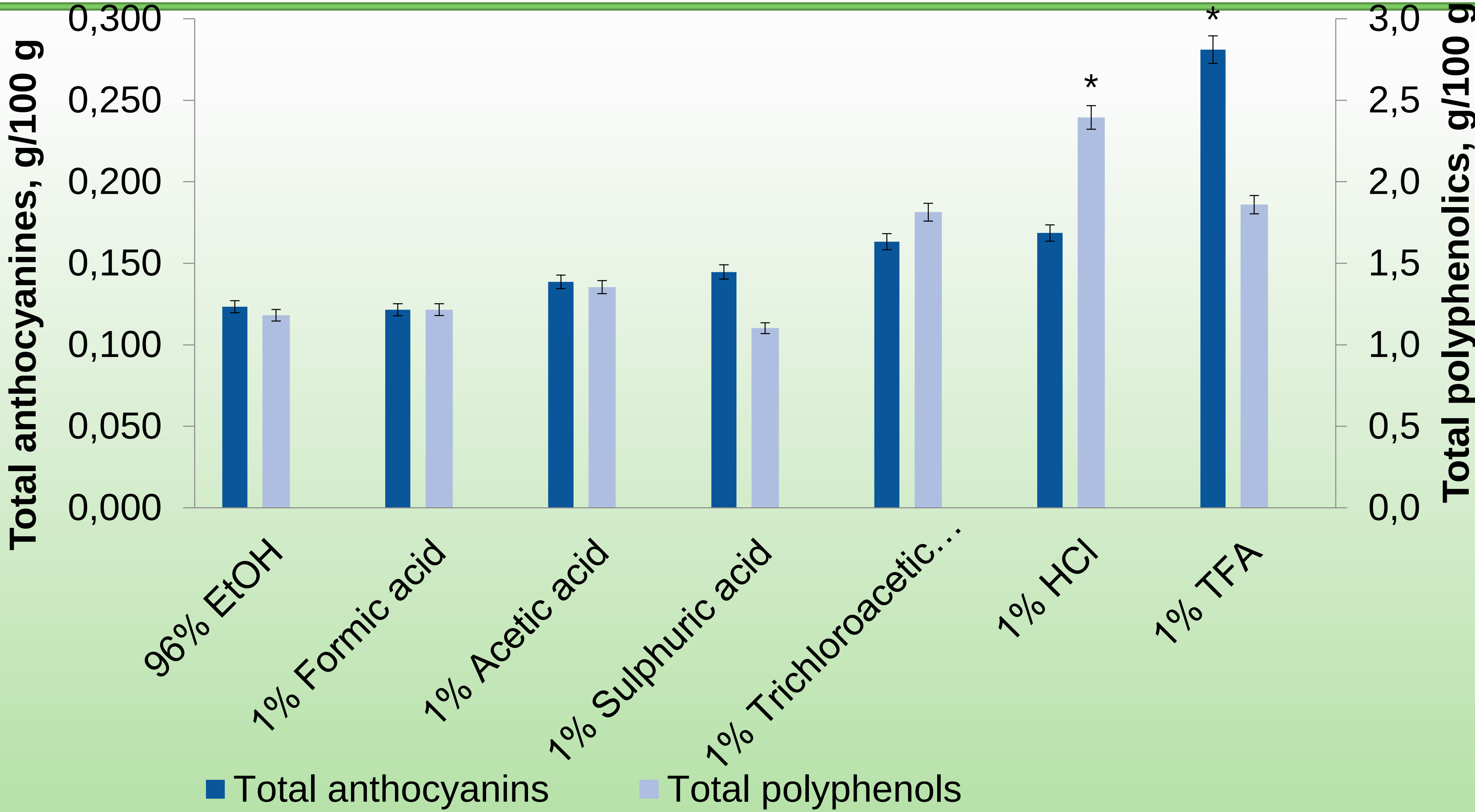
Comparison of different extraction methods

Method	Dry residue, g 100/g berry powder	Total carbohydrates g 100/g berry powder	Anthocyanins, g 100/g berry powder	Total polyphenols, g 100/g berry powder
Microwave	21.01 ± 0.86	8.8 ± 0.36	0.054 ± 0.001	1.09 ± 0.04
Soxhlet	23.88 ± 1.8	8.33 ± 0.34	0.065 ± 0.002	1.21 ± 0.05
100W ultrasound	34.05 ± 1.4	11.46 ± 0.47	0.135 ± 0.003	1.59 ± 0.07
360W ultrasound	34.53 ± 1.42	12.15 ± 0.5	0.147 ± 0.004	1.68 ± 0.07
24h shaking	33.01 ± 1.35	11.78 ± 0.48	0.098 ± 0.002	1.12 ± 0.06
Supercritical CO_2	3.41 ± 0.19	0.05 ± 0.01	ND	0.05 ± 0.01

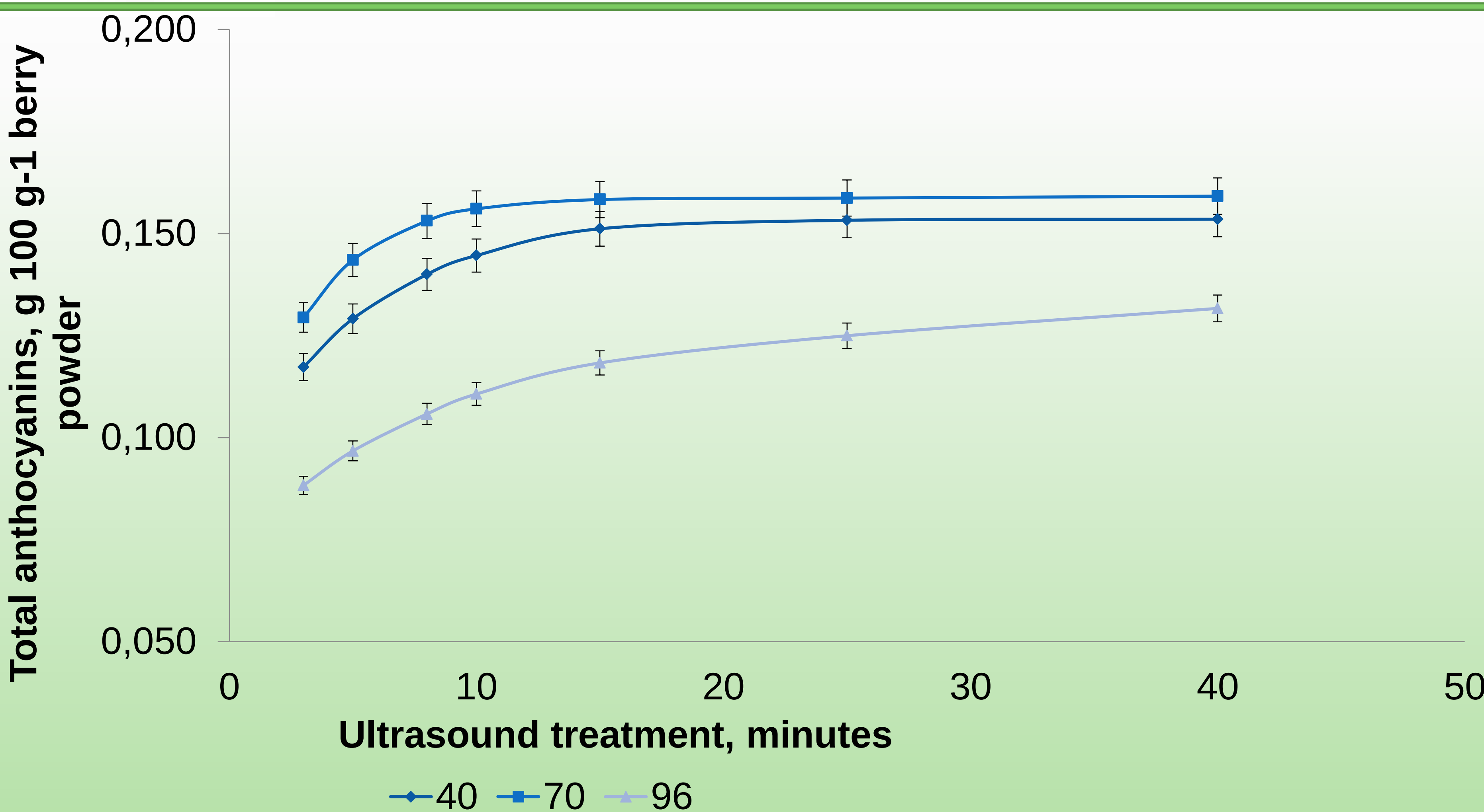
Comparison of solvent systems

Extrahent	Dry residue, g/100 g	Carbohydrates, g/100 g	Anthocyanines, g/100 g	Polyphenolics, g/100 g
CH_3 CN 49.5%, TFA 0.5%, H_2O 50%	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
Methanole 60%, acetone 30%, H_2O 10%	37.94 ± 1.56	16.86 ± 0.59	0.184 ± 0.005	2.34 ± 0.07
Methanole, HCl 1%	48.38 ± 1.98	17.93 ± 0.63	0.451 ± 0.011	4.80 ± 0.14
H_2O, HCl 1%	16.91 ± 0.69	14.82 ± 0.52	0.098 ± 0.002	0.89 ± 0.03
Ethanole 70%, HCl 1%	39.62 ± 1.59	16.85 ± 0.51	0.204 ± 0.005	3.43 ± 0.09

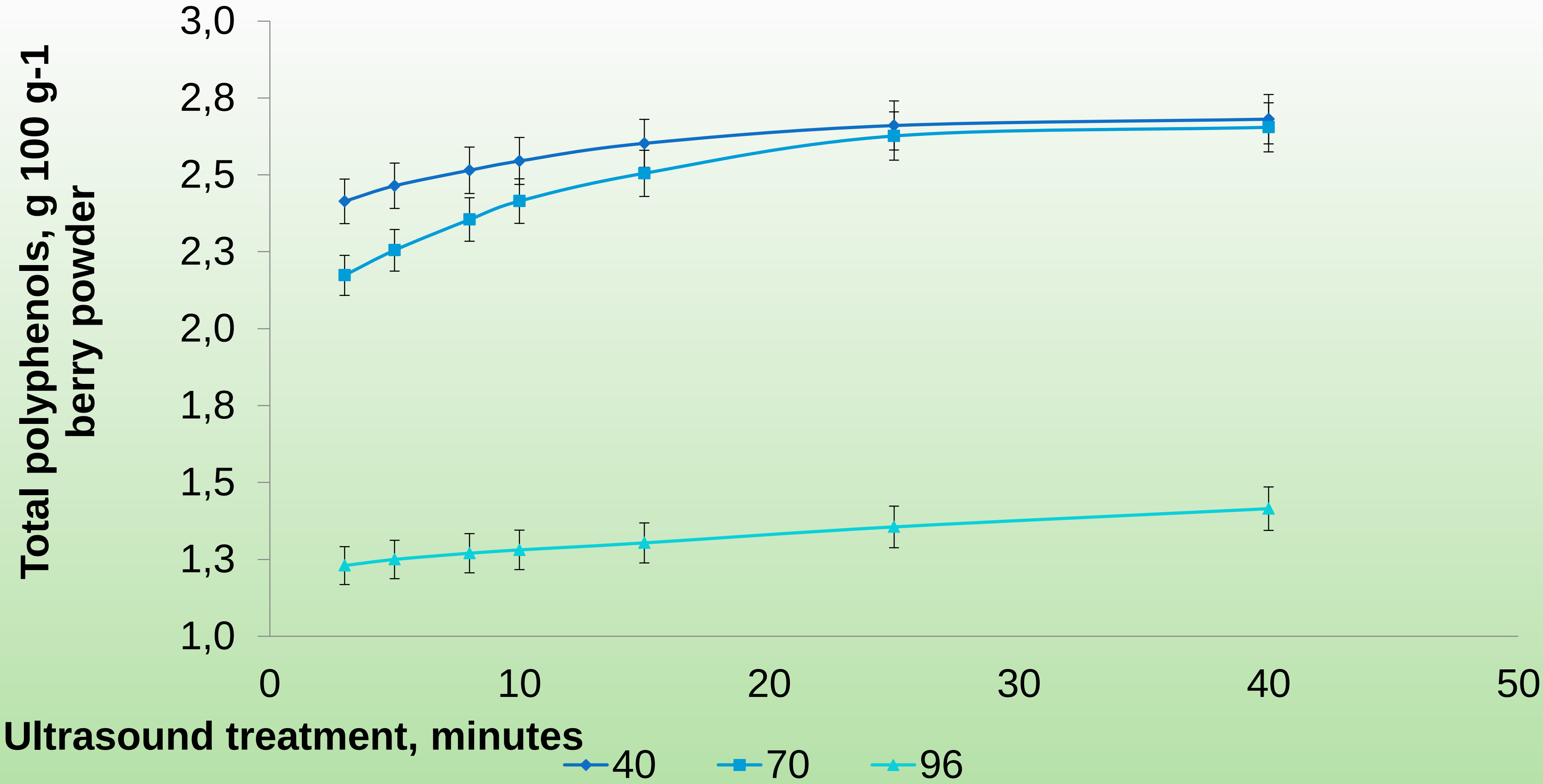
Acidification impact on total polyphenol and anthocyanin yields



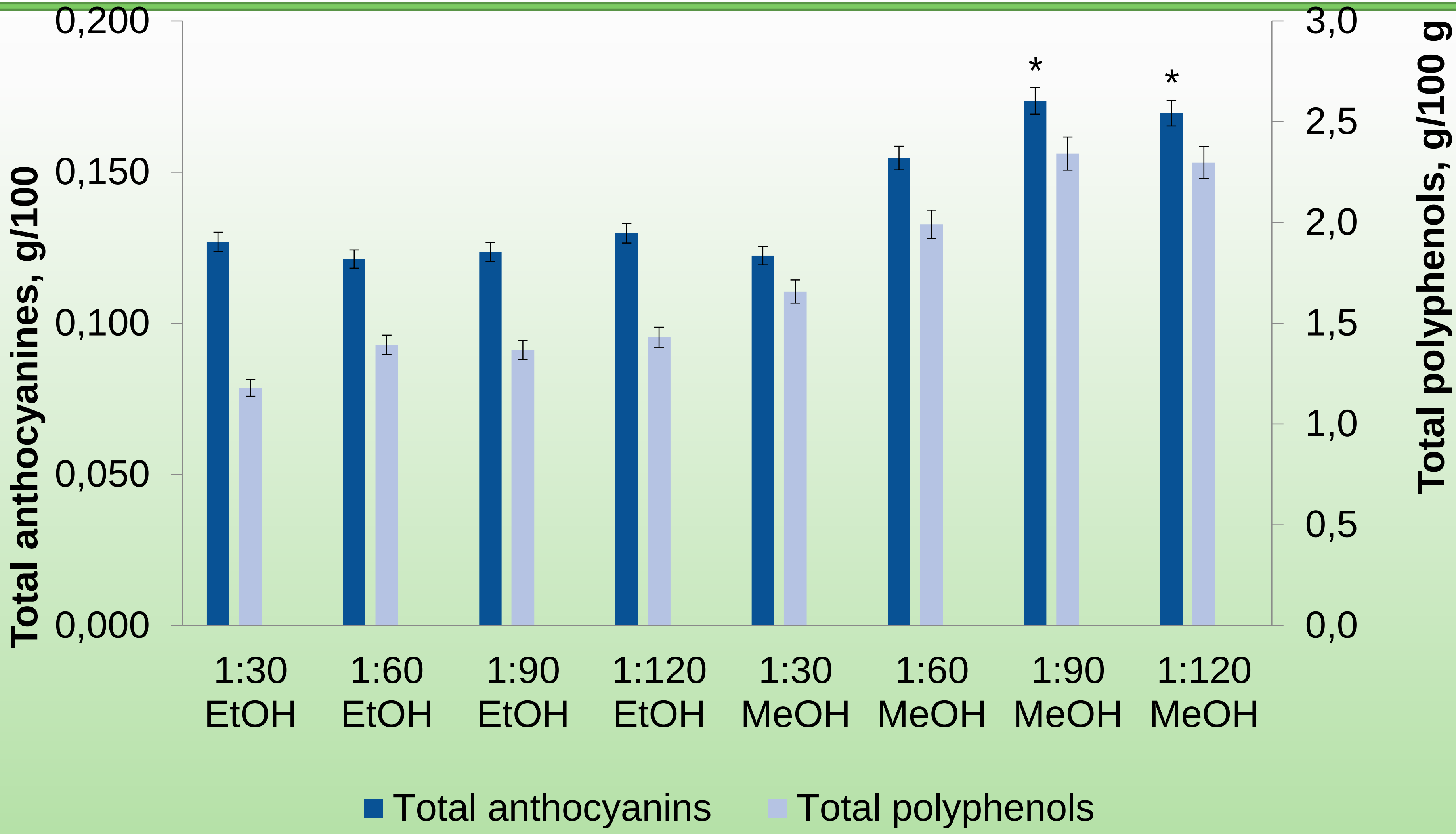
Kinetics of anthocyanine extraction from cranberry press residues



Kinetics of polyphenol extraction from cranberry press residues



Effect of the solvent/solid ratio on the extraction yield



Optimization of extraction conditions

Optimization of extraction of phenolic compounds and anthocyanins from American cranberry press residues in aqueous ethanol and methanol with TFA and formic acid was carried out using Response Surface Methodology. A two factor and three level central composite design consisting of eleven experimental runs was employed.

		Coded levels		
Independent variable	Symbol	-1	0	+1
Ethanol/Methanol, v/v %	X_1	96% / 100%	70	40
Formic acid/TFA, v/v %	X_2	10% / 1%	5% / 0.5%	1% / 0.1%

Two factor and three level Central Composite Design plan

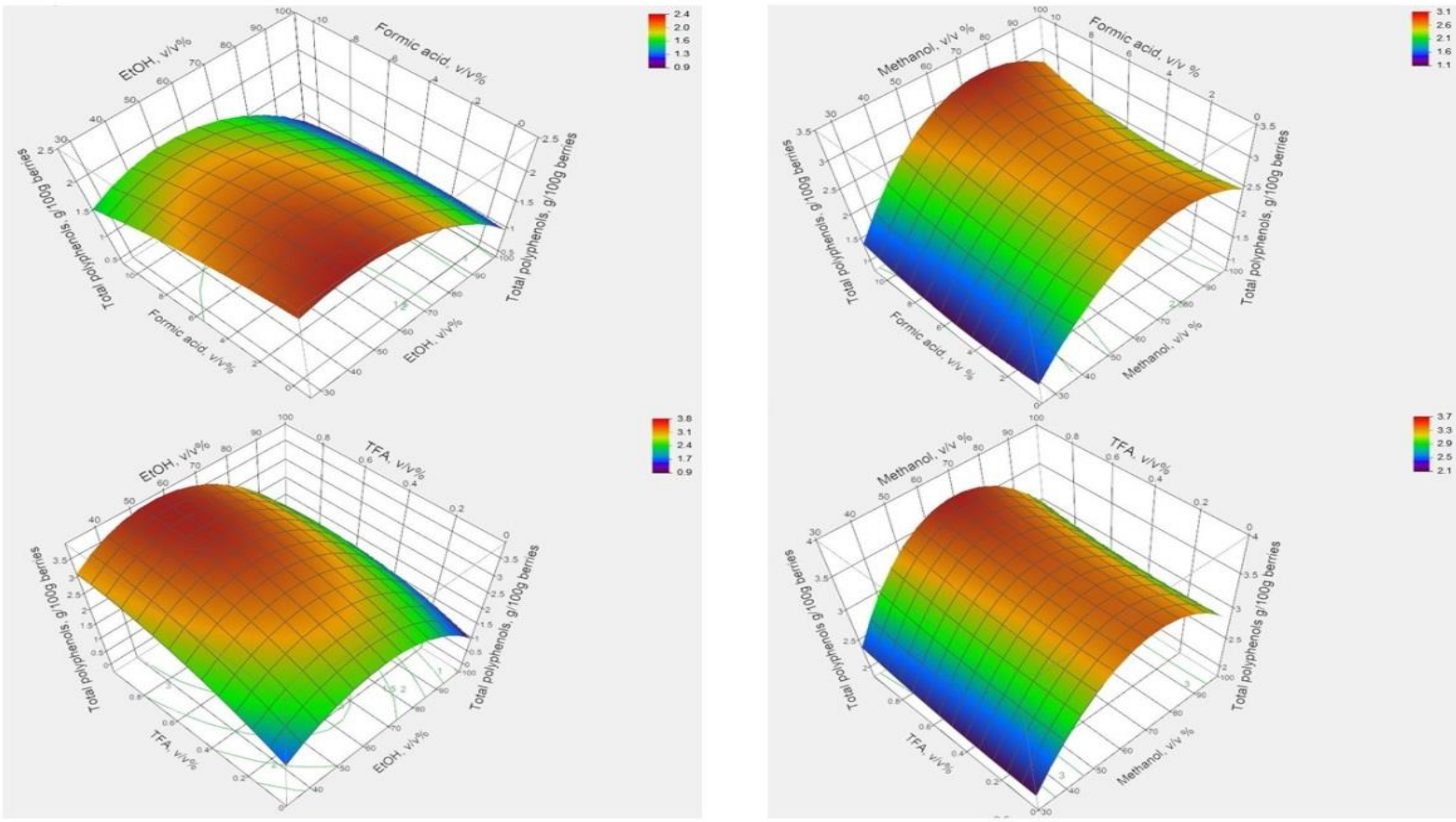
		Factor 1 (X_1)	**Factor 1 (X_2)**	
Standard order	Run order	Ethanol, v/v% (code)	TFA, v/v% (code)	Level code
1	4	40 (+1)	0.1 (+1)	+1 +1
2	9	40 (+1)	0.5 (0)	+1 0
3	6	40 (+1)	1.0 (-1)	+1 -1
4	1	70 (0)	0.1 (+1)	0 +1
5	8	70 (0)	0.5 (0)	0 0
6	11	70 (0)	0.5 (0)	0 0
7	10	70 (0)	0.5 (0)	0 0
8	7	70 (0)	1.0 (-1)	0 -1
9	3	96 (-1)	0.1 (+1)	-1 +1
10	5	96 (-1)	0.5 (0)	-1 0
11	2	96 (-1)	1.0 (-1)	-1 -1

Response surface optimization of polyphenol extraction

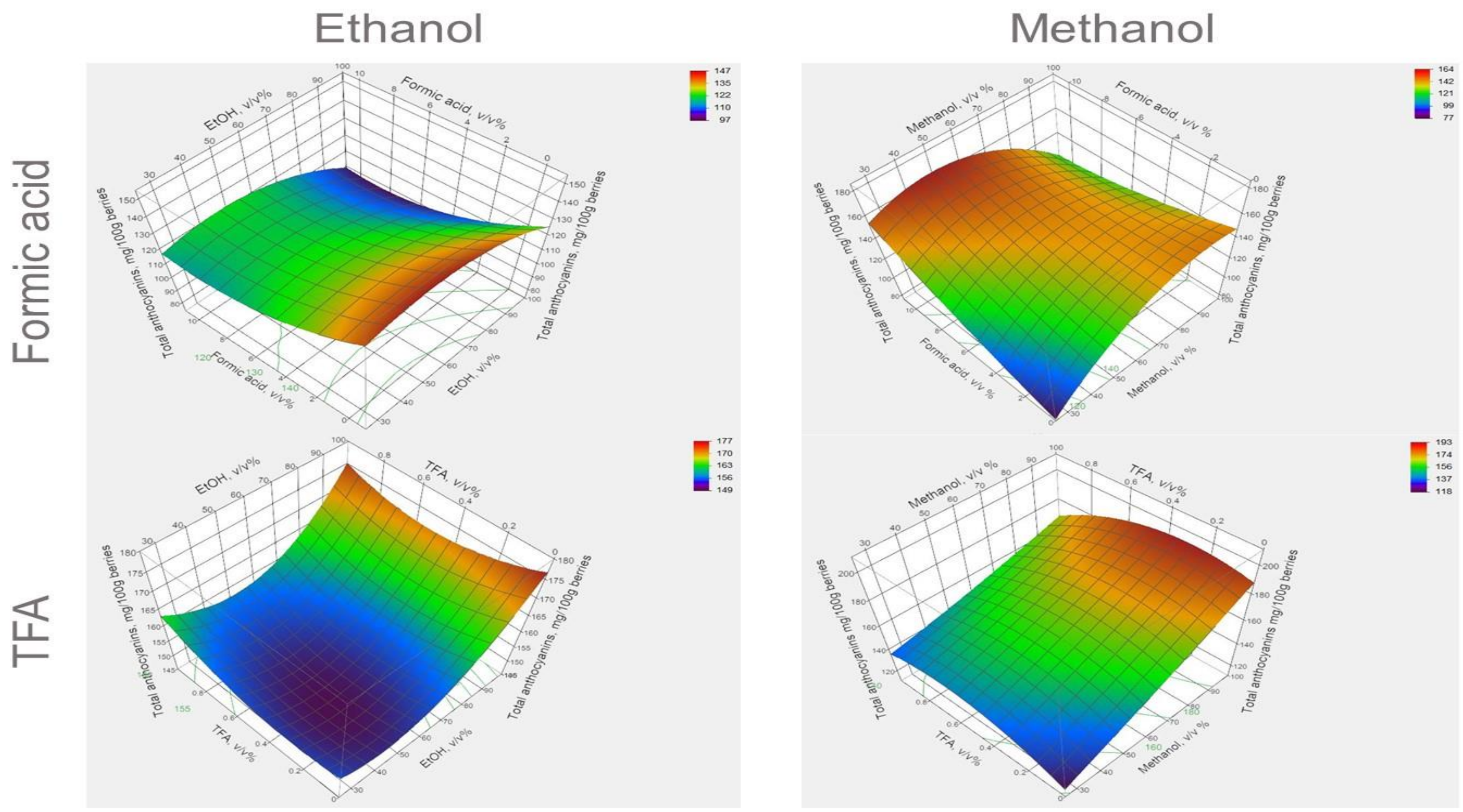
Ethanol | Methanol

Formic acid

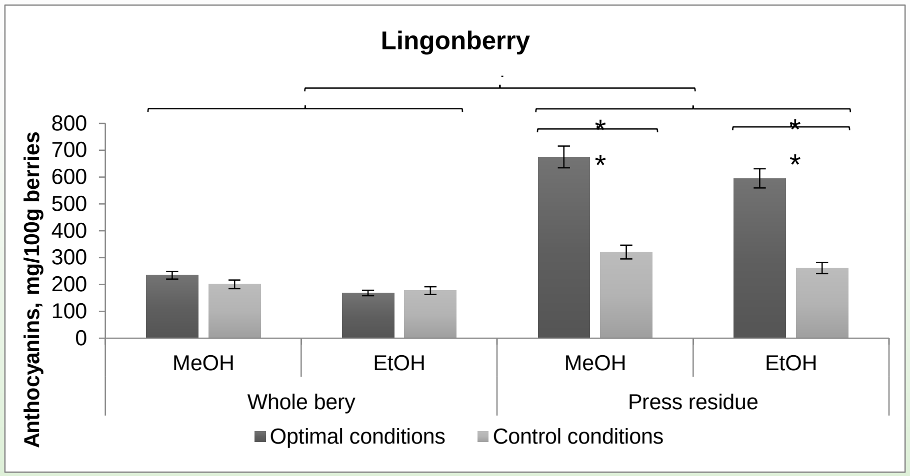
TFA

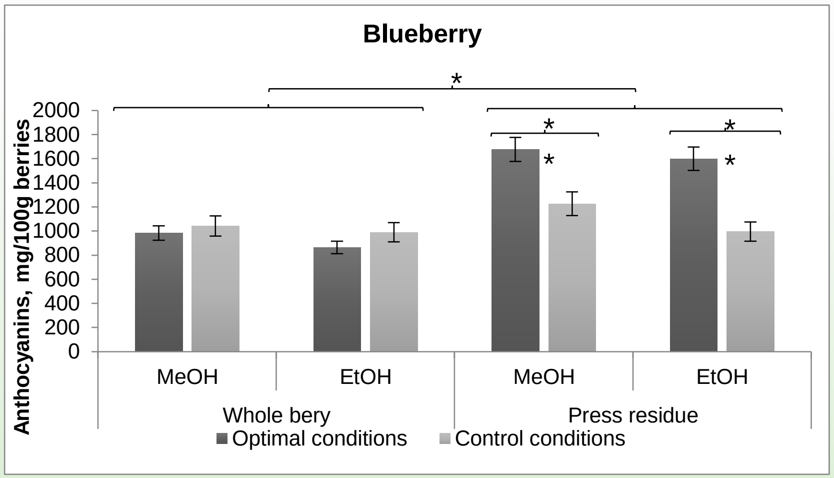


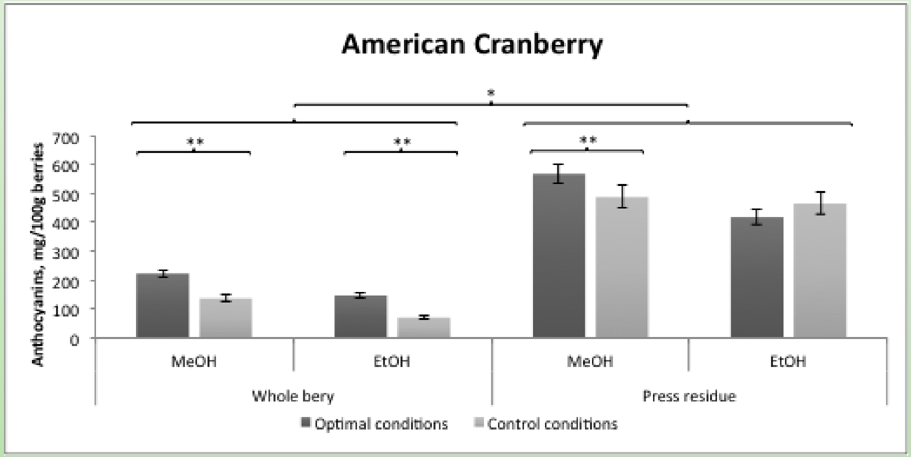
Response surface optimization of anthocyanine extraction

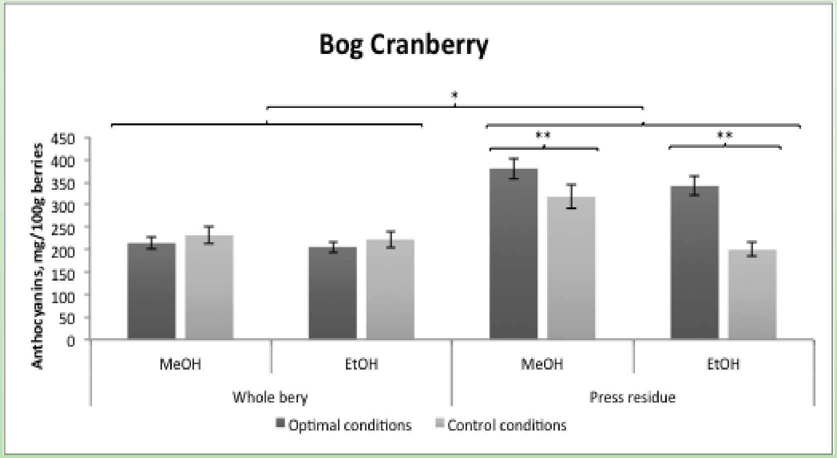


Results of optimization: whole berries *vs*. press residues









Purification of extracts to obtain purified polyphenolics

1

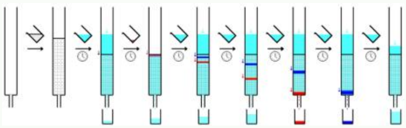
- Berry pomace
- Extraction at optimal conditions

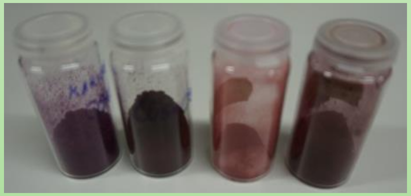
2

- Reextraction to remove lipids

3

- Column chromatography (XAD 7) to remove carbohydrates
- Evaporation, lyophilisation

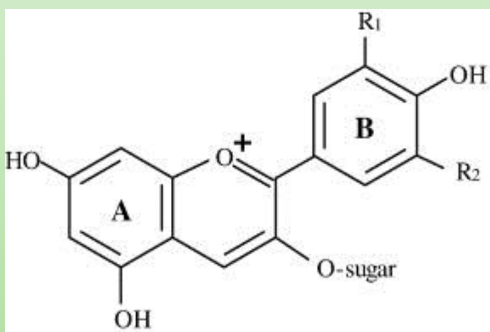




Yields and radical scavenging activity of obtained extracts

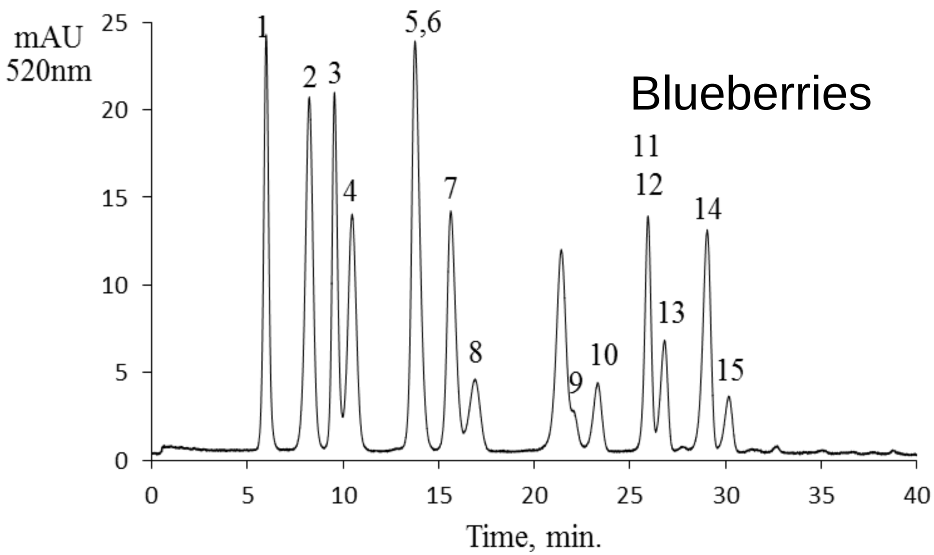
Sample	Yields, g/100g pomace	**FRAP**	**ABTS**	**DPPH**
		mM Trolox eq/1g		
Large cranberries	2.435	27.0	51.2	16.5
Highbush blueberries	3.333	24.4	35.6	11.8
Bog cranberries	3.047	28.8	53.7	15.8
Blueberries	3.211	43.7	65.8	19.2
Lingonberries	2.629	31.1	58.5	21.5
Control: ascorbic acid		20.2	38.0	14.0

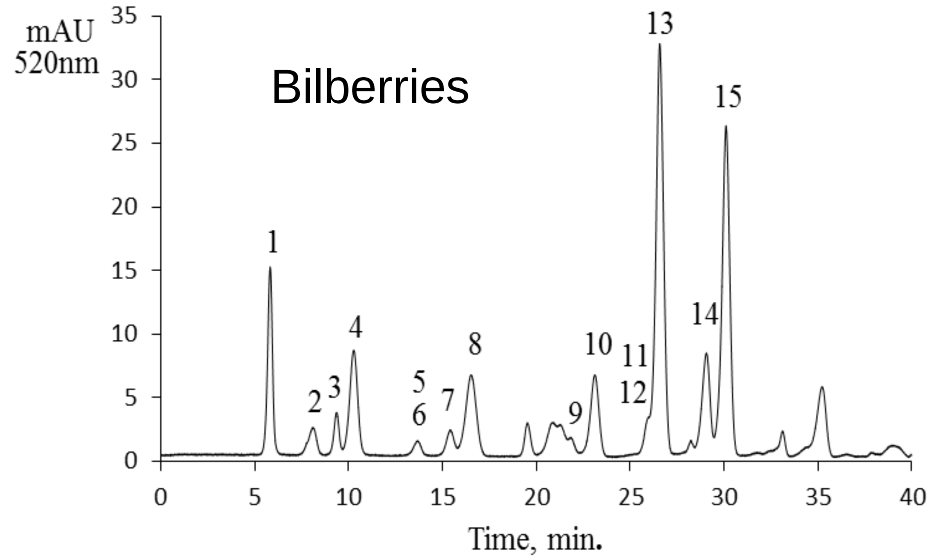
Studied anthocyanines

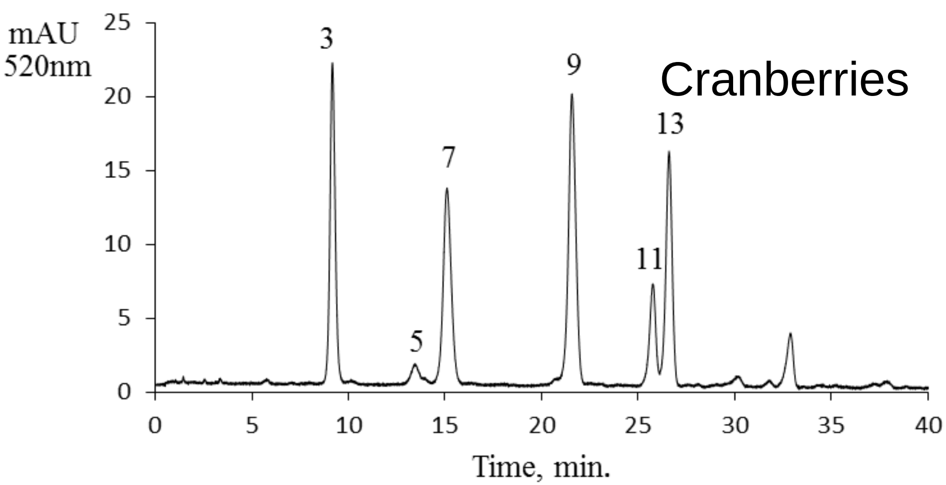


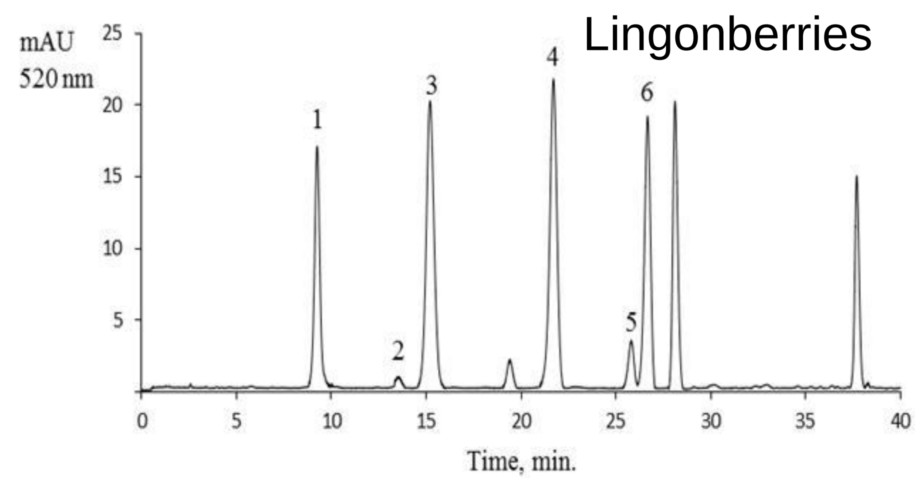
Anthocyanidin	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvinidin	OCH_3	OCH_3

Chromatograms of berry anthocyanines









Concentrations of anthocyanines in extracts (UPLC-PDA)

<table>
<tr><th>N peak</th><th>Compound</th><th>Blueberry</th><th>Billberry</th><th>Cranberry</th><th>Lingonberry</th><th>American cranberry</th></tr>
<tr><td>1</td><td>delphinidin-3-O-galactoside</td><td>31.41 ± 3.06</td><td>7.62 ± 0.64</td><td>—</td><td>—</td><td>—</td></tr>
<tr><td>2</td><td>delphinidin-3-O-glucoside</td><td>39.70 ± 3.22</td><td>1.83 ± 0.17</td><td>—</td><td>—</td><td>—</td></tr>
<tr><td>3</td><td>cyanidin-3-O-galactoside</td><td>26.50 ± 2.28</td><td>1.56 ± 0.13</td><td>9.81 ± 0.96</td><td>19.30 ± 1.89</td><td>1.73 ± 0.17</td></tr>
<tr><td>4</td><td>delphinidin-3-O-arabinoside</td><td>26.27 ± 2.55</td><td>6.47 ± 0.53</td><td>—</td><td>—</td><td>—</td></tr>
<tr><td>5</td><td>cyanidin-3-O-glucoside</td><td rowspan="2">49.18 ± 4.21</td><td rowspan="2">0.86 ± 0.81</td><td>0.71 ± 0.06</td><td>1.65 ± 0.15</td><td>0.06 ± 0.01</td></tr>
<tr><td>6</td><td>petunidin-3-O-galactoside</td><td>—</td><td>—</td><td>—</td></tr>
<tr><td>7</td><td>cyanidin-3-O-arabinoside</td><td>28.70 ± 2.34</td><td>1.15 ± 0.09</td><td>9.14 ± 0.89</td><td>5.44 ± 0.45</td><td>3.07 ± 0.31</td></tr>
<tr><td>8</td><td>petunidin-3-O-glucoside</td><td>8.95 ± 0.71</td><td>6.50 ± 0.60</td><td>—</td><td>—</td><td>—</td></tr>
<tr><td>9</td><td>peonidin-3-O-galactoside</td><td>2.73 ± 0.28</td><td>0.24 ± 0.02</td><td>12.41 ± 1.21</td><td>0.33 ± 0.04</td><td>3.04 ± 0.21</td></tr>
<tr><td>10</td><td>petunidin-3-O-arabinoside</td><td>8.55 ± 0.76</td><td>5.54 ± 0.44</td><td>—</td><td>—</td><td>—</td></tr>
<tr><td>11</td><td>peonidin-3-O-glucoside</td><td rowspan="2">20.44 ± 1.91</td><td rowspan="2">1.58 ± 0.29</td><td>3.28 ± 0.30</td><td>0.86 ± 0.07</td><td>0.36 ± 0.04</td></tr>
<tr><td>12</td><td>malvidin-3-O-galactoside</td><td>—</td><td>—</td><td>—</td></tr>
<tr><td>13</td><td>peonidin-3-O-arabinoside</td><td>9.93 ± 0.86</td><td>25.82 ± 2.45</td><td>8.18 ± 0.70</td><td>0.31 ± 0.03</td><td>2.31 ± 0.23</td></tr>
<tr><td>14</td><td>malvinidin-3-O-glucoside</td><td>26.20 ± 2.38</td><td>5.34 ± 0.49</td><td>—</td><td>—</td><td>—</td></tr>
<tr><td>15</td><td>malvidin-3-O-arabinoside</td><td>6.39 ± 0.62</td><td>19.61 ± 1.14</td><td>—</td><td>—</td><td>—</td></tr>
</table>

LC-HRMS (Orbitrap) results μg/g

Compound	Lingonberry	Cranberry	American cranberry	Blueberry	Crowberry	Highbush blueberry
4-hydroxybenzoic acid	556	116	95	80	198	76
Protocatechuic acid	2287	1151	374	3376	3668	2967
p-Coumaric acid	1374	313	272	182	277	26
Gallic acid	23	33	105	585	1872	770
Caffeic acid	747	352	103	293	67	240
Ferulic acid	2352	303	33	105	114	454
Syringic acid	21	30	23	193	589	1534
Resveratrol	0	31	73	38	35	50
Pelargonidin	0	0	0	0	0	0
Naringenin	73	27	3	10	154	6
Kaempferol	90	76	34	11	35	66
Cyanidin	0	94	0	0	0	13
(+)-Catechin	14038	4368	123	53	190	0
(-)-Epicatechin	3980	1632	242	1092	348	0
Ellagic acid	884	345	235	207	179	169
Quercetin	559	893	1208	184	458	977
Delphinidin	1382	3408	170	1079	712	574
Taxifolin	1758	2285	5608	6184	794	1500
Myricetin	2448	2641	815	3889	210	3388
Malvidin	79	124	323	229	255	10186
Chlorogenic acid	902	10833	166	2269	521	145
Quercetin-3-glucoside	1020	4382	216	913	521	528
Procyanidin A2	22414	1893	5474	301	5203	101
Procyanidin B2	5638	2898	48	3163	88	0
Rutin	451	368	0	0	292	286
Total amount of phenolics, μg/g	63078	38595	15744	24437	16780	24054

Thank you for attention!

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