Secondary raw materials of agricultural processing companies as a source of anthocyanin colorants

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Abstract. Many recent researches in vitro and in vivo proved the large therapeutic potency of non-toxic anthocyanins in anti-inflammatory, anti-infective, anti-oxidative actions. Anthocyanin is a natural phenolic colorant approved in many countries. A reason why the world market of natural food colorants is reduced is because fruit and berry raw materials are expensive. Yet the fruit and berry raw materials are extracted with significant losses and by-products. This constitutes around 23-45% of the whole amount of berries processes in the Russian Federation. Thus, a priority direction of the food industry is a development of technologies allowing to use precious berry pomace with high bioactive compounds, i.e. anthocyanins, organic acids, pectin. The aim of the research is to extract food colorant from the pomace of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* to identify individual anthocyanin pigments. The food safety and composition of the pomace of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* as raw material for food colorant extraction were found. Individual anthocyanin pigments of anthocyanin extracts were identified through the method of high-performance liquid chromatography. Cyanidin-3-galactoside was found in the extracts of berries (85,6 %) and pomaces (81,2%) of *Vaccinium vitis-idaea*. Fifteen compounds were identified in the extracts of *Vaccinium myrtillus*. The major ones were delphinidin-3-glucoside (13,4 %), delphinidin-3-galactoside (12,4 %), and cyanidin-3-glucoside for the fresh berries. As for the pigments of its pomaces, they were delphinidin-3-glucoside (15,3 %), delphinidin-3-galactoside (14,7 %), and delphinidin-3-arabinoside (10,5 %). Hence, there are more anthocyanin pigments in the extracts of pomaces, than in those of the fresh berries with identical compounds – 24,7 % more for *Vaccinium myrtillus* and 11,1 % more for *Vaccinium myrtillus*. The possibility to extract anthocyanin pigments from by-products of the local fruit and berry raw materials – i.e. of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* ones – and identify them is discussed.

1 Introduction

The color is a basic characteristic of food stuff that indirectly makes consumers choose a product and estimate its quality. «Clean label» and «Free from All» global trends are becoming popular with Russians. This stimulates demands for products made of natural raw

materials and colorants. Nearly 70% of interviewed from Western Europe study the food label before buying a product. Three quarters of them consider the “No artificial ingredients” indication crucial, according to the Mintel market research firm [1–3].

Such researches prove the opinion, that the last decade saw a fast-growing segment of consumers, taking care of their health, appear. This is what market experts and specialists on healthy nutrition figure. They are usually educated and well-informed people searching for sources of commercial and scientific information to health promotion. Advertisements are distrusted [4].

Coloring food technologies must meet many requirements. The colorant must be safe, temperature resistant, durably high-color and have no off-flavors. Chemical compound and properties of a natural colorant are inferior to those of the synthetic ones. They are less resistant to storing and production load. Though, natural plant colorants are sometimes obligatory as in case with fermented milk products. Lactic acid bacteria decompose synthetic azo dyes in several hours – the anaerobic degradation of azo dyes is a key concept for technologies reducing pollution of ecosystems. Thus, acid-tolerant natural pigments should be used to dye some products [5–8].

Besides, synthetic colorants prevailing on the Russian market are often non-conform to the main requirements. Colorants used in food are proved to cause physiological failures, allergies, and severe chronic diseases [9, 10].

Natural colorants, including anthocyanin, not only dye food products but also have health promoting properties proved in vitro and in vivo. Natural colorants proved non-toxic have anti-inflammatory, anticarcinogenic, and antibacterial potency; ease oxidative stress; prevent heart diseases, obesity, and visual impairment; controls diabetes (WHO, 1982) [11–15].

Natural anthocyanins as color additive for food and drinks are approved in many countries. Nowadays the market sees the growing demands for natural food colorants, mainly the red ones [16]. Yet the world market of natural food colorants is reduced as the technologies are complex and fruit and berry raw materials are expensive. The local fruit and berry raw materials are still extracted with significant losses and by-products (23–45 %). For example, in Altai Krai only the losses constitute around 860–870 tones, which results from a low depth of processing and lack of innovative decisions on berry marcs processing.

Purpose of the study. Thus, the economic situation and the fact of ecology deterioration make the problem of depth of processing of fruit and berry secondary raw material crucial. So, the search of new raw materials for anthocyanin colorants is relevant. The aim of the research is to extract food colorant from the pomace of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* to identify individual anthocyanin pigments.

2 Materials and methods

The subjects of the research are the samples of pomaces and ethanolic extracts of anthocyanins. The samples of pomaces of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* (fig. 1) are the wastes of technological cycle of dry extracts production from a going concert. The LLC “KIT” Altai Krai specializes in processing of local fruit and berry raw material. The feedstock is standardized in accordance with GOST 34219-2017, GOST 20450-75 и TRCU 021/2011 requirements, according to the specification. The extracts of anthocyanins were extracted from a mixture of ethyl alcohol (60%) and hydrochloric acid (1%) incubating in water bath for 1.5 hour. The solution was further cooled to room temperature and filtrated from foreign matters. The method was chosen because the extraction of anthocyanins by ethyl alcohol gives a more concentrated colorant [17]. The

extracts of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* were the control samples obtained likewise.

Vaccinium myrtillus

Vaccinium vitis-idaea

Fig. 1. Dried samples of the pomaces

Physical and chemical features of the pomaces were analyzed through standard methods. The mass concentration of the juice was found through the thermogravimetric analysis as per GOST 28561. The method presupposes that a friable or allocated on the absorbing surface sample would be dried on high temperature and controlled pressure. The mass concentration of the titratable organic acids (expressed as malic acid) was measured by potentiometric titration as per GOST 25555.0-82. The solution was subject to potentiometric titration to pH 8,1. The solution is titrated and constantly interfused with sodium hydroxide (NaOH)=0,1 mol/dm³. The interfusing is fast until pH 6,0, then slowly until pH 7,0. Four drops of a titrant are pored next several times until pH is 8,1. The amount of the titrant and the pH level are noted alongside. After the pH 8,1 is reached, no less than 4 drops of sodium hydroxide are added. The mass concentration of sugars was defined by photocolourimetry as per GOST 8756.13-87. The method is based on interaction of carbonyl group of sugars with potassium ferricyanide in basic conditions and optical density measurement of the solution obtained by photoelectrocolorimeter. The mass concentration of extractives was specified through gravimetric single-contact extraction analysis as per general pharmacopoeia article 1.5.3.0006.15. The mass concentration of pectin (protopectin) by the method as per GOST 29059-91. The method is to titrate a substance preliminary isolated from pectin substances before and after hydrolysis. The results of the titration are proportional to the amount of the free and etherified carboxyl groups. The multiplication of them by corresponding equivalents results in content of polyuronides in pectin substances of a product. The mass concentration of anthocyanins in the samples of the extracts was precised per GOST 32709-2014. The anthocyanins were identified by the method of high-performance liquid chromatography (HPLC). The ethanolic extracts were separately chromatographed in liquid chromatograph Agilent 1200 under the following conditions: the column Luna 5u C_{18} (2) 100A (250x4,6 mm 5 μm); the temperature of the heat block 25°C; the detection wavelength 543 nm; the ME CN eluent; the solution of the phosphoric acid in water (0,01%); gradient eluting. The collection of data, the processing of chromatograms and absorption spectra were realized in the AgilentChemStation. The peaks were identified according to literature data and the holding time was compared for the analogical columns [18].

3 Results and discussion

The organoleptic assessment of the samples proved that the pomaces are composed of undamaged homogeneous sphere fruits 5-7 mm in diameter. Those of the *Vaccinium myrtillus* were violet with palatability, sweetness and the strong taste of the initial raw material. The *Vaccinium vitis-idaea* ones were crimson with palatability, sweet and sour strong taste. Composition of the samples of the pomaces are presented in the table 1.

Table 1. Composition of the samples of the pomaces on a dry basis (n=3, M±m)

Pomaces	Concentration, %					
	juice	organic acids (expressed as malic acid)	sugars	extractives	pectin substances (protopectin)	anthocyanins
<i>Vaccinium vitis-idaea</i>	4,91±0,11	4,52±0,10	3,2±0,1	60±1,0	3,1±0,2	0,504±0,001
<i>Vaccinium myrtillus</i>	3,57±0,11	4,89±0,10	3,7±0,1	71±1,0	3,6±0,2	0,612±0,001

The data obtained matches with those of the literature [19].

The food colorings as well as other raw materials for foodstuff must conform to the requirements on safety points, i.a. microbiological ones proved experimentally (table 2).

Table 2. Microbiological safety points for the samples of the pomaces (n=3, M±m)

Type of raw material	Mesophilic anaerobic and optionally anaerobic microorganisms max ufc/1g	Yeast max ufc/g	Mold max ufc/g	Coliform bacteria prohibited in the product in g
Norms of TRCU 021, mg/kg, max	5,0·10 ⁴	200	500	0,1
<i>Vaccinium myrtillus</i>	<20	<15	Not found	
<i>Vaccinium vitis-idaea</i>	<10	<20	Not found	

The data of the table 2 show that the samples of the pomaces are safe. This also proves that sanitary and hygienic requirements of storage and processing of the raw material are met.

The concentration of anthocyanin colorants in the extracts may be estimated by the absorbency intensity of the dyed samples as well. Thus, this stage of the research presupposed the comparison of the anthocyanin colorants content in the extracts made of pomaces and fresh berries. Having analyzed the absorbency of the extracts of extracts and fresh berries of *Vaccinium myrtillus* and *Vaccinium vitis-idaea*, the growth of the absorbency was confirmed (table 3). This attests that the concentration of the dyed substances in the extracts of the pomaces is bigger.

Table 3. Absorbency of the extracts of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* at the wavelength 543 nm

Type of raw material	Absorbency, nm	
	Fresh berries	Pomaces
<i>Vaccinium vitis-idaea</i>	0,632	0,793

Vaccinium myrtillus	0,234	0,258
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Many chemical compounds are known to have electronic signals allowing to examine their structure. The dyed compounds, i.e. those of anthocyanins, have intense signals comprised between 400 and 600 nm. Their absorbency peaks relate directly to the structure and polymerization degree of an anthocyanin. The fig.2 presents the absorbency of anthocyanin ethanolic extracts made of pomaces of *Vaccinium vitis-idaea* (1) and *Vaccinium myrtillus* (2) and the fresh berries (3), (4) resp.

1 – pomaces *Vaccinium vitis-idaea*; 2 – fresh berries *Vaccinium vitis-idaea*;
3 – pomaces *Vaccinium myrtillus*, 4 – fresh berries *Vaccinium myrtillus*

Fig. 2. Absorbency spectra of anthocyanin ethanolic extracts of *Vaccinium vitis-idaea* and *Vaccinium myrtillus*

The fig.2 demonstrates that the extract of the pomaces of *Vaccinium vitis-idaea* has two absorbency peaks in UV 282 ± 2 nm and VIS 541 ± 2 nm. The peak of the extract of the pomaces of *Vaccinium myrtillus* is in VIS 543 ± 2 nm, UV 281 ± 2 nm, and shoulder in 370 nm. The peak of the extract of the fresh berries of *Vaccinium vitis-idaea* is in UV 279 ± 2 nm and VIS 537 ± 2 nm. The peaks of the extract of the fresh berries of *Vaccinium myrtillus* are in UV 281 ± 2 nm and VIS 543 ± 2 nm, and shoulder in 370 nm. The absorbency spectra of the ethylic extracts may have 3 peaks of adsorption in the UV area comprised between 260 and 380 nm, according to literature data. This may be used to estimate amount of acyl fragments in the structure. The degree of decarboxylation may displace the peaks, as shown in the fig.2 for the samples 3 and 4.

The data obtained experimentally enabled to figure the amount of anthocyanins on a basis of cyanidin-3,5-diglucoside in dry raw material (table 4).

Table 4. The amount of anthocyanins on a dry basis of cyanidin-3,5-diglucoside

Type of raw material	Total amount of anthocyanins, %	
	Fresh berries	pomaces
<i>Vaccinium vitis-idaea</i>	1,21	1,51
<i>Vaccinium myrtillus</i>	0,45	0,50

According to the table 4, the biggest amount of anthocyanins on a basis of cyanidin-3,5-diglucoside is in the *Vaccinium myrtillus*. More anthocyanins were found in the pomaces, as anthocyanins and pectin are mostly localized in the skin and not in the pulp. The literature data says that the amount of anthocyanins in the skin is 6,8–10,8 times over than in the pulp [20].

The compound of the anthocyanins was further identified by the method of chromatography. The results are presented in the tables 5 and 6.

Table 5. Results of the HPLC of the extracts of berries and pomaces of *Vaccinium vitis-idaea*

№	Compounds	Retention time, min		Content of samples, %	
		Berries	Pomaces	Berries	Pomaces
1	Cyanidin-3-galactoside	8,708	27,320	85,6	81,2
2	Non identified	9,748	31,089	4,9	3,2
3	Petunidin-3-galactoside	10,863	33,889	9,4	15,5

The table 5 demonstrate that the extracts have two peaks. The first one is presumably the peak 1 of cyanidin-3-galactoside and the second one is the peak 3 of petunidin-3-galactoside. These are the most concentrated anthocyanins which is also described in the literature [18, 20–22]. The quality compound of anthocyanins usually differs between botanic species and it is stable except for different geographical ranges and climate conditions. Hence, anthocyanin complex of the berries of *Vaccinium vitis-idaea* compounds glycosylated cyanidin and petunidin with residues of galactose in position C-3 of heterocycle ring C-.

Table 6. Results of the HPLC of the extracts of berries and pomaces of *Vaccinium myrtillus*

№	Compounds	Retention time, min		Content of the component, %	
		berries	pomaces	berries	pomaces
1	Delphinidin-3-galactoside	21,462	21,498	12,4	14,7
2	Delphinidin-3-glucoside	24,689	24,780	13,4	15,3
3	Cyanidin-3-galactoside	26,800	26,913	10,4	7,4
4	Delphinidin-3-arabinoside	27,856	27,980	10,5	10,5
5	Cyanidin-3-glucoside	30,788	30,912	11,6	8,5
6	Petunidin-3-galactoside	33,090	33,209	3,4	5,2
7	Cyanidin -3- arabinoside	36,752	33,618	9,4	4,9
8	Petunidin-3- glucoside	38,870	36,866	2,1	9,6
9	Delphinidin	39,933	39,004	2,9	0,7
10	Peonidin-3- galactoside	41,499	39,321	4,9	0,9
11	Petunidin -3- arabinoside	41,882	40,009	3,5	2,4
12	Peonidin -3- glucoside	42,573	41,544	0,6	3,6
13	Malvidin -3- galactoside	42,928	41,918	9,9	3,6
14	Peonidin -3- arabinoside	43,904	42,611	3,2	0,4
15	Malvidin-3- glucoside	45,557	42,958	0,6	9,6
16	Non identified	48,549	43,933	0,2	1,9
17	Non identified	49,453	45,586	0,9	0,3

Seventeen anthocyanins of the extracts of the fresh bilberries were found with 15 identified. The most concentrated ones are cyanidin-3-galactoside, petunidin-3-galactoside, malvidin -3- galactoside, delphinidin-3-arabinoside, petunidin -3- arabinoside.

Seventeen components of the extracts of pomaces of the bilberries with 15 identified. The most concentrated anthocyanins are cyanidin-3-galactoside, peonidin -3- glucoside, delphinidin-3-arabinoside, peonidin-3- galactoside, delphinidin-3- glucoside.

As shown in the table 6, the prevailing components in the extract of the fresh berries are delphinidin-3- glucoside (13,4 %), delphinidin-3- galactoside (12,4 %), and cyanidin-3-glucoside (11,6 %). As for those of the extracts of the pomaces, they are delphinidin-3-glucoside (15,3 %), delphinidin-3- galactoside (14,7 %), and delphinidin-3-arabinosid (10,5 %).

4 Conclusions

The reaches revealed that there are more anthocyanin pigments in the extracts of pomaces, than in those of the fresh berries with identical compounds – 24,7 % more for *Vaccinium myrtillus* and 11,1 % more for *Vaccinium myrtillus*. The analysis proved that compounds of the extracts of fresh berries and pomaces differ in quality and in quantity. The most concentrated anthocyanins with diverse compound are in the extracts of the berries and pomaces of *Vaccinium myrtillus*. Fifteen anthocyanins were identified in them, while in those of *Vaccinium vitis-idaea* only 2. Besides, only 2 compounds are identified in all the samples. They are cyanidin-3-galactoside (prevailing in the extracts of berries and pomaces of *Vaccinium vitis-idaea* – 85,6 % and 81,2 % resp.) and petunidin-3-galactoside. The fact suggests that the colorants made of pomaces of *Vaccinium vitis-idaea* would result in a higher anti-oxidative potency of food stuff. The research [23] shows that the antioxidative potency of the cyanidin-3- glucoside is higher than of other anthocyanins. It is 75% higher than of cyanidin-3-galactoside and 90% higher than of the peonidin-3-glucoside.

Thus, the possibility to extract and identify anthocyanin pigments from by-products of the local fruit and berry raw materials – i.e. of *Vaccinium myrtillus* and *Vaccinium vitis-idaea* ones – is presented. However, the use of natural anthocyanin coloring remains individual and depends on the kind, form and compound of a product. Nevertheless, natural anthocyanin colorants are preferable as they are multifunctional and not only dye food products and render them more attractive. Stabilization of anthocyanin colorants in the food systems, their assimilability and bioavailability need further analysis.

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