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USE OF ANTHOCYANIN ANALYSIS FOR DETECTION OF BERRY JUICE ADULTERATIONS

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Abstract. Chromatographic profiles of anthocyanins in authentic and adulterated black currant and raspberry juices were compared in this paper. HPLC apparatus with DAD detector was used for separation of anthocyanins. Analysis of adulterated juices required optimization of elution conditions. Two types of eluents were applied at gradient: A – 4.5% formic acid and B – 100% acetonitrile. There was detected 30%, 20% and 10% addition of strawberry to raspberry and black currant juices on the basis of pelargonidin-3--glucoside content. Cyanidin-3-xylorutinoside indicated the 30%, 20% and 10% addition of red currant to raspberry and black currant juices. Anthocyanin analysis is an effective method for detection of berry juice adulterations.

Key words: anthocyanins, HPLC, berry fruits, juices, adulterations

INTRODUCTION

Particular juices differ in quantity and type of anthocyanins. Analysis of anthocyanin composition is a typical method for examination of berry juice authenticity [Hofsommer and Koswig 2000, Fügel et al. 2004]. HPLC technique is the most useful for determination of anthocyanins [Landbo and Meyer 2004, Mullen et al. 2002, Tsao and Yang 2003]. After chromatographic separation of anthocyanins, characteristic chromatographic profile (fingerprint) was required which could allow juice identification (Fuchs and Koswig 1997, Goiffon et al. 1991, Hofsommer 1994, Koswig and Hofsommer 1995, Stój et al. 2001, Versari et al. 1997, 1999]. Comparison of unknown sample chromatogram with that characteristic for authentic sample let to estimate if analysed sample is authentic or adulterated [Hofsommer 1995].

It appears from the literature that there are little works upon trials of identification of berry juice adulterations on the basis of anthocyanin chromatograms. Analysis of anthocyanins was used for detection of cranberry juice adulteration with extract of grape peels [Hong and Wrolstad 1986] and black currant juice with cherry juice [Versari et al. 1997].

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The aim of the present paper was to determine the anthocyanins in authentic black currant and raspberry juices and in black currant and raspberry juices adulterated with strawberry and red currant juices. Composition of anthocyanins was assayed by HPLC method. Elution conditions were optimized for different types of juices.

MATERIALS AND METHODS

Four berry species were collected in 1998, 1999 and 2000. Strawberries (*Fragaria ananassa*) of 'Senga' cv. originated from the plantation in Zemborzyce, raspberries (*Rubus idaeus*) of 'Beskid' cv. as well as black currants (*Ribes nigrum*) of 'Ben Lomond' cv. were from the Experimental Farm in Felin and red currants (*Ribes rubrum*) of 'Rondom' cv. were collected in a garden in Klementowice.

Berry fruits were stored in the refrigerator at –28°C. Juices were made from fruits defrosted at ambient temperature in juice extractor Zelmer 277.8 and then centrifuged in centrifuge MPW 365 for 15 minutes at 4°C and 11 000 rpm.

Following adulterated juices were prepared: raspberry juice with 10, 20, 30% addition of strawberry juice, raspberry juice with 10, 20, 30% addition of red currant juice, black currant juice with 10, 20, 30% addition of strawberry juice and black currant juice with 10, 20, 30% addition of red currant juice.

Anthocyanins purification and HPLC separations were carried out according to the method described by Hong and Wrolstad [1990] with modifications.

Mini-column Sep-Pak C_{18} Waters was activated by means of washing with 5 ml of methanol and then 5 ml of 0.01% HCl. Aliquots of 1 ml of juice were introduced into the mini-column. In order to remove saccharides and organic acids, mini-column was washed with 2 ml of 0.01% HCl and anthocyanins were eluted with 2 ml of 0.01% HCl in methanol.

HPLC apparatus Gilson consisting of two pumps 306, dynamic mixer 811C, manometer 805 and detector UV-VIS DAD 170 was used for separation of anthocyanins. The chromatograph was combined with the software for data acquisition UniPoint™ due to Interface 506C system. Separation was performed on column Symmetry of 250 × 4.6 mm dimensions filled with C_{18} Waters of 5 μm particle diameter. Two types of eluent were applied at gradient: A – 4.5% formic acid and B – 100% acetonitrile. Elution gradient was following: 0-5 min, 0-5% B; 5-12 min, 5-10% B; 12-22 min, 10-15% B; 22-26 min, 15-20% B; 26-33 min, 20-0% B. Other chromatographic parameters were as follows: dosing loop of Rheodyne 7725i, injector – 20 μl, eluent flow rate – 1 ml/min and detection wavelength – 520 nm.

Calibration curves were plotted for three standards: cyanidin-3- glucoside, cyanidin--3-rutinoside and pelargonidin-3-glucoside (Extrasynthese, France). 2 mg of each standard were dissolved in 1 mL of 0.1% HCL in methanol and dilutions were prepared. Methanol solutions contained 2000, 1000, 500, 100 and 50 mg/l of particular standard.

Cyanidin-3-glucoside, cyanidin-3-rutinoside and pelargonidin-3-glucoside were identified in examined juices on the base of spectrum and retention time of available standards (Extrasynthese, France). The other anthocyanins were probably identified on the base of spectrum, elution order and literature data [Bakker et al. 1994, Goiffon et al. 1991, Hong and Wrolstad 1990, Versari et al. 1997].

Determination results were statistically worked out using t-Student test at significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

The anthocyanins for which standards are not commonly available were identified on the base of spectrum, elution order and literature data. According to the literature, the wavelength maxima (λ_{max}) in the visible range is closely related to the hydroxylation pattern of the anthocyanin. Derivatives of pelargonidin (λ_{max} = 502 nm) can be distinguished from derivatives of cyanidin (λ_{max} = 516-520 nm) and delphinidin (λ_{max} = 525--528 nm) on the basis of their different visible spectra. The nature of sugar substitution has no relevant effect on the spectrum [Hong and Wrolstad 1990, Versari et al. 1997]. The elution order of anthocyanins is correlated with their hydrophobicity. The most hydrophilic anthocyanins are eluted at first. Following anthocyanins are less hydrophilic and more hydrophobic. According to the literature, elution order of aglycones is: delphinidin < cyanidin < pelargonidin. Within an identical aglycone, addition of a second carbohydrate increases its polarity resulting in a decrease in retention time, generally. However the presence of hydrophobic methyl group in the rhamnose molecule affects cyanidin-3--rutinoside is eluted after cyanidin-3-glucoside [Bakker et al. 1994, Goiffon et al. 1991].

Results of chromatographic separations of anthocyanins indicated that strawberry, raspberry, black currant and red currant juices differed in composition of anthocyanins (Table 1-4). Strawberry juices contained: cyanidin-3-glucoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside (probably) and pelargonidin-3-glucoside acylated with acetic acid (probably), raspberry juices – cyanidin-3-sophoroside (probably), cyanidin-3-glucorutinoside (probably), cyanidin-3-glucoside and cyanidin-3-rutinoside, black currant juices – delphinidin-3-glucoside (probably), delphinidin-3-rutinoside (probably), cyanidin-3-glucoside and cyanidin-3-rutinoside and red currant juices – cyanidin-3--sophoroside, cyanidin-3-glucorutinoside, cyanidin-3-glucoside, cyanidin-3-xylorutinoside (probably) and cyanidin-3-rutinoside. It was found that pelargonidin-3-glucoside was characteristic anthocyanin in strawberry juices like in Bakker et al. [1992], Bakker et al. [1994], Goiffon et al. [1991], Hong and Wrolstad [1990], Skrede et al. [1992] and Versari et al. [1997] research. Characteristic anthocyanin of red currant juices was

Table 1. Contents of anthocyanins in strawberry juices in 1998, 1999 and 2000
Tabela 1. Zawartości antocyjanów w sokach truskawkowych w latach 1998, 1999, 2000

Year of strawberry harvest Lata zbioru truskawek	Anthocyanins – Antocyjany			
	peak 1 – cy-3-glu pik 1 – cy-3-glu mg/l	peak 2 – pg-3-glu pik 2 – pg-3-glu mg/l	peak 3* area × 10^4 pik 3* pow. × 10^4	peak 4** area × 10^4 pik 4** pow. × 10^4
1998	31	1 436	99	480
1999	67	1 678	233	261
2000	105	1 929	271	357
x	68	1 681	201	366

Cy-3-glu – cyanidin-3-glucoside, pg-3-glu – pelargonidin-3-glucoside, *pelargonidin-3-rutinoside (probably), **pelargonidin-3-glucoside acylated with acetic acid (probably).

Cy-3-glu – cyjanidyno-3-glukozyd, pg-3-glu – pelargonidyno-3-glukozyd, *pelargonidyno-3-rutynozyd (przypuszczalnie), **pelargonidyno-3-glukozyd acylowany kwasem octowym (przypuszczalnie).

Table 2. Contents of anthocyanins in raspberry juices in 1998, 1999 and 2000
Tabela 2. Zawartości antocyjanów w sokach malinowych w latach 1998, 1999, 2000

Year of raspberry harvest Lata zbioru malin	Anthocyanins – Antocyjany			
	peak 1* area × 10^4 pik 1* pow. × 10^4	peak 2 ** area × 10^4 pik 2** pow. × 10^4	peak 3 – cy-3-glu pik 3 – cy-3-glu mg/l	peak 4 – cy-3-rut pik 4 – cy-3-rut mg/l
1998	5 515	1 042	428	756
1999	1 745	1 112	218	769
2000	5 505	819	641	520
x	4 255	991	429	682

*Cyanidin-3-sophoroside (probably), **cyanidin-3-glucorutinoside (probably), cy-3-glu-cyanidin-3-glucoside, cy-3-rut – cyanidin-3-rutinoside.

*Cyjanidyno-3-soforozyd (przypuszczalnie), **cyjanidyno-3-glukorutynozyd (przypuszczalnie), cy-3-glu – cyjanidyno-3-glukozyd, cy-3-rut – cyjanidyno-3-rutynozyd.

Table 3. Contents of anthocyanins in black currant juices in 1998, 1999, 2000
Tabela 3. Zawartości antocyjanów w sokach z czarnych porzeczek w latach 1998, 1999, 2000

Year of black currant harvest Lata zbioru czarnych porzeczek	Anthocyanins – Antocyjany			
	peak 1* area × 10^4 pik 1* pow. × 10^4	peak 2** area × 10^4 pik 2** pow. × 10^4	peak 3 – cy-3-glu pik 3 – cy-3-glu mg/l	peak 4 – cy-3-rut pik 4 – cy-3-rut mg/l
1998	1 492	9 048	208	6 550
1999	881	4 879	113	3 771
2000	1 353	8 736	170	6 579
x	1 242	7 554	164	5 633

*Delphinidin-3-glucoside (probably), **delphinidin-3-rutinoside (probably), cy-3-glu – cyanidin-3-glucoside, cy-3-rut – cyanidin-3-rutinoside.

*Delfinidyno-3-glukozyd (przypuszczalnie), **delfinidyno-3-rutynozyd (przypuszczalnie), cy-3-glu – cyjanidyno-3-glukozyd, cy-3-rut – cyjanidyno-3-rutynozyd.

cyanidin-3-xylorutinoside similarly as in red currant juice investigated by Goiffon et al. [1991]. Therefore there was examined the possibility of detection of the adulterations of raspberry and black currant juices with strawberry and red currant juices on the basis of anthocyanins analysis.

There was identified an additional peak (peak 5) – pelargonidin-3-glucoside, a typical anthocyanin of strawberry juices, absent in authentic raspberry juices on chromatographic profiles of adulterated raspberry juices (Fig. 1). There was detected 30%, 20% and 10% addition of strawberry juices to raspberry juices on the basis of pelargonidin-3--glucoside content. Contents of pelargonidin-3-glucoside were 496 mg/l, 332 mg/l and 165 mg/l, respectively (14.2%, 9.2% and 4.2% of total anthocyanin peak area) (Table 5). Peak 6 – pelargonidin-3-rutinoside and peak 7 – pelargonidin-3-glucoside acylated with

Table 4. Contents of anthocyanins in red currant juices in 1998, 1999, 2000
Tabela 4. Zawartości antocyjanów w sokach z czerwonych porzeczek w latach 1998, 1999, 2000

Year of red currant harvest Lata zbioru czerwonych porzeczek	Anthocyanins – Antocyjany				
	peak 1* area × 10^4 pik 1* pow. × 10^4	peak 2** area × 10^4 pik 2** pow. × 10^4	peak 3 – cy-3-glu pik 3 – cy-3-glu mg/l	peak 4*** area × 10^4 pik 4*** pow. × 10^4	peak 5 – cy-3-rut pik 5 – cy-3-rut mg/l
1998	52	472	35	462	175
1999	68	1 167	26	1 013	517
2000	315	1 580	138	1 659	629
x	145	1 073	66	1 045	440

*Cyanidin-3-sophoroside (probably), **cyanidin-3-glucorutinoside (probably), cy-3-glu – cyanidin-3--glucoside, ***cyanidin-3-xylorutinoside (probably); cy-3-rut – cyanidin-3-rutinoside.

*Cyjanidyno-3-soforozyd (przypuszczalnie), **cyjanidyno-3-glukorutynozyd (przypuszczalnie), cy-3-glu – cyjanidyno-3-glukozyd, ***cyjanidyno-3-ksylorutynozyd (przypuszczalnie); cy-3-rut – cyjanydyno-3-rutynozyd.

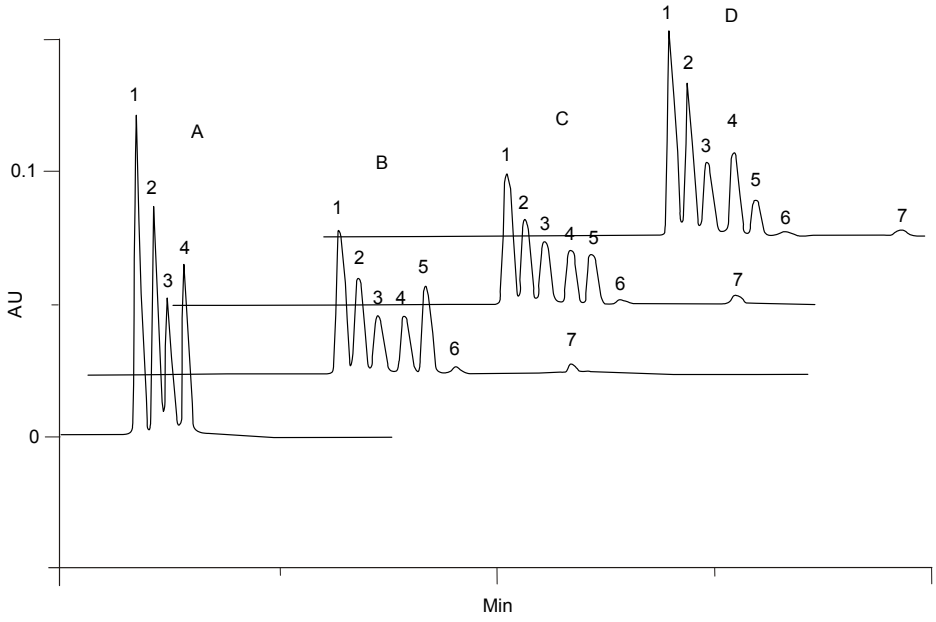

Fig. 1. Chromatograms of anthocyanins contained in raspberry juice (A) and in raspberry juices with 30% (B), 20% (C) and 10% (D) addition of strawberry juices in 1999. Peak identification in Tables 2 and 5
Rys. 1. Chromatogramy antocyjanów zawartych w sokach malinowych (A) i w sokach malinowych z 30- (B), 20- (C) i 10-procentowym (D) dodatkiem soków truskawkowych w 1999 r. Identyfikacja pików w tabeli 2 i 5

Table 5. Contents of anthocyanins in raspberry juices with addition of strawberry juices in 1998, 1999 and 2000
Tabela 5. Zawartości antocyjanów w sokach malinowych z dodatkiem soków truskawkowych w latach 1998, 1999, 2000

Addition of strawberry juices Dodatek soku truskawkowego	Year of raspberry and strawberry harvest Lata zbioru malin i truskawek	Anthocyanins – Antocyjany						
		peak 1* area × 10^4 pik 1* pow. × 10^4	peak 2** area × 10^4 pik 2** pow. × 10^4	peak 3 cy-3-glu pik 3 cy-3-glu mg/l	peak 4 cy-3-rut pik 4 cy-3-rut mg/l	peak 5 pg-3-glu pik 5 pg-3-glu mg/l	peak 6*** area × 10^4 pik 6*** pow. × 10^4	peak 7**** area × 10^4 pik 7**** pow. × 10^4
30%	1998	3 582	684	273	475	411	28	150
	1999	1 142	721	169	549	504	75	91
	2000	3 619	626	443	357	572	85	94
	x	2 781	677^t	295	460^t	496^t	63	112^t
20%	1998	4 016	757	319	482	280	16	95
	1999	1 297	790	192	609	346	32	70
	2000	4 263	763	494	397	370	53	63
	x	3 192	770	335	496^t	332^t	34	76^t
10%	1998	4 743	909	369	646	143	0	54
	1999	1 489	1 074	201	690	164	26	37
	2000	4 840	788	544	424	188	0	0
	x	3 691	924	371	587^t	165^t	9	30

*Cyanidin-3-sophoroside (probably), **cyanidin-3-glucorutinoside (probably), cy-3-glu – cyanidin-3--glucoside, cy-3-rut – cyanidin-3-rutinoside, pg-3-glu – pelargonidin-3-glucoside, ***pelargonidin-3-rutinoside (probably), ****pelargonidin-3-glucoside acylated with acetic acid (probably).

^{t}Contents or areas of anthocyanins in raspberry juices with addition of strawberry juices are significantly different at $\alpha = 0.05$ than contents or areas of the same anthocyanins in raspberry juices.

*Cyjanidyno-3-soforozyd (przypuszczalnie), **cyjanidyno-3-glukorutynozyd (przypuszczalnie), cy-3-glu – cyjanidyno-3-glukozyd, cy-3-rut – cyjanidyno-3-rutynozyd, pg-3-glu – pelargonidyno-3-glukozyd, ***pelargonidyno-3-rutynozyd (przypuszczalnie), ****pelargonidyno-3-glukozyd acylowany kwasem octowym (przypuszczalnie).

^{t}Zawartości lub powierzchnie antocyjanów w sokach malinowych z dodatkiem soków truskawkowych różnią się istotnie przy $\alpha = 0,05$ od zawartości lub powierzchni tych samych antocyjanów w sokach malinowych.

acetic acid had less significance in estimation of addition of strawberry juices to raspberry juices because of their small areas. Peak areas (contents) of anthocyanins from raspberry juices (peak 1, 2, 3 and 4) decreased in adulterated raspberry juices. Area of cyanidin-3-glucorutinoside in raspberry juices with 30% addition of strawberry juices

was statistically lower – 677 × 10^4 than in authentic raspberry juices – 991 × 10^4. Contents of cyanidin-3-rutinoside were significantly lower (P = 0.05) in raspberry juices with 30%, 20% and 10% addition of strawberry juices – 460 mg/l, 496 mg/l and 587 mg/l, respectively than in authentic raspberry juices – 682 mg/l.

Raspberry and red currant juices had four common anthocyanins: cyanidin-3-sophoroside, cyanidin-3-glucorutinoside, cyanidin-3-glucoside and cyanidin-3-rutinoside. Moreover red currant juices, unlike raspberry juices, contained cyanidin-3-xylorutinoside. Cyanidin-3-xylorutinoside (peak 4) indicated adulterations of raspberry juices with red currant juices (Fig. 2). Area of this anthocyanin presented on chromatogram of raspberry juice with 30% addition of red currant juice was 351 × 10^4 (6.7% of total anthocyanin peak area), on chromatogram of raspberry juice with 20% addition of red currant juice – 255 × 10^4 (4.7%) and on chromatogram of raspberry juice with 10% addition of red currant juice – 128 × 10^4 (2.3%) (Table 6). Peak area of cyanidin-3-glucorutinoside increased in raspberry juices adulterated with red currant juices (red currant juices contained more of this anthocyanin than raspberry juices) and peak areas of cyanidin-3--sophoroside, cyanidin-3-glucoside and cyanidin-3-rutinoside decreased in these juices. Contents of cyanidin-3-glucoside in raspberry juices with 30% and 20% addition of red currant juices were significantly lower (P = 0.05) – 304 mg/l and 345 mg/l, respectively than in raspberry juices without addition of red currant juices – 429 mg/l.

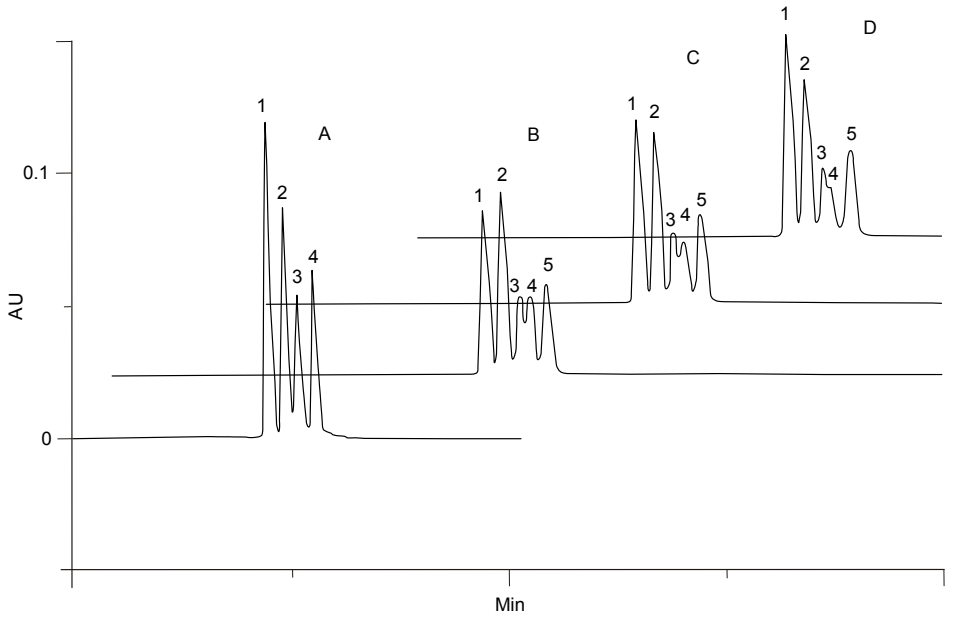

Fig. 2. Chromatograms of anthocyanins contained in raspberry juice (A) and in raspberry juices with 30% (B), 20% (C) and 10% (D) addition of red currant juices in 1999. Peak identification in Tables 2 and 6

Rys. 2. Chromatogramy antocyjanów zawartych w sokach malinowych (A) i w sokach malinowych z 30- (B), 20- (C) i 10-procentowym (D) dodatkiem soków z czerwonych porzeczek w 1999 r. Identyfikacja pików w tabeli 2 i 6

Table 6. Contents of anthocyanins in raspberry juices with addition of red currant juices in 1998, 1999 and 2000
Tabela. 6. Zawartości antocyjanów w sokach malinowych z dodatkiem soków z czerwonych porzeczek w latach 1998, 1999, 2000

Addition of red currant juice Dodatek soku z czerwonych porzeczek	Year of raspberry and red currant harvest Lata zbioru malin i czerwonych porzeczek	Anthocyanins – Antocyjany				
		peak 1* area × 10^4 pik 1* pow. × 10^4	peak 2** area × 10^4 pik 2** pow. × 10^4	peak 3 cy-3-glu pik 3 cy-3-glu mg/l	peak 4*** area × 10^4 pik 4*** pow. × 10^4	peak 5 cy-3-rut pik 5 cy-3-rut mg/l
30%	1998	3 683	715	296	147	565
	1999	1 163	1 285	129	397	665
	2000	3 599	1 051	488	510	449
	x	2 815	1 017	304[t]	351	560
20%	1998	4 101	826	357	95	636
	1999	1 323	1 247	132	346	692
	2000	4 300	1 042	547	323	520
	x	3 241	1 038	345[t]	255	616
10%	1998	4 769	924	393	59	676
	1999	1 506	1 119	143	154	726
	2000	5 129	1 064	629	172	552
	x	3 801	1 036	388	128	651

*Cyanidin-3-sophoroside (probably), **cyanidin-3-glucorutinoside (probably), cy-3-glu – cyanidin-3--glucoside, ***cyanidin-3-xylorutinoside (probably), cy-3-rut – cyanidin-3-rutinoside.

[t]Contents or areas of anthocyanins in raspberry juices with addition of red currant juices are significantly different at $\alpha = 0.05$ than contents or areas of the same anthocyanins in raspberry juices.

*Cyjanidyno-3-soforozyd (przypuszczalnie), **cyjanidyno-3-glukorutynozyd (przypuszczalnie), cy-3-glu – cyjanidyno-3-glukozyd, ***cyjanidyno-3-ksylorutynozyd (przypuszczalnie), cy-3-rut – cyjanidyno-3-rutynozyd.

[t]Zawartości lub powierzchnie antocyjanów w sokach malinowych z dodatkiem soków z czerwonych porzeczek różnią się istotnie przy $\alpha = 0,05$ od zawartości lub powierzchni tych samych antocyjanów w sokach malinowych.

Presence of pelargonidin-3-glucoside (peak 5) from strawberry juices determined adulteration of black currant with strawberry juices (Fig. 3). Content of pelargonidin-3--glucoside in black currant with 30% addition of strawberry juices was 450 mg/l (6.7% of total anthocyanin peak area), in black currant with 20% addition of strawberry juices – 324 mg/l (4.3%) and in black currant with 10% addition of strawberry juices – 179 mg/l (2.2%) (Table 7). Along with the increase of addition of strawberry juices, peak areas of anthocyanins from black currant juices (peak 1, 2, 3 and 4) decreased. Areas of delphinidin-3-glucoside and delphinidin-3-rutinoside in black currant with 30% addition of strawberry juices were statistically lower – 749×10^4 and 5144×10^4, respectively than

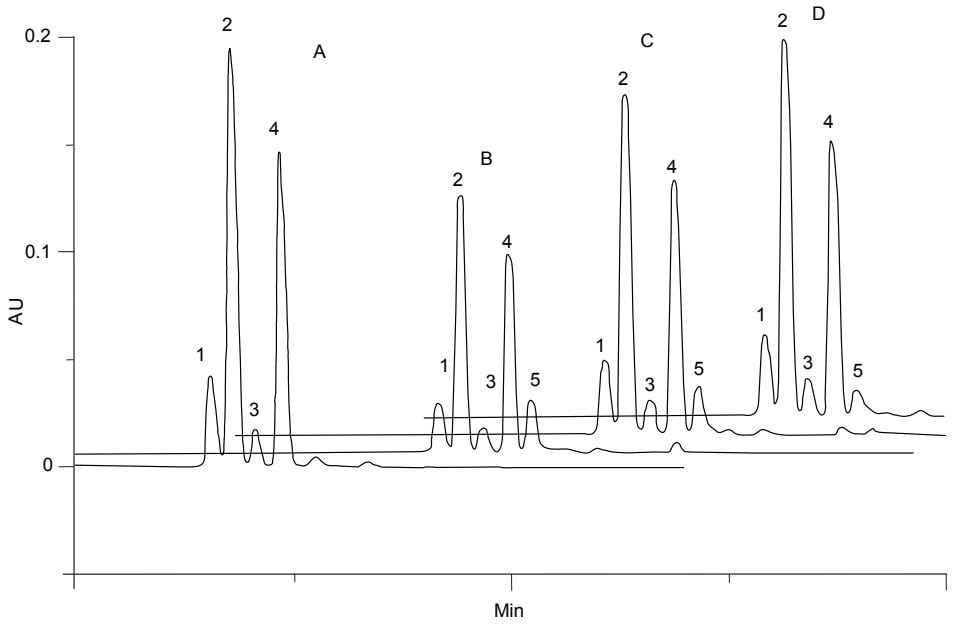

Fig. 3. Chromatograms of anthocyanins contained in black currant juice (A) and in black currant juices with 30% (B), 20% (C) and 10% (D) addition of strawberry juices in 1999. Peak identification in Tables 3 and 7

Rys. 3. Chromatogramy antocyjanów zawartych w sokach z czarnych porzeczek (A) i w sokach z czarnych porzeczek z 30- (B), 20- (C) i 10-procentowym (D) dodatkiem soków truskawkowych w 1999 r. Identyfikacja pików w tabeli 3 i 7

Table 7. Contents of anthocyanins in black currant juices with addition of strawberry juices in 1998, 1999, 2000
Tabela 7. Zawartości antocyjanów w sokach z czarnych porzeczek z dodatkiem soków truskawkowych w latach 1998, 1999, 2000

Addition of strawberry juices Dodatek soku truskawkowego	Year of black currant and strawberry harvest Lata zbioru czarnych porzeczek i truskawek	Anthocyanins – Antocyjany				
		peak 1* area × 10^4 pik 1* pow. × 10^4	peak 2** area × 10^4 pik 2** pow. × 10^4	peak 3 cy-3-glu pik 3 cy-3-glu mg/l	peak 4 cy-3-rut pik 4 cy-3-rut mg/l	peak 5 pg-3-glu pik 5 pg-3-glu mg/l
1	2	3	4	5	6	7
30%	1998	839	5 711	163	4 756	384
	1999	531	3 317	78	2 578	423
	2000	877	6 403	161	4 927	544
	x	749[t]	5 144[t]	134	4 087[t]	450[t]

Table 7 – cont.

1	2	3	4	5	6	7
20%	1998	1 213	7 495	177	5 266	271
	1999	736	4 132	111	3 188	344
	2000	913	6 572	150	5 159	358
	x	954	6 066	146	4 538	324[t]
10%	1998	1 402	8 142	187	5 837	119
	1999	822	4 588	131	3 512	211
	2000	1 212	7 571	153	5 782	208
	x	1 145	6 767	157	5 044	179[t]

*Delphinidin-3-glucoside (probably), **delphinidin-3-rutinoside (probably), cy-3-glu – cyanidin-3-glucoside, cy-3-rut – cyanidin-3-rutinoside, pg-3-glu – pelargonidin-3-glucoside.

[t]Contents or areas of anthocyanins in black currant juices with addition of strawberry juices are significantly different at $\alpha = 0.05$ than contents or areas of the same anthocyanins in black currant juices.

*Delfinidyno-3-glukozyd (przypuszczalnie), **delfinidyno-3-rutynozyd (przypuszczalnie), cy-3-glu – cyjanidyno-3-glukozyd, cy-3-rut – cyjanidyno-3-rutynozyd, pg-3-glu – pelargonidyno-3-glukozyd.

[t]Zawartości lub powierzchnie antocyjanów w sokach z czarnych porzeczek z dodatkiem soków truskawkowych różnią się istotnie przy $\alpha = 0{,}05$ od zawartości lub powierzchni tych samych antocyjanów w sokach z czarnych porzeczek.

in authentic black currant juices – 1242×10^4 and 7554×10^4. Also content of cyanidin-3-rutinoside in black currant with 30% addition of strawberry juices was significantly lower ($P = 0.05$) – 4087 mg/l than in authentic black currant juices – 5633 mg/l.

There was determined an additional peak (peak 4) – cyanidin-3-xylorutinoside, a typical anthocyanin of red currant juices, absent in authentic black currant juices on chromatographic profiles of adulterated black currant juices (Fig. 4). There was detected 30%, 20% and 10% addition of red currant to black currant juices on the basis of cyanidin-3-xylorutinoside content. Areas of cyanidin-3-xylorutinoside were 308×10^4 (2.9% of total anthocyanin peak area), 183×10^4 (1.6%) and 135×10^4 (1.1%) (Table 8). Peak areas of anthocyanins from black currant juices (peak 1, 2, 3 and 5) decreased in adulterated black currant juices. Areas of delphinidin-3-glucoside in black currant juices with 30% and 20% addition of red currant juices were significantly lower ($P = 0.05$) – 851×10^4 and 944×10^4, respectively than in authentic black currant juices – 1242×10^4. Also content of cyanidin-3-glucoside in black currant juices with 30% addition of red currant juices was significantly lower – 138 mg/l than in black currant juices without addition of red currant juices – 164 mg/l.

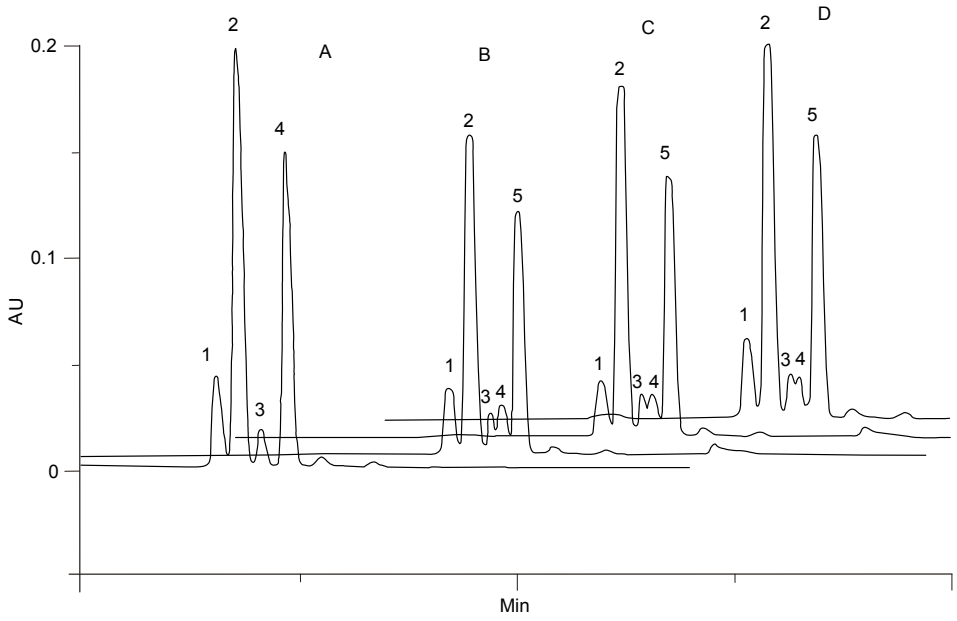

Fig. 4. Chromatograms of anthocyanins contained in black currant juice (A) and in black currant juices with 30% (B), 20% (C) and 10% (D) addition of red currant juices in 1999. Peak identification in Tables 3 and 8
Rys. 4. Chromatogramy antocyjanów zawartych w sokach z czarnych porzeczek (A) i w sokach z czarnych porzeczek z 30- (B), 20- (C) i 10-procentowym (D) dodatkiem soków z czerwonych porzeczek w 1999 r. Identyfikacja pików w tabeli 3 i 8

Table 8. Contents of anthocyanins in black currant juices with addition of red currant juices in 1998, 1999, 2000
Tabela 8. Zawartości antocyjanów w sokach z czarnych porzeczek z dodatkiem soków z czerwonych porzeczek w latach 1998, 1999, 2000

Addition of red currant juices Dodatek soku z czerwonych porzeczek	Year of black currant and red currant harvest Lata zbioru czarnych porzeczek i czerwonych porzeczek	Anthocyanins – Antocyjany				
		peak 1* area × 10^4 pik 1* pow. × 10^4	peak 2** area × 10^4 pik 2** pow. × 10^4	peak 3 cy-3-glu pik 3 cy-3-glu mg/l	peak 4*** area × 10^4 pik 4*** pow. × 10^4	peak 5 cy-3-rut pik 5 cy-3-rut mg/l
1	2	3	4	5	6	7
30%	1998	971	6 152	181	163	4 598
	1999	723	4 156	78	347	2 985
	2000	859	6 401	155	413	4 931
	x	851[t]	5 570	138[t]	308	4 171

Table 8 – cont.

1	2	3	4	5	6	7
20%	1998	1 152	7 356	201	70	5 332
	1999	703	4 590	82	216	3 286
	2000	977	7 139	157	264	5 075
	x	944[t]	6 362	147	183	4 564
10%	1998	1 316	8 228	215	57	6 203
	1999	849	4 830	87	138	3 542
	2000	1 199	7 928	170	209	5 995
	x	1 121	6 995	157	135	5 247

*Delphinidin-3-glucoside (probably), **delphinidin-3-rutinoside (probably), cy-3-glu – cyanidin-3-glucoside, ***cyanidin-3-xylorutinoside (probably), cy-3-rut – cyanidin-3-rutinoside.

[t]Contents or areas of anthocyanins in black currant juices with addition of red currant juices are significantly different at $\alpha = 0.05$ than contents or areas of the same anthocyanins in black currant juices.

*Delfinidyno-3-glukozyd (przypuszczalnie), **delfinidyno-3-rutynozyd (przypuszczalnie), cy-3-glu – cyjanidyno-3-glukozyd, ***cyjanidyno-3-ksylorutynozyd (przypuszczalnie), cy-3-rut – cyjanidyno-3-rutynozyd.

[t]Zawartości lub powierzchnie antocyjanów w sokach z czarnych porzeczek z dodatkiem soków z czerwonych porzeczek różnią się istotnie przy $\alpha = 0{,}05$ od zawartości lub powierzchni tych samych antocyjanów w sokach z czarnych porzeczek.

CONCLUSIONS

There was detected 30%, 20% and 10% addition of strawberry to raspberry and black currant juices on the basis of pelargonidin-3-glucoside content. Cyanidin-3-xylorutinoside indicated the 30%, 20% and 10% addition of red currant to raspberry and black currant juices. Anthocyanin analysis is an effective method for detection of berry juice adulterations.

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ZASTOSOWANIE ANALIZY ANTOCYJANÓW DO WYKRYWANIA ZAFAŁSZOWAŃ SOKÓW Z OWOCÓW JAGODOWYCH

Streszczenie. W pracy porównano profile chromatograficzne antocyjanów autentycznych i zafałszowanych soków z czarnych porzeczek i malin. Do rozdziału antocyjanów wykorzystano chromatograf do wysokosprawnej chromatografii cieczowej z detektorem DAD. Analiza zafałszowanych soków wymagała optymalizacji warunków elucji. Stosowano dwa eluenty w gradiencie: eluent A – 4,5-procentowy kwas mrówkowy i eluent B – 100--procentowy acetonitryl. Na podstawie zawartości pelargonidyno-3-glukozydu wykryto 30-, 20- i 10-procentowy dodatek soku truskawkowego do soku malinowego i z czarnych porzeczek. Cyjanidyno-3-ksylorutynozyd wskazywał na 30-, 20- i 10-procentowy dodatek soku z czerwonych porzeczek do soku malinowego i z czarnych porzeczek. Analiza antocyjanów jest skuteczną metodą wykrywania zafałszowań soków z owoców jagodowych.

Słowa kluczowe: antocyjany, HPLC, owoce jagodowe, soki, zafałszowania

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