

Semi-quantitative spectrophotometric determination of fruit juice adulteration by anthocyanin analysis

Semi-kvantitativ spektrofotometrisk analyse for saftforfalskning ved anthocyaninanalyse

K. KAACK

Summary

Anthocyanins of black currant, sour cherry, red currant, blackberry, elderberry, strawberry, raspberry and raspberry hybrids were separated by paper chromatography and percentage distribution was determined by direct spectrophotometry of paper strips with each anthocyanin.

The anthocyanins were identified with use of Rf-values and relative abundance by comparison

to literature values.

Percentage anthocyanin distribution was in accordance with the results given in the literature.

Adulteration of fruit juice may be detected due to alien anthocyanins. By use of absorbance of the anthocyanins an increasing amount of an alien juice can be detected because of different percentage distribution of anthocyanins in various fruit species.

Key words: Anthocyanins, adulteration, black currant, red currant, sour cherry, raspberry, blackberry, strawberry, elderberry, raspberry hybrids, quantitative determination.

Resumé

Anthocyaninindholdet i solbær, surkirsebær, ribs, brombær, hyld, jordbær, hindbær og hindbærhybrider blev bestemt ved spektrofotometrisk måling af absorbance for anthocyaninbånd fra papirkromatografisk separation.

Anthocyaninerne blev identificeret ved brug af Rf-værdier og angivelser for relativt indhold, som er angivet i litteraturen.

Procentfordeling for anthocyaninerne var i god overensstemmelse med litteraturangivelser.

Forfalskning af frugtsaft kan afsløres ved brug af anthocyaninmønstret for en frugtart. Bestemmelse af absorbance for de enkelte anthocyaniner kan også anvendes, selv om de samme anthocyaniner forekommer. Dette skyldes stærkt varierende anthocyaninfordeling i frugtarterne.

Nøgleord: Anthocyaniner, forfalskning, kvantitativ analyse, solbær, ribs, surkirsebær, hindbær, hyld, jordbær, brombær og hindbærhybrider.

Introduction

Blue and red fruit has a characteristic anthocyanin pattern (Table 1).

The different patterns of the fruit species and the different contents of the anthocyanins are useful for detection of adulteration of fruit juice or fruit juice concentrates.

Quantitative analysis can be carried out by use of high pressure chromatography which is a rather expensive method and by paper chromatography which is inexpensive. Paper chromatography is easy to perform and may be useful as a control method for the processing in-

dustry.

The aim of this experiment was to separate the anthocyanins in pure and adulterated fruit juices. Further was it the aim to calculate the percentage anthocyanin distribution after spectrophotometric determination of anthocyanin absorbance directly on the paper strips.

Analysis of anthocyanin muster for detection of adulteration has been applied earlier (8, 9, 14, 15, 21, 22). Difficulties appeared with certain fruit combinations and the detection limit has been rather high.

Table 1. Anthocyanins in fruit. *Anthocyaniner i frugter.*

Species <i>Art</i>	Anthocyanin <i>Anthocyanin</i>	Abbreviation <i>Forkortelse</i>	References <i>Referencer</i>
Strawberry <i>Jordbær</i>	Pelargonidin-3-glucoside	PE3G	11, 19, 23, 28, 32, 36
	Cyanidin-3-glucoside	CY3G	
Sour cherry <i>Surkirsebær</i>	Cyanidin-3-glucoside	CY3G	5, 6, 12, 17, 20, 23, 26, 30
	Cyanidin-3-rutinoside	CY3R	
	Cyanidin-3-sophoroside	CY3So	
	Peonidin-3-rutinoside	PE3R	
	Cyanidin-3-glucosylrutinoside	CY3GR	
Raspberry <i>Hindbær</i>	Cyanidin-3-glucoside	CY3G	1, 4, 10, 13, 18, 23, 24, 34
	Cyanidin-3-rutinoside	CY3R	
	Cyanidin-3-sambubioside	CY3Sa	
	Cyanidin-3-sophoroside	CY3So	
	Cyanidin-3-xylosylrutinoside	CY3XR	
	Cyanidin-3-glucosylrutinoside	CY3GR	
Black currant <i>Solbær</i>	Delphinidin-3-glucoside	DE3G	3, 13, 21, 22, 23, 31
	Cyanidin-3-glucoside	CY3G	
	Delphinidin-3-rutinoside	DE3R	
	Cyanidin-3-rutinoside	CY3R	
Red currant <i>Ribs</i>	Delphinidin-3-glucoside	DE3G	21, 22, 23
	Cyanidin-3-glucoside	CY3G	
	Cyanidin-3-rutinoside	CY3R	
	Cyanidin-3-sambubioside	CY3Sa	
	Cyanidin-3-sophoroside	CY3So	
	Cyanidin-3-xylosylrutinoside	CY3XR	
	Cyanidin-3-glucosylrutinoside	CY3GR	
Elderberry <i>Hylde</i>	Cyanidin-3-glucoside	CY3G	2, 27
	Cyanidin-3,5-diglucoside	CY3G5G	
	Cyanidin-3-sambubioside	CY3Sa	
	Cyanidin-3-glucosylrutinoside	CY3GR	
Blackberry <i>Brombær</i>	Cyanidin-3-glucoside	CY3G	34
	Cyanidin-3-rutinoside	CY3R	
Apple <i>Æble</i>	Cyanidin-3-galactoside	CY3Ga	7, 16, 29, 33
	Cyanidin-3-arabioside	CY3A	
	Cyanidin-7-arabioside	CY7A	

Materials and methods

Strawberry (*Fragaria × ananassa* cv. 'Senga sengana'), blackberry (*Rubus fruticosus*), red raspberry (*Rubus idaeus*), black raspberry (*Rubus occidentalis*), raspberry- and blackberry hybrids (*R. idaeus × R. occidentalis*), blackberry-, red raspberry- and black raspberry hybrid (*R. fruticosus × R. idaeus × R. occidentalis*), black currant (*Ribes nigrum*), red currant (*R. rubrum*), sour cherry (*Prunus cerasus*), and elderberry (*Sambucus nigra* cv. 'Sambucus nigra') were grown in 1987 at the Research Centre for Horticulture, Institute of Pomology.

The fruit was stored at -25°C until the manufacture of juice. 200 g of thawed fruit was pressed in a tincture press by increasing the pressure to 200 kg/cm^2 over a period of one hour. Sodium benzoate (0.2 p.c.) Potassium sorbate (0.1 p.c.) were added to the juice which was then pasteurised (10 min, 80°C) in 50 ml bottles, and cooled to 20°C in tap water. Pure and adulterated juice with 4, 8, 12 and 20 p.c. of other juices was stored at 3°C until analysis.

Anthocyanins from 10 ml juice were precipitated with 10 ml 8 p.c. neutral Pb-acetate, and 0.5 ml concentrated NH_3 . The supernatant was discarded after centrifugation and the precipitate was cleaned twice by suspension in 80 p.c. methanol and centrifugation. The anthocyanins were dissolved in 10 ml n-butanol and 1 ml concentrated HCl. A separation funnel was used to separate the anthocyanins from the supernatant by application of 100 ml petroleum ether. The yield was 1 ml concentrated anthocyanin solution of which 200 microliter was applied as a stroke on What-

mann No. 3 (140 × 460 mm) chromatography paper. After ascending separation (16 hours) in water: concentrated HCl: formic acid: n-butanol (80:40:1:8), and drying (20°C) the Rf-values were calculated before cutting the chromatography paper into strips for each anthocyanin. The strips were placed in a spectrophotometer (Shimadzu MPS-2000) and absorbance at 530 nm was measured and used for calculation of percentage anthocyanins.

Linear regression between absorbance values and p.c. alien fruit juice was calculated.

Anthocyanins were identified by use of Rf-values from the literature and comparison of chromatograms from the fruit species.

Results

Rf-values ($\times 100$) of anthocyanins in eight fruit species are presented in Table 2.

Percentage anthocyanins in blackberry, red raspberry, raspberry- and blackberry hybrids, black raspberry and a hybrid ('Brandywine') of red raspberry, black raspberry and blackberry is presented in Table 3. The accuracy of the method may be evaluated by comparison with the values in parentheses.

On average the contents were 13 p.c. DE3G, 12 p.c. CY3G, 36 p.c. DE3R and 39 p.c. CY3R with standard deviations of 2-3 p.c. in black current varieties (Fig. 1).

CY3GR and CY3R are the most abundant anthocyanins in sour cherry. The average contents were 7 p.c. CY3G, 25 p.c. CY3R, 7 p.c. PE3R, 13 p.c. CY3So and 48 p.c. CY3GR with standard deviations of 2, 9, 2, 2, 7 p.c. respectively (Fig. 2).

Table 2. Rf-values ($\times 100$) of anthocyanins in eight fruit species.

Rf-værdier ($\times 100$) for anthocyaniner i otte frugarter.

Anthocyanins <i>Anthocyaniner</i>	Black currant <i>Solbær</i>	Blackberry <i>Brombær</i>	Sour cherry <i>Kirsebær</i>	Red currant <i>Ribs</i>	Elderberry <i>Hyld</i>	Red raspberry <i>Hindbær</i>	Black raspberry <i>Sort hindbær</i>	Strawberry <i>Jordbær</i>
DE3G	10	—	—	15	—	—	—	17
CY3G	17	24	18	23	26	19	24	26
DE3R	28	—	—	—	—	—	—	—
CY3R	38	41	40	35	—	37	45	—
CY3G5G	—	—	—	—	44	—	—	—
PE3R	—	—	48	—	—	—	—	—
CY3Sa	—	—	—	54	61	55	59	—
CY3So	—	—	67	68	—	68	—	—
CY3XR	—	—	—	81	—	48	—	—
CY3Sa5G	—	—	—	—	83	—	—	—
CY3GR	—	—	87	88	—	86	82	—

Table 3. Percentage anthocyanins of blackberry, red raspberry, raspberry hybrid and blackberry. Values in brackets are calculated from mg/100 g of anthocyanins found by Torre & Barritt (34).

Procentfordeling af anthocyaniner i brombær, røde hindbær, hindbær × brombær og sorte hindbær. Værdierne i parentes er beregnet efter mg/100 g fundet af Torre & Barritt (34).

Species Art	Variety Sort	Percentage anthocyanins Procent anthocyaniner						
		CY3G	CY3R	CY3G5G	CY3Sa	CY3So	CY3XR	CY3GR
<i>R. fruticosus</i>	'Black Satin'	86 (100)	14 (0)	-	-	-	-	-
<i>R. idaeus</i>	'Glen Clova'	25 (23)	13 (7)	-	-	38 (49)	-	24 (21)
	'Meeker'	25 (25)	10 (7)	-	-	49 (47)	-	16 (21)
<i>R. idaeus</i> ×	'Boysenberry'	20 (26)	1 (3)	2 (0)	-	60 (44)	-	17 (26)
<i>R. occidentalis</i>	'Loganberry'	22 (22)	5 (7)	5 (0)	-	52 (48)	-	16 (24)
	'Tayberry'	42	43	-	-	3	-	12 -
	'Tummelberry'	7	10	-	-	36	-	47 -
<i>R. occidentalis</i>	'Cumberland'	30 (21)	37 (39)	-	11 (13)	-	22 (27)	-
	'Monger'	30 (30)	50 (34)	-	5 (13)	-	15 (23)	-
<i>R. fruticosus</i> ×	'Brandywine'	16	26	-	6	18	-	34
<i>R. idaeus</i> ×								
<i>R. occidentalis</i>								

Red raspberry varieties contained five anthocyanins (Fig. 3). CY3Sa was not detected in the varieties 'Canby', 'Glen Clova', 'Haida', 'Malling Admiral', 'Meeker' and 'Zenith'. CY3R could not be detected in 'Veten' and CY3GR was not detected in 'Nootka', 'Camenzind', 'Skeena',

'Glen Esk' and 'Ruku'. On average for the varieties containing the anthocyanin the content were 28 p.c. CY3G (n=15), 10 p.c. CY3R (n=14), 11 p.c. CY3Sa (n=9), 43 p.c. CY3So (n=15), and 19 p.c. CY3GR (n=10) with standard deviations of 6, 4, 8, 13 and 10 p.c. In the varieties 'Willamette'

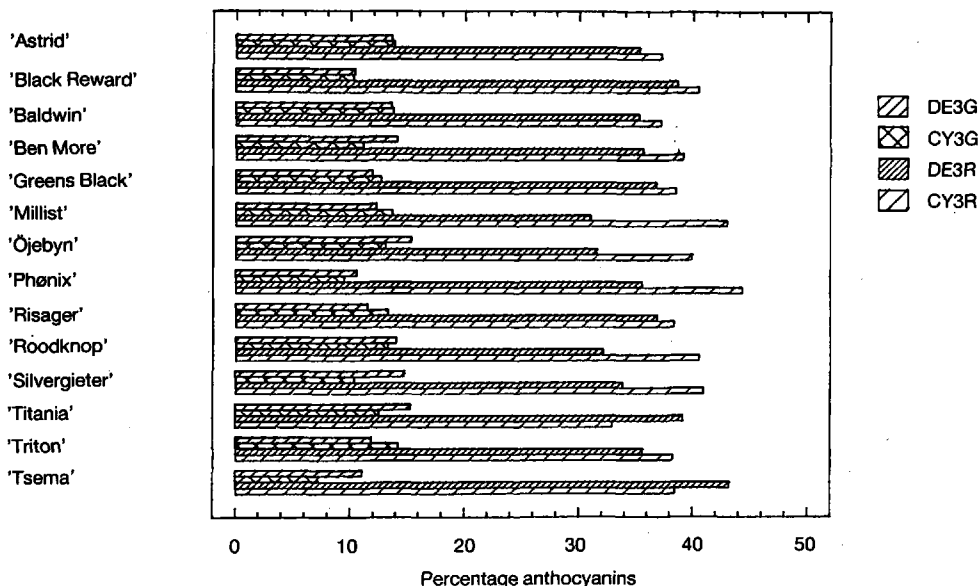


Fig. 1. Percentage anthocyanins of black currant cultivars.
Procentfordeling anthocyaniner i solbær.

'Dubbel Gorsem Kriek'
 'Wloszakowice 66'
 'Hiszpanka Pozna'
 'Jareniowka'
 'Wisnia serocka'
 'Wielcin K'
 'Przytoezvo'
 'Stevnsbær Viki'
 'Stevnsbær 14-26'
 'Stevnsbær 14-28'
 'Stevnsbær 18-31'
 'Stevnsbær 20-29'
 'Stevnsbær 22-26'
 'Stevnsbær 29-8'
 'Stevnsbær 29-12'

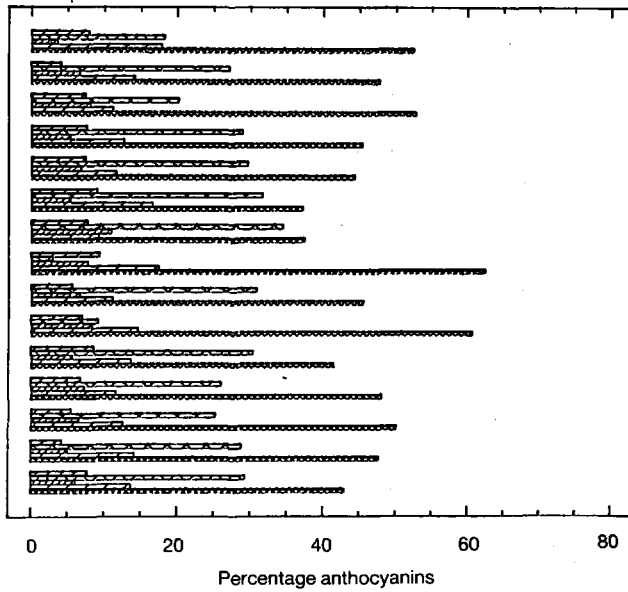


Fig. 2. Percentage anthocyanins of sour cherry cultivars.
Procentfordeling anthocyaniner i surkirsebær.

'Canby'
 'Glen Clova'
 'Haida'
 'Malling Admiral'
 'Meeker'
 'Zenith'
 'Rucami'
 'Malling Jewell'
 'Joy'
 'Veten'
 'Nootka'
 'Camenzind'
 'Skeena'
 'Glen Esk'
 'Ruku'

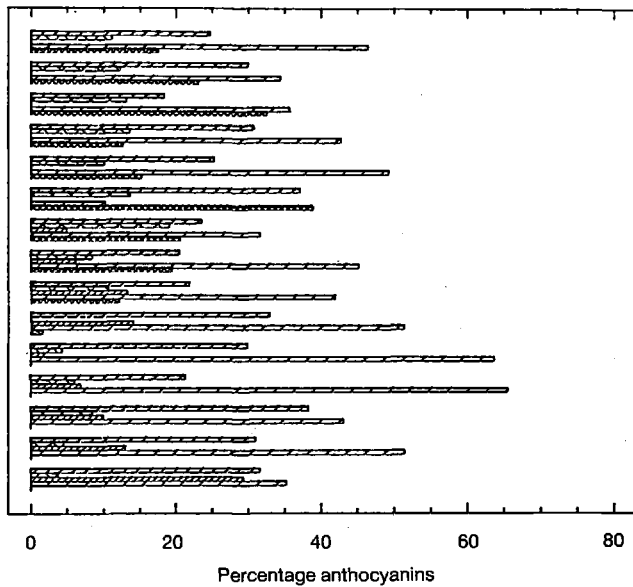


Fig. 3. Percentage anthocyanins of red raspberry cultivars.
Procentfordeling anthocyaniner i røde hindbær.

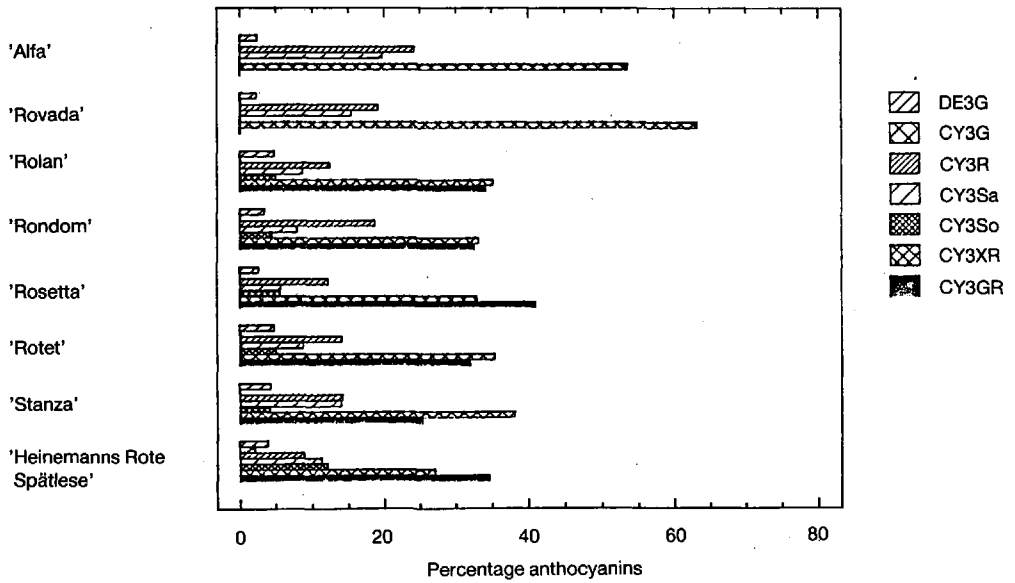


Fig. 4. Percentage anthocyanins of red currant cultivars.
Procentfordeling anthocyaniner i ribs.

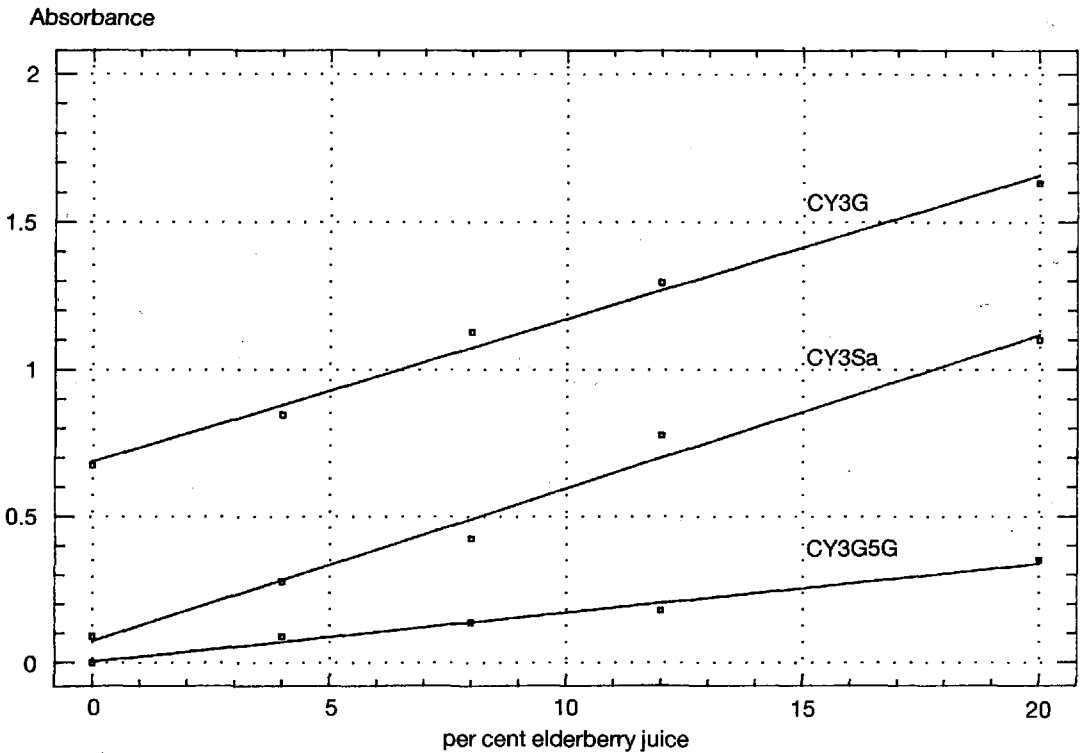


Fig. 5. Absorbance of certain anthocyanins in red raspberry juice adulterated with elderberry juice.
Absorbance for nogle anthocyaniner i hindbærsaft fortyndet med hyldesaft.

and 'Meeker', 24 p.c. CY3G, 8 p.c. CY3R, 62 p.c. CY3So, and 19 p.c. CY3GR (1) were found.

On average for the red currant varieties containing the anthocyanin the content were 4 p.c. DE3G (n=8), 2 p.c. CY3G (n=1), 15 p.c. CY3R (n=8), 11 p.c. CY3Sa (n=8), 6 p.c. Cy3So (n=6), 40 p.c. CY3XR, and 33 p.c. CY3GR with standard deviations of 4, 0, 5, 5, 3 and 12 p.c. respectively (Fig. 4). Only one variety ('Heinemanns Rote Spätlese') contained CY3G. CY3So and CY3GR was not detected in 'Alfa' and 'Rovada'.

An example from determination of CY3G, CY3Sa and CY3G5G in red raspberry juice adulterated with elderberry juice is presented in (Fig. 5). The absorbance of CY3G, CY3G5G and CY3Sa increases with elderberry juice concentration. This is due to respectively much lower, very much lower and zero content of these anthocyanins in red raspberry.

Further results from analysis of adulterated juice are presented in Table 4 as correlation coefficients for the relationship between absorbance and per cent alien fruit juice.

Discussion

The anthocyanin concentration increases with ripening of red raspberry (1) sour cherry (5, 35) and black currant (31), but the percentage distribution is almost constant. Black currant contained four anthocyanins. Compared to literature values (31) higher and lower content were found for CY3G and DE3R respectively. The standard deviation was very low, which means that the percentage distribution is almost constant, even between varieties. Five anthocyanins were detected in sour cherry with a percentage distribution in accordance with earlier results (6). Different percentage distribution and higher standard deviations between varieties, have been reported (6, 35).

The anthocyanin pattern was more complicated in red raspberry. Some varieties did not contain CY3Sa. This was also found earlier (1) in the varieties 'Canby', 'Glen Clova', 'Malling Admiral' and 'Meeker'. In several varieties of red raspberry and black raspberry the results were in accordance with literature values (1, 34). Red currant had from four to seven anthocyanins. The varieties with six anthocyanins belong to the petraeum group which descends from wild growing *Ribes petraeum* (25) which contains mostly

CY3XR and CY3GR. The wild growing *Ribes alpinum* contains mostly CY3G and CY3GR (25) which shows that 'Alfa' and 'Rovada' descends from this specie.

Adulteration of red raspberry juice with elderberry juice may (Fig. 5) be detected by measurement of absorbance for CY3G5G, CY3Sa or CY3G. The concentrations of CY3Sa and CY3G are the most abundant anthocyanins in elderberry (2) which according to Table 4 is useful for detection of adulteration of other juices with elderberry juice. Detection of adulteration of juice of black currant, blackberry or strawberry with juice containing anthocyanins with higher Rf-values is easy.

Adulteration of red raspberry juice with juice of black currant or sour cherry may be detected by analysis for DE3G-DE3R-CY3R and CY3R-CY3GR respectively. The reasons are either nonoccurrence or lower concentration in red raspberry. For similar reasons the combinations DE3R-CY3R, CY3So-CY3GR or CY3G-CY3So-CY3GR may be used for detection of adulteration of red currant juice with juice of black currant, sour cherry or red raspberry. Adulteration of juice of sour cherry with juice of elderberry, black currant, red raspberry or red currant may be detected with analysis for the following anthocyanin combinations CY3G-CY3G5G-CY3Sa, DE3G-DE3R, CY3So or CY3Sa respectively.

From these results it is obvious that quantitative anthocyanin analysis based on paper chromatography and spectrophotometric determination of absorbance of different anthocyanin combinations are useful for detection of adulteration.

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Table 4. Correlation coefficient for the relation between absorbance of anthocyanins and p.c. adulteration.
Korrelationskoefficienter for sammenhæng mellem absorbance og tilsat saftprocent.

		DE3G	CY3G	DE3R	CY3R	CY3G5G	CY3Sa	CY3So	CY3XR	CY3Sa5G	CY3GR
Red raspberry	Elderberry*										
<i>Hindbær</i>	<i>Hylde</i>	-	0.98	-	-	0.97	0.98	-	-	0.99	-
	Black Currant										
	<i>Solbær</i>	0.99	-	0.97	0.98	-	-	-	-	-	-
	Sour Cherries										
	<i>Kirsebær</i>	-	-	-	0.96	-	-	-	-	-	0.99
Red currant	Elderberry										
<i>Ribs</i>	<i>Hylde</i>	-	0.86	-	-	-	0.72	-	-	-	-
	Black currant										
	<i>Solbær</i>	-	-	0.99	0.98	-	-	-	-	-	-
	Sour cherries										
	<i>Kirsebær</i>	-	-	-	-	-	-	0.87	-	-	0.92
	Red raspberry										
	<i>Hindbær</i>	-	0.94	-	-	-	-	0.97	-	-	0.93
Sour cherry	Elderberry										
<i>Kirsebær</i>	<i>Hylde</i>	-	0.98	-	-	0.98	0.95	-	-	-	-
	Black currant										
	<i>Solbær</i>	0.93	-	0.97	-	-	-	-	-	-	-
	Red raspberry										
	<i>Hindbær</i>	-	-	-	-	-	-	0.75	-	-	-
	Red currant										
	<i>Ribs</i>	-	-	-	-	-	0.84	-	-	-	-

*) 0, 4, 8, 12, 20 p.c. juice from these fruit species was used for adulteration.

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