Danish Research Service for Plant and Soil Science
Research Centre for Horticulture
Department of Food Science and Technology
DK-5792 Årslev

Report no. 2092

Processing of anthocyanin colourant from elderberry (*Sambucus nigra* L.) pomace

Fremstilling af anthocyaninekstrakt fra pressekage af hyldebær (Sambucus nigra *L.*)

K. KAACK

Summary

Experiments with application of citric acid have been carried out with the purpose of processing anthocyanin colourants from elderberry pomace. The experiments encompassed varying concentrations of citric acid, different ratios of solvent: pomace and multiple extractions. With the usual pressing of elderberry addition of citric and hydrochloric acid had an increasing (25%) effect on the anthocyanin content in the juice.

With the first extraction of pomace the anthocyanin content of the extracts increased linearly with the citric acid concentration. By the following extraction stages the effect of citric acid was minimal.

For practical purposes a ratio of 1:1 of solvent: pomace is applicable. After normal pressing of elderberry the solvent containing 8–10 w/w% citric acid can be mixed with the pomace and the pressing can be repeated after 10 minutes.

By application of a solvent with 8–10 w/w% citric acid the first extracts had an anthocyanin content of the same magnitude as in juice processed by the usual pressing. Two or three pressings may be carried out, but the content of anthocyanin decreases by increasing number of extraction stages.

The contained extracts can be concentrated before storage. If solvents with 8–10 w/w% citric acid are applied a final citric acid concentration of 40–50 w/w% can be obtained.

Four anthocyanins cyanidin-3-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-sambubioside and cyanidin-3-sambubioside-5-glucoside present in elderberries were separated by paper chromatography and the percentage distribution of the anthocyanins was determined by spectrophotometric analysis of the paper chromatograms.

The percentage distribution of the four anthocyanins was independent of the number of extraction stages and of the citric acid concentration applied by extraction. By increasing the citric acid concentration higher anthocyanin stability was obtained.

Key words: *Sambucus nigra* L., elderberries, colourant, anthocyanin, extraction, anthocyanin stability.

Resumé

Med henblik på anvendelse ved produktion af hjemmevinsæt er der blevet udført forsøg med fremstilling af anthocyanin-ekstrakter af hyldebærpressekage. Der er udført forsøg med anvendelse af citronsyreopløsninger samt varierende forhold mellem opløsningsmiddel og pressekage ved multiple ekstraktioner. Forsøgene viste, at der opnås 25 pct. højere anthocyaninkoncentration i saften, hvis der tilsættes citronsyre eller saltsyre ved den egentlige saftfremstilling. Der var stor effekt af citronsyretilsætning ved ekstraktion af pressekage. Ved første ekstraktion var der en betydelig effekt af stigende citronsyrekoncentration på anthocyaninindholdet i ekstrakten.

Når ekstraktionen skal udføres i praksis, vil det utvivlsomt være optimalt at beholde presseresten fra den egentlige saftpresning i saftpressen og derpå tilsætte citronsyreopløsning, foretage omrøring og derpå gentage presningen efter 10 minutters ekstraktionstid. Det kan muligvis være økonomisk fordelagtigt at udføre 2–3 ekstraktioner.

Hvis ekstrakten skal koncentreres før eventuel opbevaring, vil det næppe være fordelagtigt at øge citronsyrekoncentrationen over 8–10 w/w pct. Dette vil give en citronsyrekoncentration på 40 – 50 w/w pct. i koncentratet.

Stigende citronsyrekoncentration gav øget anthocyaninstabilitet.

Anthocyaninerne cyanidin-3-glucosid, cyanidin-3,5-diglucosid, cyanidin-3-sambubiosid og cyanidin-3-sambubiosid i saft og ekstrakt blev identificeret ved papirkromatografi, og indholdet blev bestemt ved spektrofotometrisk analyse af papirkromatogrammerne. Procentfordelingen for de fire anthocyaniner i ekstrakten var den samme som i saft fra den egentlige saftpresning, og den var uafhængig af citronsyrekoncentrationen og antallet af ekstraktioner.

Nøgleord: *Sambucus nigra* L., hyld, farvestof, anthocyaniner, ekstraktion, anthocyaninstabilitet.

Introduction

By processing elderberry juice an anthocyanin concentration of 70% of the concentration in the fruits is obtained in the juice (10). This means that the pomace, which amounts to about 20% of the raw fruits, is very rich in anthocyanins.

Anthocyanin colourants have been processed from grape and cranberry pomace by extraction with ethanolic or methanolic solutions of hydrochloric acid (5,6,7,11) and from wine and elderberry pomace by use of solvents with malic or citric acid (2,3,12).

The aim of this paper is to exploit the possibilities of processing an anthocyanin colourant for home wine sets by extraction of elderberry pomace with citric acid, which normally is included in the formulation for this product.

Materials and methods

Fruits harvested in 1989 at the optimum harvest time were stored at –25°C until use in four experiments. A mixture of equal amounts of fruits from five cultivars ('Sambu', 'Sampo', 'Samdal', 'Samyl' and 'Samidan') were applied in all the experiments carried out.

Experiment number one encompassed juice processing by addition of citric acid and hydrochloric acid to the fruits immediately before coarse grinding in a Waring blender and pressing in a Tincture press. For each experimental level 250 g of fruits were applied. During the first 30 minutes of the pressing the piston moved slowly downwards without increasing pressure and a considerable runoff of juice occured. Then the pressure was increased to 500 bar for two hours. The juice was collected in two 100 ml jars and pasteurized by heating in a water bath at 80°C for 10 minutes.

In experiment number two pomace from juice processed with a juice yield of 82 w/w % was extracted with 0,4,6,7 and 8 w/w % citric acid solutions in solvent:pomace (S:P) ratios of 2, 4 and 8. Five gramme of pomace was extracted three times with 10, 20 or 40 gramme of citric acid solutions with the above mentioned concentrations. The extraction time was 10 minutes. Clear supernatant extracts were obtained by centrifugation immediately after the extraction time had expired. The extracts were pasteurized as described for experiment number one.

Experiment three encompassed five successive extractions of elderberry pomace in a ratio of 1:1 using solutions with 2, 4, 6 and 8 w/w % citric acid. After the expired extraction time the mix-

ture of solvent and pomace was pressed by use of a Tincture press as described for experiment one.

Two determinations of Rf-values and percentage distribution of the four anthocyanins were carried out.

In experiment four determination of the anthocyanin degradation rate was carried out. Normal pressing juice and extracts from experiment number three were heated on a water bath at 80°C up to three hours. At appropriate time intervals samples were taken for anthocyanin analyses. For each experimental level two samples were taken at each time interval.

The content of anthocyanins in juices and extracts was determined by use of the spectrophotometric pH-differential method described by *Wrolstad* (13), and the percentage distribution of anthocyanins by direct spectrophotometric analyses as described by *Kaack* (9).

Analysis of variance was used to evaluate the effect of extraction parameters on percentage anthocyanin distribution, yield of extracts and of kg extract per ton of elderberries.

The relationship between anthocyanin content and the extraction parameters was evaluated by use of regression analysis, which also was used for calculation of anthocyanin degradation rate constants. In the figures and tables only significant ($P<0.05$) relationships are presented.

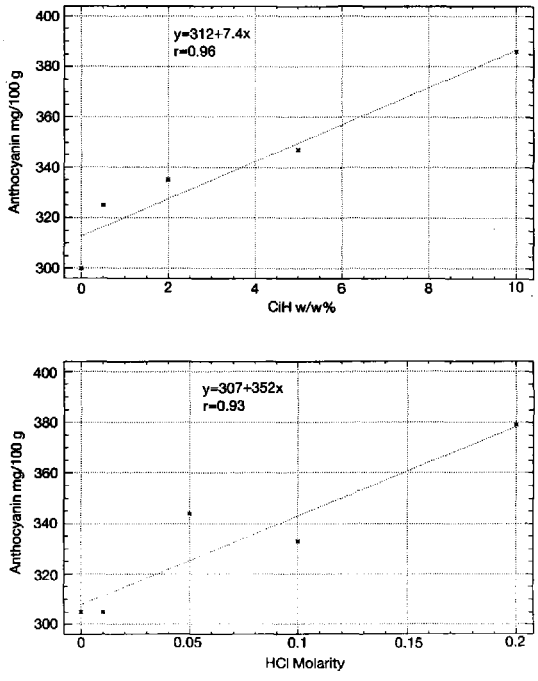

Fig. 1. Anthocyanin content in juice processed by addition of citric and hydrochloric acid (HCl) to the fruits before grinding and pressing. Experiment number one.

Anthocyaninindhold i saft fremstillet ved tilsætning af citronsyre (CiH) og saltsyre til frugterne før formaling og presning. Forsøg nr. 1.

Results and discussion

Results from determination of the effect of addition of acids to the fruits before grinding and pressing is shown in Fig. 1.

Increase of the concentration of citric acid (CiH) or hydrochloric acid had an significant increasing effect on the concentration of the anthocyanin content in the juice.

At the high concentration of both acids the increase in the anthocyanin content was about 25%.

Table 1. Rf×100 and average percentage of anthocyanins in juices processed with addition of citric (CiH) or hydrochloric (HCl) acid before grinding and pressing. Experiment number one.

Rf×100 og gennemsnitlig procentfordeling for anthocyaniner i saft fremstillet ved tilsætning af citronsyre (CiH) eller saltsyre (HCl) før presning. Forsøg nr. 1.

Anthocyanin	Rf×100		Anthocyanin % *Anthocyanin, pct* CiH	HCl	LSD
Cyanidin-3-glucoside	Cy3G	18	47	49	2
Cyanidin-3,5-diglucoside	Cy3,5G	37	7	7	1
Cyanidin-3-sambubioside	Cy3Sa	53	36	34	2
Cyanidin-3-sambubioside-5-glucoside	Cy3Sa5G	78	10	10	1

Addition of citric acid or hydrochloric had no effect on the juice yield, which on average was 82 w/w %.

The percentage distribution of the four anthocyanins in the juice processed by normal processing of the fruits was not adversely affected by the type or the concentration of the acids. Table 1 shows the average percentage of anthocyanins in juice processed by addition of citric and hydrochloric acid before grinding and pressing of elderberries.

Fig. 2 shows the anthocyanin content in juices from experiment number two from multiple extraction of elderberry pomace with 0-8 w/w % citric acid solutions. The concentration of anthocyanin increased significantly with increasing citric acid concentration and decreased significantly with increasing solvent:pomace ratio. From Fig. 2 it was quite clear that a low solvent: pomace ratio and a high concentration of citric acid are advantageous as pointed out by *Brønnum-Hansen* and *Flink* (3).

Fig. 3 shows that the content of anthocyanin in extracts of pomace increased significantly with increasing concentration of citric acid. At the first extraction step the content of anthocyanin increased linearly with the citric acid concentration. The increase at the later extraction steps could be described by multiplicative mathematical models as shown in Fig. 3.

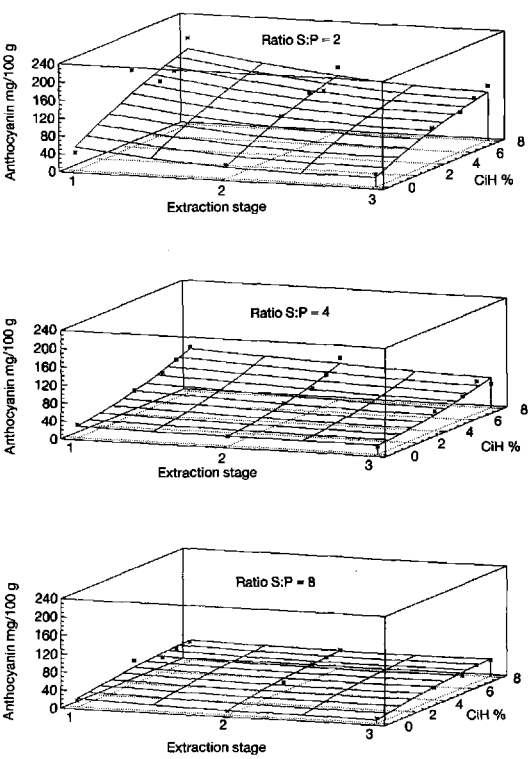

Fig. 2. Anthocyanin content in extracts processed from pomace by three multiple extractions using solvents with 0, 2, 4, 6, 7, 8 w/w % citric acid and S:P ratios of 2, 4, and 8. Experiment number two.
Anthocyaninindhold i ekstrakter fremstillet af pressekage ved tre successive ekstraktioner med 0, 2, 4, 6, 7, 8, w/w pct. citronsyreopløsninger og S:P-forhold på 2, 4 og 8. Forsøg nr. 2.

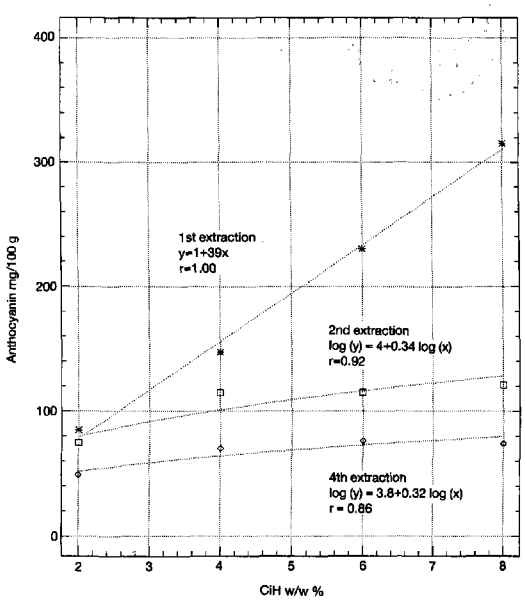

Fig. 3. Anthocyanin content in extracts from 1st, 2nd and 4th extraction of pomace with citric acid solutions and S:P ratio 1. Experiment number three.
Anthocyaninindhold i ekstrakter fra første, anden og fjerde ekstraktion af pressekage med citronsyreopløsninger og S:P-forhold på 1. Forsøg nr. 3.

At citric acid concentrations above 4 w/w % a considerably lower content of anthocyanin was obtained after the second to fifth extraction step. By comparison with Fig. 1 it was concluded that by application of 8 w/w % citric acid an anthocyanin concentration (320 mg/100 g) at the

Table 2. Percentage yield by combined extraction and pressing of pomace. Experiment number three.
Procent udbytte ved kombineret ekstraktion og presning af pressekage. Forsøg nr. tre.

	Extraction step *Ekstrationstrin*					
	1	2	3	4	5	LSD
Yield of extract w/w % *Ekstraktionsudbytte w/w pct.*	105	92	92	87	87	6
kg extract/ton elderberries *kg ekstrakt/ton hyld*	189	166	166	157	157	10

same level as in juice can be obtained by extraction of the pomace.

The yield of extracts in experiment three was not affected by the citric acid concentration, but decreased as shown in Table 2 with the number of extraction steps. From the above mentioned results the yield of juice and extracts from five extractions was calculated as shown in Table 2.

As shown in Table 3 the concentration of citric acid had no effect on the percentage distribution of anthocyanins in extracts from experiment number three. Thus it may be concluded that the quality of the extracts is equal to the quality of juice obtained by pressing. Compared to the values of percentage anthocyanin distribution given in the literature (1,4) higher contents of the minor anthocyanins (CY3,5G, Cy3Sa5G) and lower contents of the major anthocyanins (Cy3G, Cy3Sa) should have been obtained. Earlier applications (9) have shown a very good agreement betwen results obtained from HPLC analyses and the method applied in this experiment.

Fig. 4 shows the results from heating pressing juice with and without addition of citric acid. The degradation rate for anthocyanin in juice without added citric acid is of the size of order found earlier for elderberry juice (10). By increase of the concentration of citric acid considerably lower degration rate constants were found. This is a result of the low pH-value and has been found earlier (8). From this result it may be expected that degradation of anthocyanin during concentration of extracts may decrease with increasing citric acid concentration.

Degradation of anthocyanins may be described by use of mathemathical models almost according to a first order reaction. This is shown below where c is the anthocyanin concentration, a is a constant, b the degradation rate constant (1/min) and t the time in minutes.

$$\log (c) = a + bt \qquad 1)$$

The results from determination of degration rate constants (b) of the first three multiple extracts of pomace with citric acid solutions at 80°C are shown in Table 4. The pH of juice from normal pressing was 3.9.

Table 3. Values of Rf×100 and percentage distribution of anthocyanins in the extracts obtained by extraction with citric acid. Experiment number three.
Værdier af Rf×100 og procentfordeling af anthocyaniner i ekstrakter fra ekstraktion med citronsyre. Forsøg nr. tre.

			Citric acid w/w % *Citronsyre w/w pct.*				
	Rf×100	Literature *Litteratur*	2	4	6	8	LSD
			Anthocyanin % *Antocyanin pct.*				
Cy3G	22	66	49	51	55	52	5
Cy3,5G	40	1	4	3	3	4	2
Cy3Sa	57	32	38	40	37	39	2
Cy3Sa5G	81	1	9	5	5	5	2

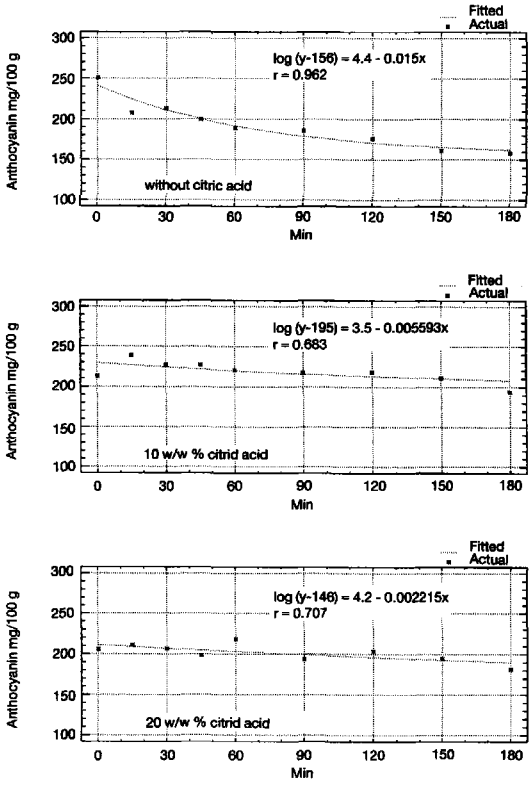

Fig. 4. Content of anthocyanin in elderberry juice with 0, 10, 20 w/w % citric acid heated at 80°C. Experiment number four.

Indhold af anthocyanin i hyldebærsaft med 0, 10, 20, w/w pct. citronsyre opvarmet ved 80°C. Forsøg nr. 4.

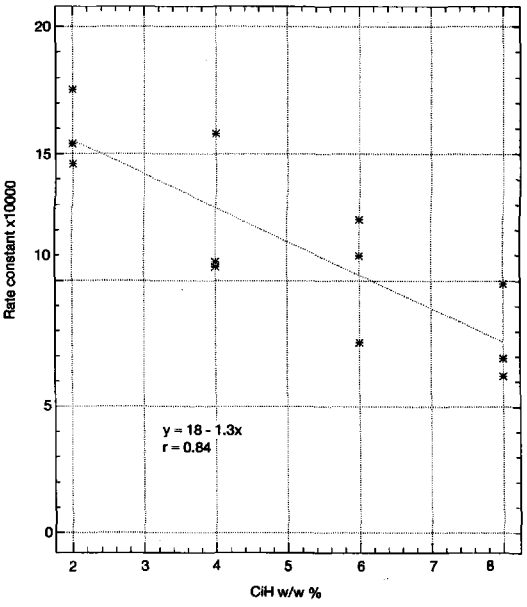

Fig. 5. Anthocyanin degradation constants of pomace extracts in relation to citric acid concentration. Experiment number four.

Anthocyaninnedbrydningskonstanter for pressekageekstrakter i forhold til citronsyrekoncentration. Forsøg nr. 4.

In Fig. 5 the rate constants multiplied by –10000 are plotted against the citric acid concentration of the solvent used by extraction. From this figure it can be concluded that the degration rate decrease by increasing citric acid concentration in the solvent and the extract.

Table 4. Rate degradation constants determined by heating of the extracts in a water bath at 80°C. The values in parentheses are correlation coefficients from regression analyses according to equation (1).

Hastighedskonstanter for nedbrydning af anthocyaniner i ekstrakter fra anvendelse af citronsyreopløsninger til ekstraktion. Værdierne i parenteserne er korrelationskoefficienter fra regressionsanalyse ved brug af udtrykket (1).

Stage *Trin*	Citric acid w/w % *Citronsyre w/w %*			
	2	4	6	8
	Reaction rate constants (–10000×b) *Hastighedskonstanter (–10000×b)*			
1	18 (0.87)	16 (0.87)	12 (0.85)	10 (0.87)
2	15 (0.92)	11 (0.91)	8 (0.82)	7 (0.78)
3	15 (0.92)	11 (0.94)	11 (0.98)	6 (0.80)
pH (average) *pH (gns.)*	2.7	2.4	2.2	2.3

Processing of a colourant from elderberry pomace can be carried out in connection with normal pressing. After mixing of the pomace with an equal amount of a 8-10 w/w % citric acid solvent pressing may be repeated after an extraction time of five minutes.

The content of anthocyanin in the first extracts are of the same magnitude as in the juice processed by usual methods.

A second or third extraction may be carried out but application of a citric acid solution is not necessary at the last extraction stage because enough acid remains in the pomace after the previous extraction. If concentration of extracts is to be carried out a citric acid concentration of 40-50 w/w % will be obtained in the concentrate.

References

1. *Brønnum-Hansen, K. & Hansen, S.H.* 1983. High-performance liquid chromatographic separation of anthocyanins of *Sambucus nigra* L. J. Chromatography 262, 385-392.
2. *Brønnum-Hansen, K., Jacobsen, F. & Flink, J.M.* 1985. Anthocyanin colourants from elderberry *(Sambucus nigra L.)*. 1. Process considerations for production of the liquid extract. J. Fd. Technology 20, 703-711.
3. *Brønnum-Hansen, K. & Flink, J. M.* 1985. Anthocyanin colourants from elderberry *(Sambucus nigra L.)* 2. Process considerations for production of a freeze dried product. J. Fd. Technology 20, 713-723.
4. *Brønnum-Hansen, K. & Flink, J. M.* 1986. Anthocyanin colourants from elderberry *(Sambucus nigra L.)*. IV. Further studies on production of liquid extracts, concentrates and freeze dried powders. J. Fd. Technology 21, 605-614.
5. *Chiriboga, C. & Francis, F. J.* 1970. An anthocyanin recovery system from cranberry pomace. J. Amer. Hort. Soc. 95, 233-236.
6. *Chiriboga, C. & Francis, F.J.* 1973. Ion exchange purified anthocyanin pigments as a colourant for cranberry juice cocktail. J. Fd. Sci. 38, 464-467.
7. *Fuleki, T. & Francis, F.J.* 1968. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanins in cranberries. J. Fd. Sci. 33, 72-77.
8. *Havlikova, L. & Mikova, K.* 1985. Heat stability of anthocyanins. Z. Lebensm. Unters. Forsch. 181, 427-432.
9. *Kaack, K.* 1988. Semi-quantitative spectrofotometric determination of fruit juice adulteration by anthocyanin analyses. Tidsskr. Planteavl 92, 279-287.
10. *Kaack, K.* 1989. Juice processsing from elderberry *(Sambucus nigra L.)*. Tidsskr. Planteavl 93, 365-368.
11. *Metvier, R.P., Francis, F.J. & Clydesdale, F.M.* 1980. Solvent extraction of anthocyanins from wine pomace. J. Fd. Sci. 45, 1099-1100.
12. *Philip, T.* 1974. An anthocyanin recovery system from grape wastes. J. Fd. Sci. 39, 359.
13. *Wrolstad, R.E.* 1976. Color and pigment analysis in fruit products. Bullletin Nr. 624. Corvallis, OR: Oregon Agricultural Station.

Manuscript received 5 July, 1990.