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Black chokeberry *(Aronia melanocarpa)* for manufacture of a food colorant

Sortrøn (Aronia melanocarpa) *til fremstilling af farvestoffer til levnedsmidler*

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Summary

A field experiment with 5 cultivars of chokeberry harvested at different maturity stages, has been carried out.

4 years after planting the average fruit yield was 5.7 g/bush. This mean a yield of about 11 ton/hectare (2000 bushes/hectare). The variation in fruit weight from 0.67 to 0.88 g/fruit is not of practical importance because harvesting can be carried out by use of a black currant combiner and because the fruits probably is useful mostly for processing of juice as a colorant.

The content of soluble solids was 18-22 g/100 g which is a high value compared to other fruits. *A. melanocarpa* "Kashamachi" had the highest content followed by 'Aron' and *A. melanocarpa* "Estland" with 19-20 g/100 g. The content of soluble solids was only 18 g/100 g in *A. melanocarpa* "Mandschurica" and 'Viking'.

All the cultivars had almost equal average content of anthocyanins and titratable acids. The average content of titratable acid was 11 g/100 g which is at the level as for apples and other berries. In chokeberries the average content of anthocyanins was at the same level as in elderberries, about 750-950 mg/100 g.

The anthocyanins in chokeberry is more heat stable than anthocyanins from strawberry and black currant, but less stable compared to anthocyanins from elderberry and grapes. It is not yet possible to explain the differences in anthocyanin stability from the chemical structure of the anthocyanins in the actual fruits. Probably increasing glycosylation causing decreasing hydrolysis rates is the most important reason for differences in stability of colorants.

The degradation of anthocyanins follow a reaction of first order. By use of the Arrhenius-equation the activation energy was calculated to 16-20 kcal/mole.

When chokeberry is applied as colorant for processing of plum juice a satisfactory colour and no deterioration in flavour is obtained by use of 10% chokeberry juice.

Key words: *Aronia melanocarpa,* black chokeberry, yield, anthocyanins, harvesting time, colorant, stability.

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Resumé

Der er i 3 år blevet udført dyrkningsforsøg med 5 sorter af sortrøn. Der blev høstet på forskellige tidspunkter i 2 år.

Desuden er der blevet udført et sammenlignende opvarmningsforsøg med bestemmelse af stabiliteten af anthocyaniner fra sortrøn, solbær, hyld, jordbær og druer. Endvidere er der blevet udført et forsøg med henblik på bestemmelse af, om saft fra sortrøn kan anvendes som farvegivende komponent i blommesaft uden at give den snerpende smag, der er karakteristisk for sortrøn.

Frugtudbyttet var i gennemsnit for alle sorter det fjerde år 5,7 kg/busk. Dette giver omkring 11 t/ha med 2000 buske/ha.

Frugtvægten, der varierede fra 0,67 til 0,88 g/frugt, er ikke særlig afgørende, da sortrøn kan høstes med en solbærhøstmaskine og fordi bærrene fortrinsvis anvendes til fremstilling af saft.

Indholdet af opløseligt tørstof var 18-22 g/100 g, hvilket er forholdsvis højt sammenlignet med indholdet i andre bærarter.

A. melanocarpa "Kashamachi" havde det højeste indhold af opløseligt tørstof på 22 g/100 g, efterfulgt af 'Aron' og *A. melanocarpa* "Estland" med 19-20 g/100 g. Indholdet var kun 18 g/100 g i *A. melanocarpa* "Mandschurica" og 'Viking'.

Der var ikke væsentlige sortsforskelle på indholdet af titrerbar syre og anthocyanin.

Indholdet af titrerbar syre var i gennemsnit kun 11 g/kg, hvilket svarer til indholdet i æbler og andre frugtarter. Anthocyaninindholdet i sortrøn var i gennemsnit af samme størrelsesorden som i hyldebær, dvs. 750-950 mg/100 g.

Anthocyaninerne i sortrøn er mere varmestabile end anthocyaniner fra jordbær og solbær, men de er mindre varmestabile end anthocyaniner fra druer og hyldebær. Det har ikke været muligt at forklare denne forskel i varmestabilitet ud fra forskelle i anthocyaninernes kemiske struktur. Det er sandsynligt, at de væsentlige forskelle er varierende glycosylering, der giver forskelle i hydrolysehastighed.

Nedbrydningen af anthocyaniner sker efter reaktioner af første orden. Ved brug af Arrhenius-ligningen blev aktiveringsenergien for anthocyaniner fundet at være 16-20 kcal/mole.

Når saft af sortrøn anvendes som farvegivende komponent i blommesaft, skal der anvendes 10% saft af sortrøn for at opnå en tilfredsstillende intens rød farve, hvilket ikke giver snerpende afsmag.

Nøgleord: *Aronia melanocarpa,* sortrøn, frugtudbytte, høsttidspunkt, anthocyaniner, naturfarvestof, stabilitet.

Introduction

The ban within the EEC of synthetic colorants such as orcine (E121), écarlate (E125), and poncea R (E126) for use in the food industry in many countries has evoked interest for processing of natural colorants (1).

Black chokeberry, *Aronia melanocarpa,* is native to eastern North America and is grown for fruit production in eastern Europe and the Soviet Union (17, 25, 28). In the western Europe this species is used today only for ornamental purposes. Black chokeberry deserves more attention since it is a very healthy culture which can be grown mostly without any application of pesticides and it can be harvested with a black currant harvester (8). In addition the berries can be an extremely good natural anthocyanin source (32, 33).

Especially the anthocyanins processed from waste from manufacture of grapes is applied as a colorant (1), but recently experiments to evaluate the possibility for application of other fruit such as elderberry has been carried out (23).

Waste products from manufacture of grape wines or elderberry juice are cheap raw materials for processing of food colorants, but chokeberry may have some lucrative qualities as a natural colorant.

The aim of this paper is to determine the level of fruit yield and quality of chokeberry and to evaluate the possibilities of application of chokeberry juice as a food colorant.

Materials and methods

Field experiment

In spring 1988 plants of the 5 cultivars 'Aron', *A. melanocarpa* "Kashamachi", *A. melanocarpa* "Mandschurica", *A. melanocarpa* "Estland" and 'Viking' were planted in the experimental field at Department of Pomology at a distance of 3x1.5 m giving 2000 plants/ha (10 per cent waste acreage).

No pesticides or fungicides were used. Each year 104 kg N, 20 kg P and 105 kg K were supplied. Pruning was done yearly in order to shape bushes for mechanical harvest.

First harvest was carried out when the fruits turned black. The following 5 harvests was carried out with intervals of one week.

Handpicking was done in 1989 and 1990. In 1991 mechanical harvesting with a black currant harvester (Aunslev) was combined with handpicking. The berries were frozen and stored at minus 25°C.

Chemical analyses

The content of anthocyanins were determined by spectrophotometry (Shimadzu MPS 2000) by use of the method given by *Wrolstad* (48) and the content was expressed as cyanidin-3-glucoside.

Analyses for content of soluble solids and titratable acids as citric acid were carried out by use of refractometry (Bausch & Lomb refractometer) and titration (Mettler DL titrator) with 0.1 N NaOH respectively.

Juice processing

Enzymation was carried out for black currants and mixtures of plums and chokeberries only. For each kg mash 0.3 ml of Pektolase LX (Grindsted Products) was applied and the treatment time after heating to boiling was 2 hours at 40°C.

The pressing was carried out using a Tincture Press by increasing the pressure to 200 kg/cm^2 during one hour.

Colour stability

Stability of anthocyanins from chokeberries, black currants, strawberries and elderberries were determined in 1991 by use of fruits harvested and frozen in 1990.

Before processing of single fruit juices for the colour stability test, chokeberries were mixed in a Waring blender with equal amounts of water, and elderberries were mixed with half amount of water. Black currants were processed without dilution with water. Enzymation of the mash was carried out for black currants only. For each kg of mash 0.3 ml of Pektolase LX (Grindsted Products) was applied and the treatment time was 2 hours at 40°C.

The stability of anthocyanins from chokeberry, black currants, elderberry, and grape extract was determined in apple concentrate diluted to 10 w/w% soluble solids and in a model solution containing 10 w/w% sucrose 0.3 w/w% citric acid where pH was adjusted to the value 3.3 as found for diluted apple juice.

The apple juice and the model solution were coloured by addition of the processed juices or the grape concentrate soluted in the model solution to obtain almost equal surface colour. The juices and the grape concentrate were diluted to almost equal concentration of anthocyanin (cyanidin-3-glucoside) and the amount added to juices was adjusted to obtain visual acceptable juice colours.

In the heating or storage experiment 1, only juice of strawberries and of chokeberry soluted in apple juice and model solution was heated at 5, 12, 20, and 30°C. The experimental design for heating experiment 2 is shown in table 1. For each treatment bottles with 25 ml juice were stored in cooling cabinets (5 to 40°C) or kept in water baths (50 to 80°C).

The degradation rate b for the anthocyanins was determined by nonlinear regression by use of the following equation were y is the anthocyanin concentration, x the treatment time and a0 and a1 are constants.

$$y = a0 + EXP(a1 - bx) \qquad 1)$$

$$\log (y - a0) = a1 - bx \qquad 2)$$

For each heating temperature the differences between the b-values was evaluated by t-tests.

The energy of activation (Ea) cal/mole and the entropy (S) were calculated by use of the Arrhenius equation (3), where R and T is the gas constant and absolute temperature respectively.

$$\log b = - Ea/RT + \log (S) \qquad 3)$$

Table 1. Experimental design for heating experiment 2 with apple juice or model solution coloured by addition of single fruit juices or grape extract soluted in model solution respectively.
Forsøgsplan for opvarmningsforsøg 2 med æblejuice og syntetisk saft, der var tilsat henholdsvis rene frugtsafter eller drueekstrakt opløst i modelopløsning.

°C *°C*	Days *Dage*
5	1, 5, 10, 15, 22, 26
12	1, 5, 10, 15, 22, 26
20	1, 5, 10, 15, 22, 26
30	1, 3, 5, 9, 15, 22, 26
40	1, 1.25, 1.5, 2, 3, 5, 9, 12, 15, 17, 19, 22, 26
	Hours *Timer*
50	1, 2, 4, 6, 11.25, 24, 48, 72, 96, 120, 150
60	0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 12.6, 25, 48, 72, 96, 127
	Minutes *Minutter*
70	10, 20, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360
80	5, 10, 15, 20, 30, 40, 60, 75, 90, 120, 150, 180, 300, 360

The energy of activation was calculated by multiplication of the slopes (Ea/R) from equation (3) with the gas constant R,

$$Ea = R(\text{slope}) \qquad 4)$$

Mixed juices
The suitability of chokeberry as a colorant for plum juices was tested by processing the chokeberry cultivars 'Viking', *A. melanocarpa* "Estland" and *A. melanocarpa* "Mandschurica" together with plums from the cultivars 'Kirkes' and 'Opal' picked at the optimum harvest time in 1991. The chokeberries and plums were frozen up to processing of mixtures containing 0, 1, 2, 5 and 10 per cent chokeberry juice. The taste and colour were evaluated.

Results

Field experiment

All 5 cultivars were flowering for 2 weeks in May and harvesting was carried out in September.

Table 2 and 3 shows the fruit yield and fruit weight respectively. The first year of bearing no difference between cultivars was found. In the second year the yield of 'Aron' and *A. melanocarpa* "Kashamachi" was significantly lower than the yield of *A. melanocarpa* "Mandschurica", *A. melanocarpa* "Estland" and 'Viking'. The third year it was not possible to make statistical analysis because all plants from one cultivar were mechanical harvested in one operation. Totally the most productive cultivars were *A. melanocarpa* "Mandschurica", 'Viking' and *A. melanocarpa* "Estland".

The largest fruits were produced by the cultivars *A. melanocarpa* "Mandschurica" and 'Viking' while the smallest fruits were produced by the cultivar *A. melanocarpa* "Kashamachi".

Table 4 shows the average values of soluble solids, titratable acid calculated as citric acid and anthocyanin calculated as cyanidin-3-glucoside. A. melanocarpa "Kashamachi" had the highest content of soluble solids followed by 'Aron' and A. melanocarpa "Estland". There was no difference between cultivars in content of titratable acid.

The content of anthocyanin was highest in 'Viking' and 'Aron', followed by *A. melanocarpa* "Kashamachi", *A. melanocarpa* "Estland" and *A. melanocarpa* "Mandschurica". The content of anthocyanin was influenced more by the year than by the cultivar.

The values for all varieties of soluble solids, titratable acid and anthocyanin in relation to harvest days are shown in Fig. 1, from which it appears that the content of soluble solids and anthocyanins increased during ripening, while the content of titratable acid decreased.

Colour stability
Examples from changes in anthocyanin content during storage or heating in experiment 2 is shown in Fig. 2.

Table 2. Yield, kg/bush. Average of 6 bushes per cultivar. The bushes were planted in spring 1988.
Udbytte kg/busk. Gennemsnit for 6 buske pr. sort. Buskene blev plantet i foråret 1988.

Cultivar *Sort*	1989	1990	1991	Total *I alt*
'Aron'	1.2	2.0	6.4	9.6
A. melanocarpa "Estland"	1.0	4.8	5.3	11.1
A. melanocarpa "Kashamachi"	1.6	2.1	5.5	9.2
A. melanocarpa "Mandschurica"	1.3	5.3	5.6	12.2
'Viking'	1.2	4.6	5.8	11.6
LSD*	n.s.	1.2	-**	-

* LSD= Least significant difference.

** It is not possible to make statistical analysis for fruit yield in 1991 since all plants from one cultivar were harvested mechanical in one operation.

Table 3. Fruit weight, g/fruit. Average 1989-90.
Bærvægt, g/frugt. Gennemsnit for 1989-90.

Cultivar *Sort*	g/fruit *g/frugt*
'Aron'	0.82
A. melanocarpa "Estland"	0.80
A. melanocarpa "Kashamachi"	0.67
A. melanocarpa "Mandschurica"	0.88
'Viking'	0.87
LSD	0.06

From these results it is obvious that degradation of anthocyanins takes several months or many days at low temperatures and only a few hours at high temperatures.

Fig. 3 shows that the effect of temperature on degradation rate of anthocyanins in strawberries and chokeberries can be described by Arrhenius plots.

In order to determine the effect of temperature and type of "juice" (apple juice, model solution) on the degradation rate constants for anthocyanins from different fruits, a multiple

Table 4. Soluble solids, titratable acid and anthocyanin. Average of 6 harvest days.
Opløseligt tørstof, titrerbar syre og anthocyanin. Gennemsnit for 6 høstdage.

Cultivar *Sort*	Soluble solids *Opl. tørstof*	Titratable acid *Titrerbar syre*	Anthocyanin *Anthocyanin*	
	g/100 g	g/kg	mg/100 g	
	1990	1990	1989	1990
'Aron'	19.6	11.0	771	967
A. melanocarpa "Estland"	19.4	11.0	741	923
A. melanocarpa "Kashamachi"	21.8	10.9	748	944
A. melanocarpa "Mandschurica"	17.9	11.0	725	912
'Viking'	17.9	10.9	811	966
LSD	0.2	0.1	37	39

Fig. 1. Content of soluble solids, titratable acids and anthocyanine as a function of harvest time. Average for 5 cultivars harvested in 1990.
Indhold af opløseligt tørstof, titrerbar syre og anthocyanin som funktion af høsttid. Gennemsnit for 5 sorter høstet i 1990.

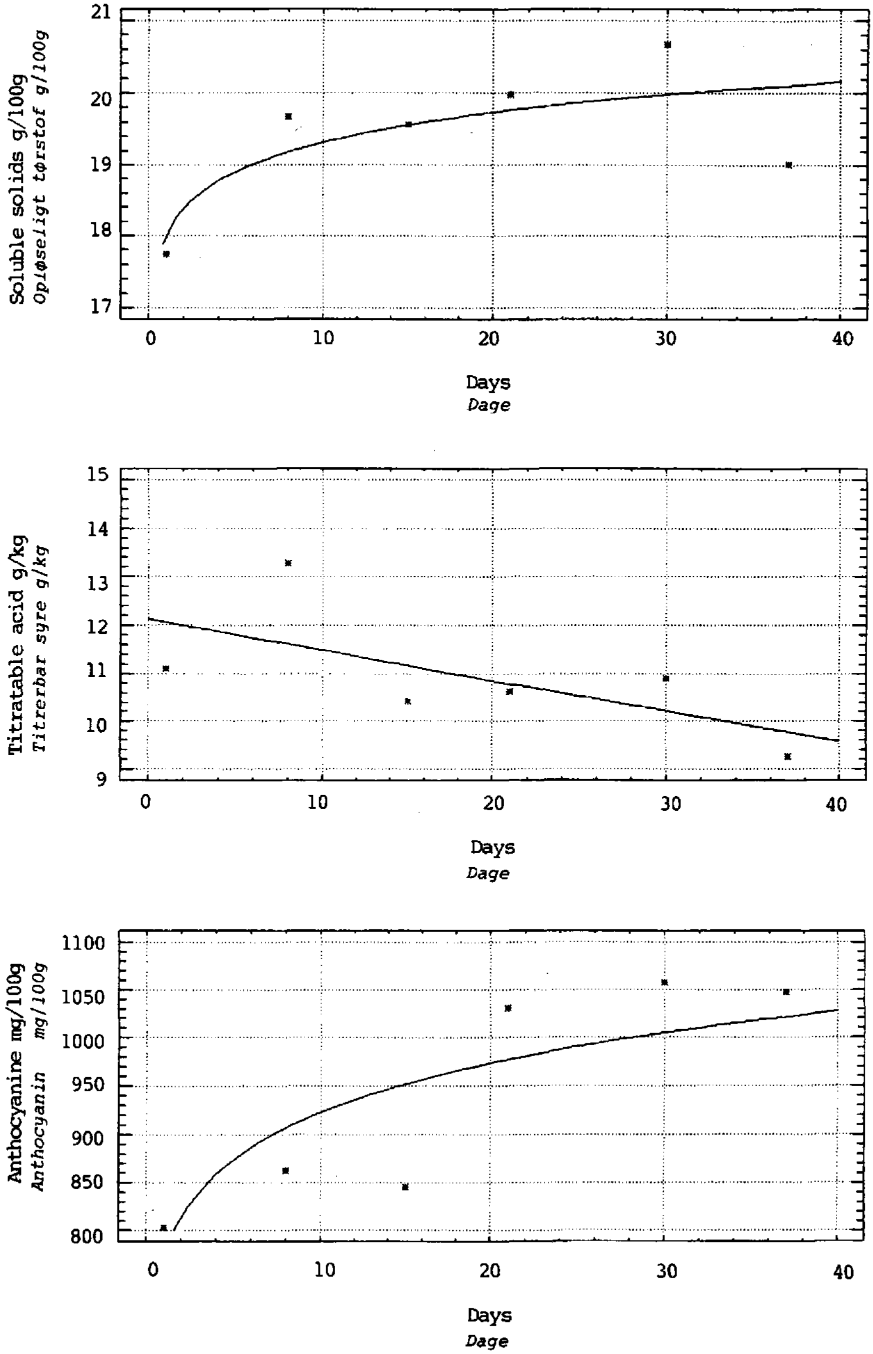
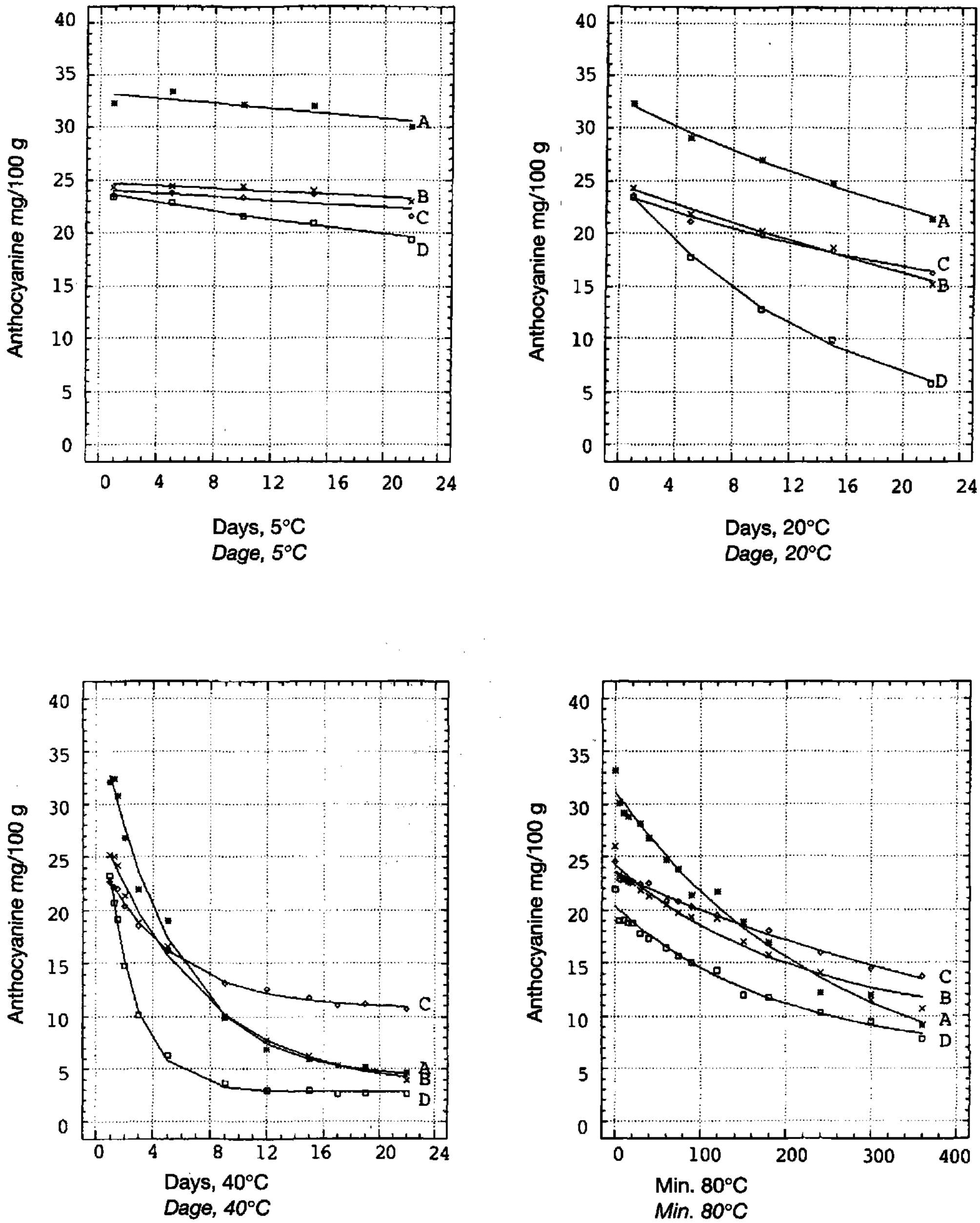


Fig. 2. Content of anthocyanine in 4 juices at 4 temperatures.
Indhold af anthocyanin i 4 safter ved 4 temperaturer



A * = Chokeberry
Sortrøn

B x = Elderberry
Hyldebær

D = Black currants
Solbær

C = Grapes
Drue

Table 5. Results from analyses of variance of the log b values from experiment 1. Ea, b and r is the energy of activation, rate constant and correlation coefficient respectively.
Resultater fra variansanalyse på log b bestemt i forsøg 1. Ea, b og r er henholdsvis aktiveringsenergi, hastighedskonstant og korrelationskoefficient.

Fruit *Frugt*	log b	Ea kcal/mole	r
Strawberry	– 1.37	15.5	0.985
Chokeberry	– 2.76	19.7	0.995
LSD	0.22	1.5	

Table 6. Results from analyses of variance of the log b values from experiment 2. Ea, b and r is the energy of activation, rate constant and correlation coefficient respectively.
Resultater fra variansanalyse på log b bestemt i forsøg 2. Ea, b og r er henholdsvis aktiveringsenergi, hastighedskonstant og korrelationskoefficient.

Fruit *Frugt*	log b kcal/mole	Ea	r
Black currant	– 8.10	16.4	0.992
Chokeberry	– 8.73	17.9	0.960
Grape	– 9.01	18.5	0.982
Elderberry	– 8.98	19.1	0.979
LSD	0.33	1.3	

analyses of variance on the values of the logarithm to the degradation rate constants was carried out. The results from this analyses for experiment 1 is shown in Table 5. No effect of type of "juice" or interaction between fruit and temperature was found.

The degradation rate was significantly highest for strawberry and increased as shown in Fig. 3 by increasing temperatures. Similar results from experiment 2 is shown in Table 6.

The average degradation rate were equal in apple juice and model solution. The rate of degradation of anthocyanins was highest for black currant and lowest for grapes and elderberry, while the degradation rate for anthocyanins from chokeberry was in between these.

Fig. 4 shows that the effect of temperature on the degradation rate can be described by Arrhenius plots. By increasing temperatures increasing degradation rates was found. The degradation rate was equal for grape and elderberry anthocyanins.

Mixed juice

Juice of the plum cultivar 'Kirkes' is red while juice of the plum cultivar 'Opal' is yellow. Mixture with chokeberry juice for both cultivars gave juices with a strong intense red colour. The best colour was obtained by using 10% chokeberry juice for both plum cultivars. The colour of the mixed juices with 10% chokeberry reminded of a mixture of black currant and strawberry. In juices with 10% chokeberry a week astringency could be detected, but the taste was still acceptable.

Discussion

Field experiment

The yield of chokeberry is 5-8 t/ha in foreign countries (8, 17, 29, 38). With an yield of 5.7 kg/bush and 2000 bushes/ha a fruit yield of 11.4 t/ha can be obtained in Denmark 4 years after planting. The most productive cultivars were *A. melanocarpa* "Mandschurica", *A. melanocarpa* "Estland" and 'Viking'.

In black currant the fruit yield the third year after planting is about 50% of full production (45). Since chokeberry are comparable to black currant this indicate that full production in chokeberry for the most productive cultivars probably will be more than 15 t/ha. This shows that chokeberry is more productive than elderberry (18) from which a similar colorant can be processed (23).

The differences in fruit size is of minor importance because chokeberry can be efficiently harvested by use of a black currant harvester.

Soluble solids, titratable acid

As shown in Fig. 1 did the content of soluble solids and anthocyanins increase with ripening while the content of titratable acid decreased.

Fig. 3. Log of degradation rate as a function of the reciprocal value of the absolute temperature for juices processed from strawberry and chokeberry. Experiment 1.
Log til nedbrydningshastigheden som funktion af den reciprokke absolutte temperatur. Forsøg 1.

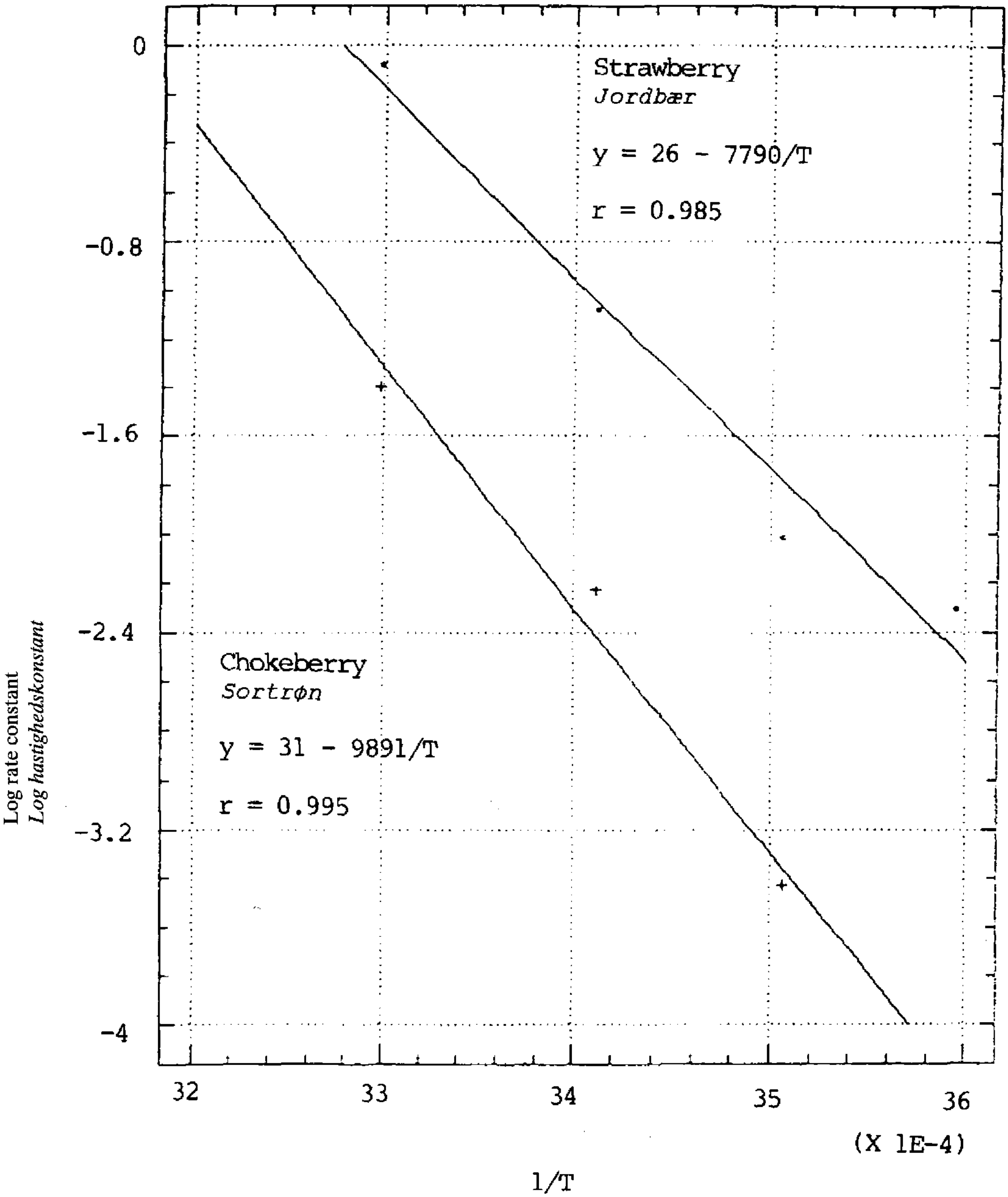
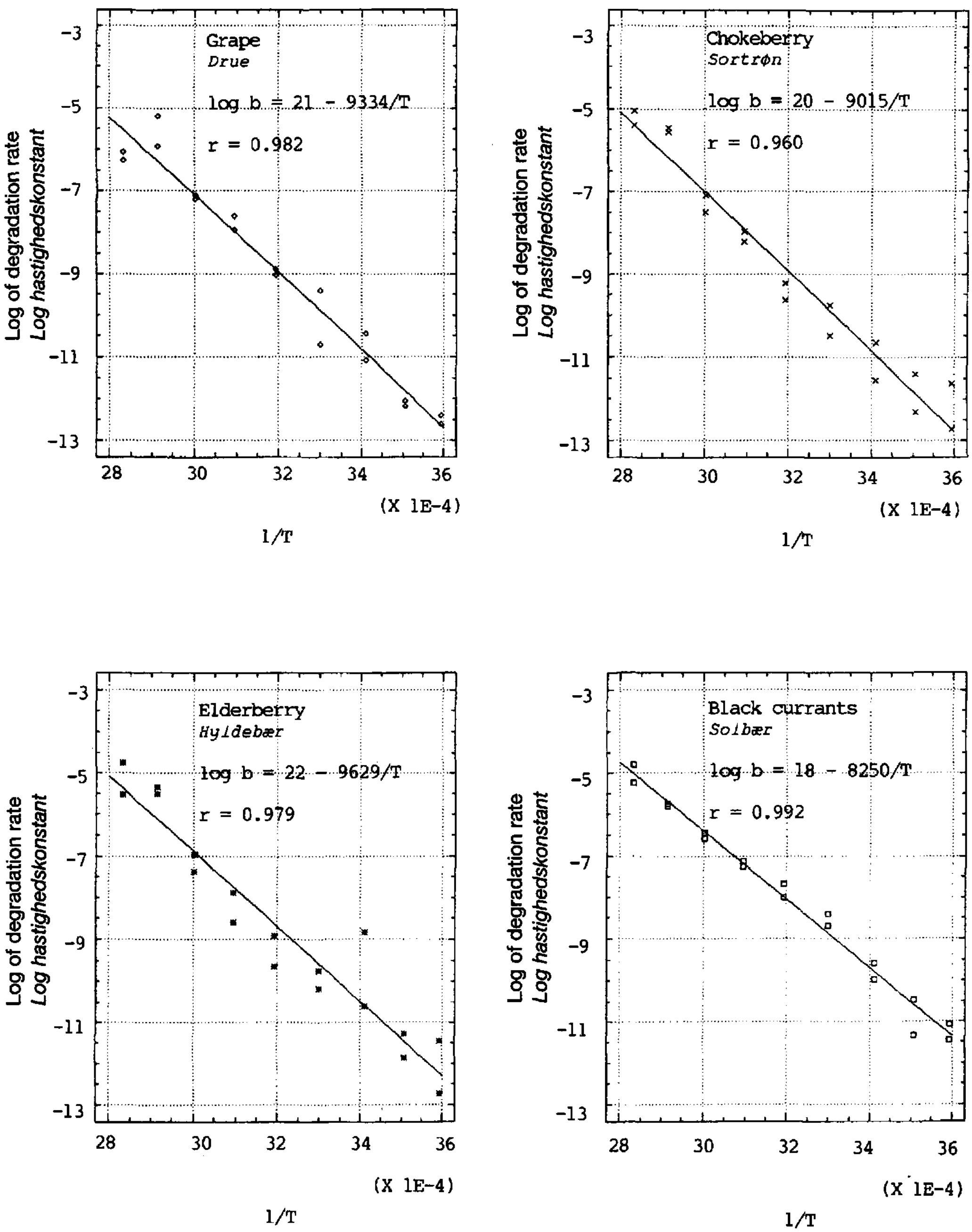


Fig. 4. Log of degradation rate as a function of the reciprocal value of the absolute temperature. Experiment 2.
Log til nedbrydningskonstanten som funktion af den reciprokke absolutte temperatur. Forsøg 2.



Similar patterns have been found for other fruits such as elderberry and cherries (5, 14, 22).

Chokeberry contained at maximum content of anthocyanins 20% soluble solids and 9 g/kg of titratable acid. This is in accordance with earlier results mentioned in the literature with 13 to 21% soluble solids and 7 to 12 g/kg titratable acids (24, 28, 32, 33). The content of anthocyanins is of the same order as in elderberry (18). The content of soluble solids and titratable acid are of the levels as found in sour cherries and apples respectively.

Compared to other fruits such as black currant and oranges chokeberry has a low content of vitamin C (17, 24, 32).

Juice yield by pressing was found to be 75%, which is of the same order as for cherries and elderberries (20, 21, 24, 28).

Colour stability

A satisfactory visual colour was obtained if a concentration of anthocyanins corresponding to 25 mg/100 g was applied. The storage or heating temperature has an enormous effect on the degradation rate of the anthocyanins. From Fig. 2 it appears that the half life time is from several months at 5°C to 2 hours at 80°C.

Anthocyanins are sugar derivatives of anthocyanidins which are polyhydroxy- and polymethoxy-derivatives of 2-phenylbenzopyrilium (flavylium) salts with different substitutions in the 3'and 5' positions of the secondary ring of the molecules. Position 3 in the primary ring of the anthocyanins always are substituted with a sugar moiety, while the position 5 is either -OH or a sugar moiety. The structure of the most frequently occurring anthocyanins in fruits is shown in table 7 (9, 43).

Table 7. Substitutions of the most frequently occurring anthocyanins.
Kemisk struktur hos hyppigt forekommende anthocyaniner.

	Position in the molecule *Position i molekylet*	
	3'	5'
Pelargonidin	-H	-H
Cyanidin	-OH	-H
Peonidin	-OCH3	-H
Delphinidin	-OH	-OH
Petunidin	-OCH3	-OH
Malvidin	-OCH3	-OCH3

The degradation of anthocyanins from grapes, elderberry, black currants, strawberry and chokeberry followed first order reactions as described earlier (40, 41).

The sugar moiety can be acylated with phenolic acids. With 2 or more acyl residues such as in the anthocyanins of Cineria, *Ipomea tricolor, Sambucus racemosa* and *Brassica oleracea* higher heating stability is obtained (2, 13, 26, 27, 42, 49).

Removal of the sugar moieties by hydrolysis of the anthocyanins to anthocyanidins decreases the stability of the anthocyanins very much (12, 15, 40). Therefore differences in hydrolysis rate of the anthocyanins may be of significant importance for the colour stability. It has been shown that galactosidic anthocyanins of cranberry are more stable than arabinosidic ones during storage of the juice (37). Diglucosides are more stable than monoglucosides (34, 44).

Strawberry contain almost only pelargonidin-3-glucoside which compared to cyanidin-3-glucoside is very unstable (6, 30, 36, 41, 47). The reason for this is the hydroxylation in the 3'-position of the anthocyanidin which stabilize the molecule.

Black currants contain delphinidin-3-glucoside (13%), cyanidin-3-glucoside (12%), delphinidin-3-rutinoside (36%) and cyanidin-3-rutinoside (39%) (4, 19, 35). The higher stability of anthocyanins from black currants can be explained by the higher degree of hydroxylation or differences in hydrolysis rates.

Chokeberry contain cyanidin-3-arabinoside (30%), cyanidin-3-galactoside (65%), cyanidin-3-glucoside (2%) and cyanidin-3-xyloside (3%) (31). The higher stability of anthocyanins compared to black currant anthocyanins may be explained by the higher concentrations of galactosidic anthocyanins which earlier has been found to require longer hydrolysis time (37).

The elderberries contain cyanidin-3-sambubioside-5-glucoside (1%), cyanidin-3,5-diglucoside (1%), cyanidin-3-sambubioside (33%) and cyanidin-3-glucoside (65%) (35). Compared to juice of elderberry the rate of anthocyanin degradation was greater in juice of chokeberry but it was claimed that juice of chokeberry because of less browning had better colouring properties (32).

Grapes contain-3,5-diglucosides, 3-monoglucoside, 3-(6-O-p-coumaryl-glucoside)-5-glucosides, and the 3-(6-O-p-coumaryl-glucoside) of cyanidin, delphinidin, petunidin, peonidin and malvidin (7, 46). Higher stability of colorants processed from elderberry and grapes may be explained by the greater number and more complex sugar moieties in the anthocyanins probably requiring longer hydrolysis time just as found by comparison of hydrolysis rates for mono- and diglucosides from grapes (34, 44).

As an example chokeberry was mixed with 2 cultivars of plum for juice processing. Adding 10% chokeberry improved the colour of the juices for both cultivars and did not affect the taste.

Activation energy

The activation energy for the anthocyanins has been estimated to be 21-30 kcal/mole (10, 16, 39, 41). In this experiment the values of activation energy were 16-20 kcal/mole.

Flavour

The raw fruits of chokeberry has a puckery flavour (11). Of this reason pure juice of chokeberry may have an unsatisfactory flavour (29). Of 48 aroma compounds identified mainly the carbonyls, esters and some of the aromatic compounds may be of great significance (11).

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