ANTHOCYANINS OF THE SASKATOON BERRY (AMELANCHIER ALNIFOLIA NUTT): Interactions with physical and chemical parameters and colour intensification of the pigment extracts

by

RICHARD C. GREEN

A thesis submitted to the faculty of Graduate Studies
in partial fulfillment of the requirements
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ANTHOCYANINS OF THE SASKATOON BERRY (*AMELANCHIER ALNIFOLIA* NUTT): INTERACTIONS WITH PHYSICAL AND CHEMICAL PARAMETERS AND COLOUR INTENSIFICATION OF THE PIGMENT EXTRACTS

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DEDICATION

To my parents, Mrs. Shirley E. Green and the late Mr. William H. Green.

ABSTRACT

The relationships among levels of anthocyanins, total phenolic content, pH, titratable acidity, sugars and colour of eight cultivars of saskatoon berries (Amelanchier alnifolia), harvested at three stages of maturity, were studied. Anthocyanins, total phenolic compounds, sugars, sugar-acidity ratios and anthocyanins-phenolics ratios differed among the cultivars and increased with ripening of the berries. Titratable acidity and pH also varied with the cultivar but showed little change with berry development. Anthocyanin contents ranged from 25 to 179 mg/100 g of berries and were comparable to those of other commercial fruit. The highest pigment levels were found in the cultivars Honeywood, Northline and Porter. Significant correlation ($P < 0.05$) with anthocyanins was obtained for total phenolics content ($r = 0.874$), titratable acidity ($r = 0.823$), pH ($r = -0.826$), sugar-acidity ratio ($r = -0.847$), colour density ($r = 0.583$) and % colour due to tannin ($r = -0.743$). The relationships among surface colour and compositional factors of the berries showed low significance. The results suggest that high levels of anthocyanin in saskatoon berries are associated with high total phenolic compounds and acids and low pH and sugar-acidity ratios.

The influence of acetaldehyde and catechin on colour of aqueous solutions of cyanidin 3-glucoside and saskatoon berry aqueous and alcohol extracts was also investigated. Pigment systems were analyzed periodically during storage at 23°C in the dark for absorbance

spectra, high performance liquid chromatography profiles and Hunterlab colour values. Presence of acetaldehyde in the fruit extracts and both acetaldehyde and catechin in the cyanidin 3-glucoside model system caused a marked increase in colour intensity during storage. Visible spectra of the colour intensified samples showed both a bathochromic shift and an increase in absorbance. The HPLC profiles of the acetaldehyde treated berry extracts revealed the appearance of a new peak and the decrease of two anthocyanin peaks. Chromatograms of the cyanidin 3-glucoside solution containing both acetaldehyde and catechin displayed six new peaks and loss of the anthocyanin. Addition of catechin alone had no significant effect on the colour of the model and fruit pigment extract systems. Results indicated that colour intensification was due to molecular condensation involving catechin, acetaldehyde and anthocyanin as well as other phenolic components in the berry extracts. Hue angle of the extracts accurately measured the colour intensification reaction.

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1. INTRODUCTION

The colour of a food greatly influences our overall judgement on the worth of the product from both an aesthetic and a safety point of view. It plays an important role in taste thresholds, flavour identification, pleasantness, acceptability and ultimately food choice.

Processed foods as well as some raw foods often are insufficiently coloured and it may be necessary to add colouring substances to these products. Until recently, food colourants consisted of the widely available, easily synthesized and inexpensive azo-dyes. In the past two decades, however, there has been interest in the use of naturally occurring plant colourants in place of these synthetic food dyes, which have become controversial from a health safety point of view. Among the natural pigments, the anthocyanins are the most widespread. These are water-soluble pigments responsible for nearly all the red, purple and blue colours of flowers and fruits. Yet, in spite of their wide occurrence, these pigments have had only limited use in the food industry. The reason for this may be threefold: anthocyanins are not stable chemically and are susceptible to discolouration by various chemical and physical agents used in food processing and storage, they are difficult to purify and they have not been available commercially in large quantities. Alternatively, some naturally occurring plant and food components such as acetaldehyde and various flavonoids have been reported to form stable, intensely coloured complexes with anthocyanins. The mechanisms of formation, structures and properties of these colour

augmentation compounds, however, are not clearly understood. Thus, improved knowledge of these aspects of anthocyanins could be beneficial in stabilizing the colour of food products containing red pigments and increasing the potential of anthocyanins as food colourants.

The saskatoon berry (Amelanchier alnifolia Nutt.) is a western Canadian wild fruit which is coloured by anthocyanin pigments. Recently, there has been increasing interest in the commercial cultivation and utilization of this fruit. Approximately 200 hectares of saskatoons are currently grown throughout western Canada and products such as saskatoon jelly, syrup and wine are manufactured and marketed in Alberta, British Columbia and Saskatchewan. These developments suggest that the saskatoon berry may become an established western Canadian commercial fruit. Information on the physico-chemical properties of saskatoon berries is limited and further knowledge in this area could aid their commercial development. The anthocyanin pigments of saskatoon berries are important aesthetically and economically because they are located in the skin and flesh of the fruit and their stability is of significance in the marketability of the fresh berries as well as the processed products such as jam, jelly, syrup, juice and wine. For this reason and because of the increased interest in the use of natural colourants in place of synthetic dyes, an investigation into the anthocyanins and other constituents of saskatoon berries was initiated.

In this study the following aspects were investigated: 1) influence of saskatoon berry ripeness on anthocyanin level and other

physical and chemical parameters; 2) relationships of anthocyanin levels to other physico-chemical properties of the most promising saskatoon berry cultivars and 3) the effect of acetaldehyde and catechin, two possible polymerizing and colour intensifying agents, on the anthocyanins of saskatoon berries.

2. LITERATURE REVIEW

2.1 SASKATOON BERRIES

The saskatoon (Amelanchier alnifolia Nutt.) is a fruit bearing shrub native to the southern Yukon and Northwest Territories, the Canadian prairies and the northern prairies of the United States (Harris, 1972). It is extremely adaptable and grows under a wide range of environmental conditions. Saskatoon plants begin to bear fruit when they are two to four years old and with proper management can yield 8 to 10 tonnes of fruit per hectare. The fruit is usually referred to as a berry but is actually a pome (Harris, 1972; Olson and Steeves, 1982). Saskatoon berries were originally used as a major food source by the native people and early settlers of the North American prairies and until recently, could be picked only in the wild. In the past two decades, however, there has been increasing interest in utilizing this berry as a western Canadian fruit crop. To date over 200 ha of saskatoon orchards have been planted and several new cultivars have been developed and propagated vegetatively (Olson and Steeves, 1982; Anonymous, 1985).

2.1.1 Composition of Saskatoon Berries

The nutritional value of saskatoon berries on a dry weight basis is shown in Table 1. Saskatoon berries contain higher levels of protein, fat and fiber than most other fruit. Although the bioavailability of the minerals is not known, these berries also appear to be an excellent

Table 1. Nutritional composition of saskatoon berries[a]

Nutrient	Composition (% dry wt.)	Nutrient	Composition (ppm dry wt.)
Protein	9.7 ± 1.3[b]	Iron	67.03 ± 11.65
Fat	4.2 ± 0.5	Sodium	31.83 ± 7.67
Fiber	19.0 ± 3.0	Manganese	67.05 ± 11.79
Calcium	0.44 ± 0.06	Copper	7.23 ± 0.70
Phosphorous	0.16 ± 0.02	Zinc	16.50 ± 2.78
Potassium	1.22 ± 0.16	Barium	34.76 ± 4.85
Magnesium	0.20 ± 0.03	Molybdenum	0.38 ± 0.01
Sulfur	0.06 ± 0.02	Aluminum	74.45 ± 13.22
		Carotene	29.70 ± 5.00

[a] Results pertain to the Smoky cultivar.
[b] Standard deviation of three samples
Source : Mazza, 1982

source of manganese, potassium, copper and carotene (Mazza, 1982). Tuba et al. (1944) indicated that fresh saskatoon berries might be a useful source of vitamin C. Panther and Wolfe (1972), however, reported a negligible ascorbic acid content and that an ascorbic acid oxidizing enzyme system was present in the berries.

Chemical parameters of saskatoon berries, cv. Smoky, have been investigated. Total solids content ranges from 20 to 29.4 % fresh weight and values of 15.9 - 23.4 % and 10 % have been reported for soluble solids (% sucrose) and reducing sugars, respectively (Mazza et al., 1978; Mazza 1980a, 1982). Wolfe and Wood (1971) found that the sugar content, consisting mainly of fructose and glucose, increases slowly as the fruit matures and then accelerates markedly before ripening. Results also indicated that the fructose content decreases rather markedly (25 %) after the fruit ripens while the glucose content remains unchanged. Berry pH values range from 4.2 - 4.4 and titratable acidity values (% malic acid) from 0.36 - 0.49 % (Wodak and Wolfe, 1971; Mazza et al., 1978; Mazza, 1980a). The predominant acid in saskatoon berries is malic (Wolfe and Wood, 1971) and the predominant aroma component is benzaldehyde (Mazza and Hodgins, 1985). There are at least four anthocyanins in ripe saskatoon berries; cyanidin 3-galactoside and 3-glucoside account for about 61 % and 21 % of the total anthocyanins, respectively (Mazza, 1986).

2.1.2 Processing and Marketing

A preliminary processing evaluation of saskatoon berries was carried out in the mid-sixties by staff of the Department of Food Science, University of Alberta, who determined sugars, acids and vitamin C contents of several selections of saskatoon berries (Wolfe and Wood, 1971; Panther and Wolfe, 1972). In the early 1970's, the Alberta Native Fruit Pilot Project was initiated through the cooperative efforts of the Alberta Department of Agriculture, Agriculture Canada at Beaverlodge and the Peace Country Small Fruit Growers Society (Mazza, 1980b). Problem areas investigated by that project's cooperators were seedling and fruit production, production efficiency, fresh market development and processed products development.

Mazza (1979) examined the marketability of a saskatoon jelly product. In this study, jelly formulae for saskatoon berry and two other native Alberta fruits were developed and test marketed with a honey product. Over 91 % of the respondents indicated they would purchase the product again and over 28 % rated the product superior. Although these results pertained to a gift pack containing saskatoon jelly, the highly positive consumer response suggested that production of native fruit products could be commercially viable. Subsequently, syrup and wine formulae were developed and in the past five years saskatoon jelly, syrup and several brands of wine have reached commercial production and marketing (Figure 1).

Figure 1. Commercial products of saskatoon berry.

2.2 ANTHOCYANINS

2.2.1 Occurrence

Anthocyanins are the most widespread natural colourants. They are synthesized by almost all the higher plants, with particularly frequent occurrence in the angiosperms (Harborne, 1967). Structure of the anthocyanins is related to genetic factors and their distribution has taxonomic significance (Harborne, 1976). These pigments accumulate primarily in the epidermal cells of flowers and fruits but are also found in other plant parts such as roots and leaves (Harborne, 1967, 1976; Timberlake and Bridle, 1975, 1982; Brouillard, 1983). Anthocyanin levels in some commercial fruits are shown in Table 2. Usually more than one type exists in the plant and the combination of those that are present exerts an additive effect on colour.

2.2.2 Biosynthesis

The biosynthesis of anthocyanins can be seen in the general metabolism of flavonoids (Grisebach, 1982) (Figure 2). A common precursor for all flavonoids is the chalcone 4, 2', 4', 6-tetrahydroxy-chalcone (naringenin chalcone), produced from 4-coumaroyl-CoA and three molecules of malonyl-CoA. The 4-coumaroyl-CoA is supplied by general phenylpropanoid metabolism which involves the conversion of L-phenyl-alanine (a product of the shikimic acid pathway) to activated cinnamic acids (Hahlbrock and Grisebach, 1975). Malonyl-CoA is supplied by the

Table 2. Anthocyanin levels in commercial fruits.

Fruit	Anthocyanin level (mg/100g fresh wt.)	Reference
Blackberry (*Rubus* sp.)	82 - 326	Torre & Barritt, 1977
Blueberry (*Vaccinium cyanococcus*)	250 - 542	Ballinger *et al.*, 1979
Highbush blueberry (*V. corymbosum*)	25 - 490	Ballinger *et al.*, 1972
Cranberry (*V. macrocarpon*)	63 - 75	Sapers *et al.*, 1983
Cranberry (*V. oxycoccus*)	25 - 100	Fuleki & Francis, 1968b
Crowberry (*Empetrum nigrum* coll.)	300 - 560	Linko *et al.*, 1983
Elderberry (*Sambucus nigra*)	20 - 100	Bronnum-Hansen *et al.*, 1985
Grape (*Vitis vinifera*) skin	4.5 - 22	Yokotsuka *et al.*, 1984
Black grape (*Vitis vinifera* sp.)	50 - 300	Peynaud & Ribereau-Gayon, 1971
Concord grape (*V. labrusca*)	30 - 120	Watada & Ab, 1975

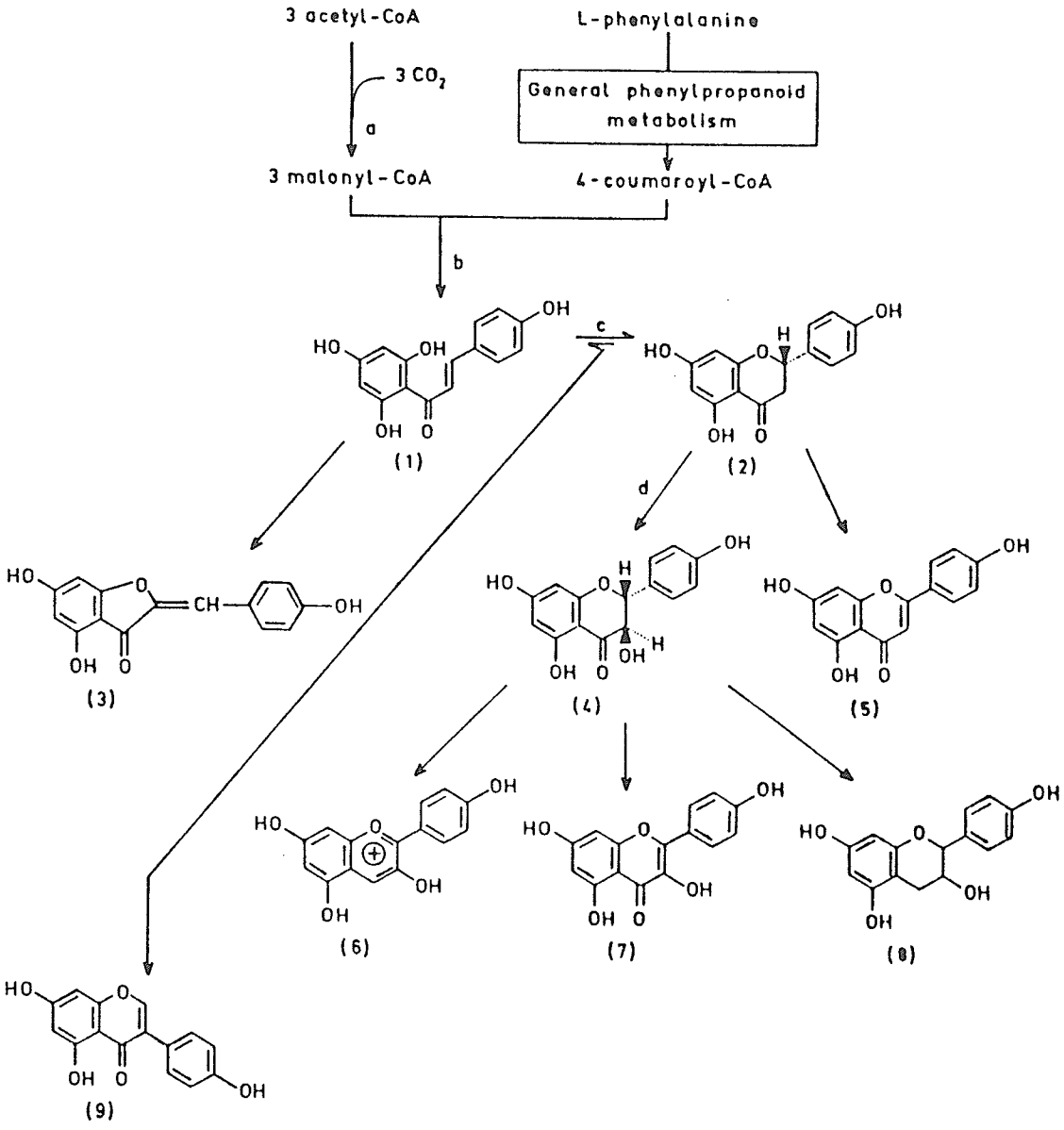

Figure 2. Biosynthetic pathway of the flavonoids as proposed by Grisebach (1982). Acetyl-CoA carboxylase (a); chalcone synthase (b); chalcone isomerase (c); flavanone 3-hydroxylase (d). Chalcone (1); flavanone (2); aurone (3); dihydroflavonol (4); flavone (5); anthocyanidin (6); flavonol (7); catechin (8); isoflavone (9).

acetyl-CoA carboxylase reaction. The chalcone is isomerized to a flavanone and the middle ring is modified to produce other classes of flavonoids. Anthocyanin biosynthesis proceeds by the pathway chalcone ---> flavanone ---> 3-hydroxyflavone ---> anthocyanidin. The anthocyanidin formed is immediately glycosylated by a glycosyl transferase enzyme to produce the anthocyanin. Further glycosylation and acylation can proceed in a stepwise manner through the action of glycosyltransferases and acyltransferases, respectively (Grisebach, 1982).

The control of anthocyanin production is thought to depend largely on genetic inheritance (Slinkard and Singleton, 1984; Teusch et al., 1986) but is also influenced by many external environmental factors. Increased light exposure has been shown to produce higher levels of anthocyanins (Kliewer, 1970; Knobloch et al., 1982; Grisebach, 1982) while environmental temperature has demonstrated an inverse relationship with the pigment content (Kliewer, 1970; McClure, 1975). Other environmental factors affecting anthocyanin accumulation in plants are plant hormones, mechanical damage, pathogenic attack and the nutritional quality of the soil (McClure, 1975).

2.2.3 Chemical Structure

The anthocyanins are part of the C-15 group of compounds known collectively as the flavonoids and are glycosides of anthocyanidins (or aglycones). The basic anthocyanidin chemical structure is the 2-phenylbenzopyrylium cation or flavylium cation (Figure 3). Individual

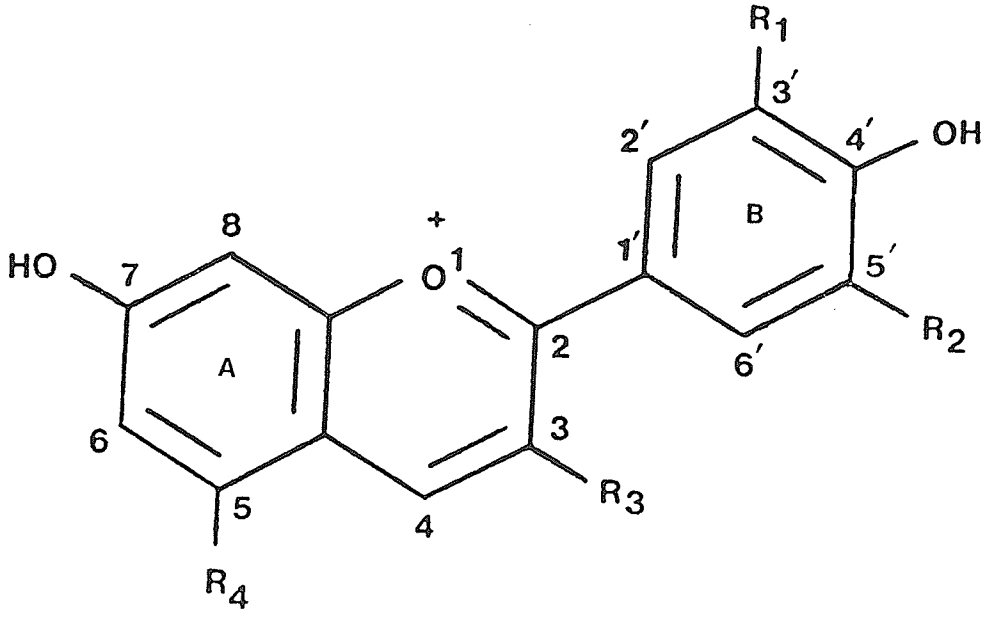

Figure 3. The flavylium cation. R_1 and R_2 are H, OH or OCH_3, R_3 is a glycosyl or H and R_4 is OH or a glycosyl.

anthocyanidins differ in the number and position of hydroxyl groups on the molecule and the degree of methylation of these hydroxyls. An increase in the number of hydroxyl groups tends to deepen the colour to a more bluish shade whereas a higher degree of methylation increases redness (Harborne, 1967). Sixteen naturally occurring anthocyanidins have been identified (Table 3). Those which occur most frequently in plants are cyanidin, delphinidin, malvidin, pelargonidin and peonidin (Harborne, 1967; Timberlake and Bridle, 1975). Since each anthocyanidin may be glycosylated and acylated by different sugars and organic acids, at different positions, the number of anthocyanins is 15-20 times greater than the number of anthocyanidins (Hrazdina, 1981; Mazza and Brouillard, 1987a).

With the exception of the yellow 3-deoxyanthocyanins (i.e. those originating from apigeninidin, luteolinidin and tricetinidin) a sugar always replaces the 3-hydroxyl of the anthocyanidin. Additional glycosylation has been shown to occur at the hydroxyls at C-5, C-7, C-3', C-4' and C-5' (Yoshitama, 1977; Yoshitama and Abe, 1977; Stirton and Harborne, 1980). The most common sugars of glycosylation are glucose, galactose, rhamnose and arabinose (Timberlake, 1980). Di- and trisaccharides formed by combinations of these four monosaccharides may also occur (Brouillard, 1982). Glycosylation is believed to confer solubility and stability (Harborne, 1979) and plays an important role in acylated anthocyanins since the acyl residues are linked to the sugars. The acylating agents most frequently encountered belong to the hydroxylated cinnamic acid series and include p-coumaric, caffeic, sinapic and

Table 3. Naturally occurring anthocyanidins.

Flavylium salt	Substitution pattern (R)					
	3	5	6	7	3'	5'
Apigeninidin (Ap)	H	OH	H	OH	H	H
Luteolinidin (Lt)	H	OH	H	OH	OH	H
Tricetinidin (Tr)	H	OH	H	OH	OH	OH
Pelargonidin Pg)	OH	OH	H	OH	H	H
Aurantinidin (Au)	OH	OH	OH	OH	H	H
Cyanidin (Cy)	OH	OH	H	OH	OH	H
Peonidin (Pn)	OH	OH	H	OH	OMe	H
Rosinidin (Rs)	OH	OH	H	OMe	OMe	H
Delphinidin (Dp)	OH	OH	H	OH	OH	OH
Petunidin (Pt)	OH	OH	H	OH	OMe	OH
Pulchellidin (Pl)	OH	OMe	H	OH	OH	H
Europinidin (Eu)	OH	OMe	H	OH	OMe	OH
Malvidin (Mv)	OH	OH	H	OH	OMe	OMe
Hirsutidin (Hs)	OH	OH	H	OMe	OMe	OMe
Capensinidin (Cp)	OH	OMe	H	OH	OMe	OMe

From Mazza and Brouillard, 1987a.

ferulic acids (Albach et al., 1965; Saito et al., 1971; Goto et al., 1979, 1982, 1983a; Brouillard, 1981, 1983). Recently, anthocyanins acylated with aliphatic dicarboxylic acids, usually malonic acid , have been identified (Cornuz et al., 1981; Bridle et al., 1984; Harborne and Boardley, 1985; Takeda et al., 1986). It has been proposed that the acyl groups may stabilize the anthocyanin by interacting with the positively charged pyrylium ring to protect it from nucleophilic attack by water (Brouillard, 1981; Goto et al., 1982) or by favoring complexation of the acylated anthocyanin with another molecule (Hoshino et al., 1980a).

2.2.4 Significance in Food

The biological function of anthocyanins is to impart an appealing colour to attract insects, birds and animals to plants for purposes of pollination and seed dispersal (Harborne, 1976). Colour is also an important attribute for providing an attractive appearance to food. It has been reported that liking or disliking a food is conditioned by its colour; attractive foods are sought out as pleasure-giving while unattractive foods are avoided (Meggos, 1984).

Processing conditions for production of food or beverage products often result in either the alteration or removal of the natural pigments, yielding an insufficiently coloured final food product and in some fabricated foods, colour may even be completely lacking. In these situations the natural or characteristic colour must be restored or

imparted in order for the product to be appetizing and therefore, food colourants are usually added. A food colour additive can generally be defined as any material which when added or applied to a food, is capable of imparting a colour thereto (Health and Welfare Canada, 1981; FDA, 1982; Meggos, 1984). Health and Welfare Canada (1981) stipulates that food colours are used in the following situations: to replace natural colours which have been destroyed or altered by heat or by preservatives; to ensure uniformity of colour from batch to batch where raw materials of varying colour intensity have been used; to intensify the natural colour of products when it is believed consumers prefer stronger colours; to restore colour to products in which the natural colours are affected by light; to give "eye appeal" to foods which otherwise might look less attractive or less appetizing. With the development of processed foods, the widely available, easily synthesized and inexpensive azo-dyes came to be used in manufacturing food products such as carbonated and still beverages, dry mixes, baked goods, confections and dairy products.

In recent years, however, a large number of medical studies have suggested that the synthetic food colours may be toxic, carcinogenic or detrimental to mental and physical health (Yoshimoto et al., 1977, 1979, 1984; IFT, 1986). As a result, a trend toward the use of natural food colourants in place of the synthetic compounds has developed. Francis (1984) reports that the total number of patents for food colourants doubled for the years 1976-81 in comparison with the years 1971-75. Patents in the natural colourant class more than doubled whereas patents

for synthetic colourants were equal. Also, the applications for crude plant extracts and novel plant pigments showed substantial increase.

The anthocyanins are potential replacements for the red, purple and blue synthetic colourants. Since the banning of Amaranth (FD&C Red No. 2) in 1976 in the United States (IFT, 1986), the importance of minimizing the loss of anthocyanins in foods and utilizing these pigments as food colourants has greatly increased.

The utilization of anthocyanins as food colourants holds the following advantages: They have been consumed by humans and animals for countless generations without adverse effects to health; they are brightly coloured, especially in the red region; and they are water-soluble, which simplifies their incorporation into aqueous food systems (Francis, 1984). The current limitations to the use of anthocyanins are that they are not very stable chemically, they are difficult to purify (Francis, 1975) and their tinctorial strength is generally weaker than that of the synthetic dyes (Riboh, 1977). However, recent findings on the influence of chemical structure, pH and copigments on anthocyanin stabilization may increase further their potential as food colourants (Timberlake and Bridle, 1977; Brouillard and Dubois, 1977; Brouillard, 1983; Goto et al., 1983b; Mazza and Brouillard, 1987b, c).

The stability of anthocyanins is of significance in the marketability of fresh fruit and processed products such as jams, jellies, syrups, juices and wines (Fuleki, 1969; Watada and Ab, 1975; Ballinger et al.,

1974; Francis, 1975). Red food colourants can be prepared from anthocyanin extracts of commercial fruits such as grapes (Main et al., 1978), miracle fruit (Buckmire and Francis, 1978), blueberries (Francis, 1985), cherries (McLellan and Cash, 1979), cranberries (Chiriboga and Francis, 1973) and elderberries (Bronnum-Hansen et al., 1985).

2.2.5 Stability in Foods

Anthocyanins are relatively unstable in harvested fruits, processed fruit products and other foods in which they exist. Their stability varies with the aglycone structure as well as the degree of glycosylation and acylation and is strongly influenced by physical and chemical factors.

The most important physical factors are temperature and light. The effect of temperature on anthocyanin degradation has been extensively studied (Skalski and Sistrunk, 1973; Timberlake, 1980; Sistrunk and Morris, 1982; Maccarone et al., 1985; Morris et al., 1986). The mechanism responsible for thermal degradation is believed to be associated with the structural transformation of anthocyanins which is endothermic from the left to right direction (Brouillard and Delaporte, 1978; Brouillard, 1982):

$$\underset{\text{base (Blue)}}{\text{Quinoidal (A)}} \rightleftharpoons \underset{\text{cation (Red)}}{\text{Flavylium } (AH^+)} \rightleftharpoons \underset{\text{(Colourless)}}{\text{Pseudobase (B)}} \rightleftharpoons \underset{\text{(Colourless)}}{\text{Chalcone (C)}}$$

Thus, increasing temperature causes a shift in anthocyanin structure to

the colourless forms.

Exposure to light has also been reported to increase the rate of anthocyanin loss in grapes (Palamidis and Markakis, 1975), grape juice (Flora, 1977), cranberry (Sapers et al., 1983; Attoe and von Elbe, 1981), and red cabbage (Sapers et al., 1981). The photodegradation mechanism for the pigments of these fruits, however, remains to be determined.

Chemical factors which influence the stability of anthocyanins are levels of pH, oxygen, ascorbic acid, sulfur dioxide, sugars and their degradation products, enzymes and nonanthocyanin phenolic compounds. It has been shown that anthocyanins are more stable in acidic solutions than in neutral or alkaline conditions and that lowering the pH contributes far more colour stability to anthocyanins than any other method (Sistrunk and Cash, 1974; Markakis, 1982; Simard et at., 1982). The nature of the chemical structures which anthocyanins can adopt with changing pH has been clarified (Brouillard and Dubois, 1977; Brouillard and Delaporte, 1977; Brouillard et al., 1978, 1979; Brouillard, 1982; Mazza and Brouillard 1987b). At room temperature in acidic aqueous solutions, four anthocyanin species can exist in equilibrium: the quinoidal base (A), the flavylium cation (AH^+), the pseudobase or carbinol (B) and the chalcone (C) (Figure 4). Interconversion between these four structures takes place according to Scheme 1 (Brouillard and Delaporte, 1977; Brouillard, 1982).

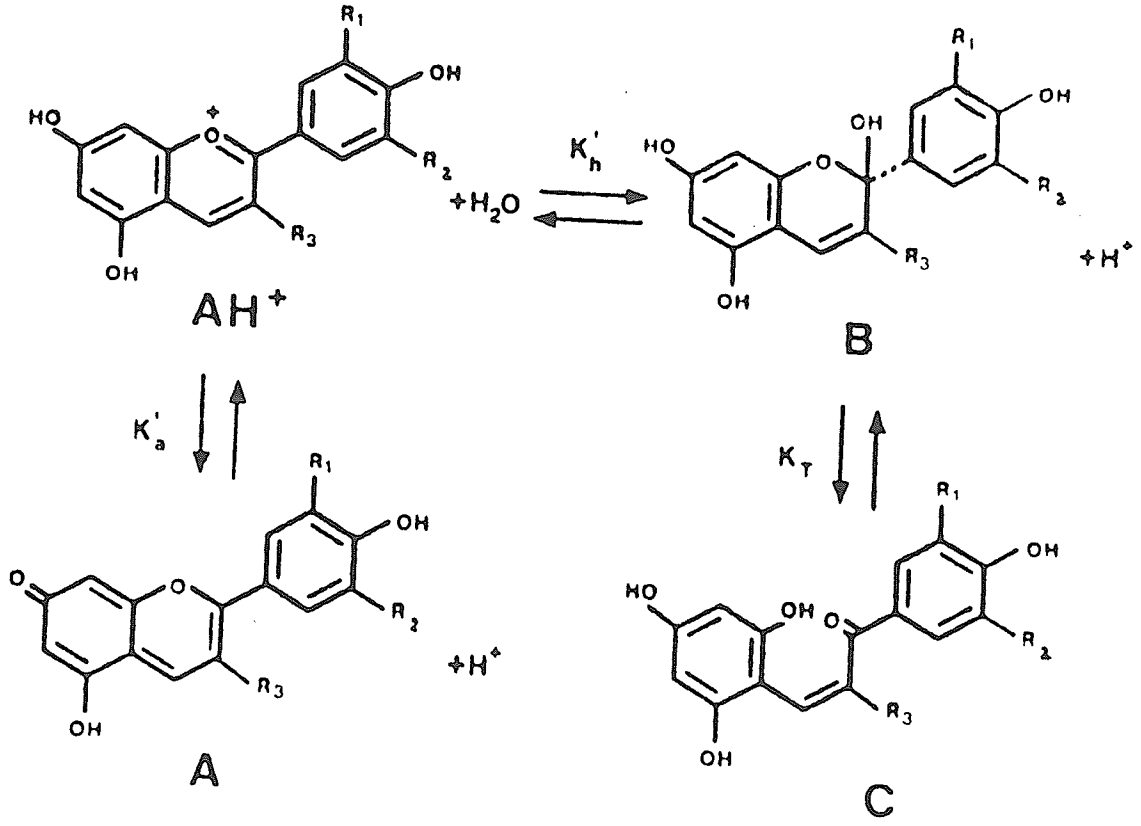

Figure 4. Structural transformations of anthocyanins. A = quinoidal base, AH^+ = flavylium cation, B = pseudobase and C = chalcone. R_1 is usually glycosyl, R_2 and R_3 are H, OH or OCH_3. (From Mazza and Brouillard, 1987a).

Scheme 1

$AH^+ \overset{K_a}{\rightleftharpoons} A + H^+$ Acid-base equilibrium (1)

$AH^+ + H_2O \overset{K_h}{\rightleftharpoons} B + H^+$ Hydration equilibrium (2)

$B \overset{K_T}{\rightleftharpoons} C$ Ring-chain tautomeric equilibrium (3)

The K_a, K_h and K_T are the equilibrium constants for the acid-base, hydration and ring-chain tautomeric equilibria, respectively. The $K_a = ([A]/[AH^+])a_{H+}$, $K_h = ([B]/[AH^+])a_{H+}$, $K_T = [C]/[B]$ and a_{H+} is the activity of the hydronium ion ($pH = -\log a_{H+}$). The relative amounts of AH^+, A, B and C at equilibrium vary with pH and structure of the anthocyanin. At pH values below 2.0 the anthocyanin usually exists primarily in the form of the red (R_3 = O-sugar) or yellow (R_3 = H) flavylium cation (AH^+). Increasing the pH causes a rapid proton loss to yield the blue quinoidal forms (A). In long-standing slightly acidic solutions, the hydration of the flavylium cation (AH^+) occurs to give the colourless carbinol or pseudobase (B). This, in turn, at an even slower rate, can irreversibly shift to the open chalcone form (C), which is also colourless. In alkaline media the chalcone is formed quite rapidly (Brouillard, 1982). Such a result is of practical importance, as it indicates that alkaline conditions should be avoided in the processing of foods containing anthocyanins. Conversion of the colourless to coloured forms is possible; however, it takes approximately 12 h for the

chalcone form of 3,5-diglucosides and 6-7 h for the chalcone form of 3-glucosides to reach equilibrium with the corresponding flavylium ion form at 25°C (longer at lower temperatures) (Brouillard, 1982).

The presence of oxygen can increase the lability of anthocyanins to other degradation factors (Attoe and von Elbe, 1981). Daravingas and Cain (1965) found greater retention of anthocyanin in raspberries canned under nitrogen or vacuum than under air. Similarly, Clydesdale et al. (1978) reported that the stability of Concord grape pigments used as colourants of a dry beverage mix was greatly enhanced by flushing the package with nitrogen.

Ascorbic acid has been shown to contribute to anthocyanin degradation (Starr and Francis, 1973; Shrikhande and Francis, 1974; Calvi and Francis, 1978). Under anoxic conditions the degradation of anthocyanins and ascorbic acid is thought to occur through a direct condensation mechanism (Poei-Langston and Wrolstad, 1981). In the presence of oxygen, hydrogen peroxide formed from the aerobic degradation of ascorbic acid is believed to decolourize the pigment. Other studies, however, have shown contradictory results to the ascorbic acid induced degradation of anthocyanins. Sistrunk and Gascoigne (1983) found that ascorbic acid caused browning in grape juice but did not affect the anthocyanin content. In the presence of ascorbic acid, Kallio et al. (1986) observed a slight increase in the stability of anthocyanins when oxygen was not removed and a decrease in stability in crowberry juice samples flushed with nitrogen.

Sugars have been found to accelerate anthocyanin breakdown and sugar degradation products, namely furfural and 5-hydroxymethylfurfural, which are formed when sugars are heated with acids, are even more effective (Tinsley and Bockian, 1960; Daravingas and Cain, 1965, 1968; Debicki-Pospisil et al., 1983). It is believed that the sugar degradation products condense with anthocyanins to form brown coloured products (Eskin, 1979).

Sulfur dioxide has a reversible bleaching effect on anthocyanins (Palamidis and Markakis, 1975; Markakis, 1982). The negative bisulfite ion adds directly to the anthocyanin at position two (Jurd, 1964) or four (Timberlake and Bridle, 1968), producing a colourless sulfonic acid.

Enzymatic systems are capable of decolourizing anthocyanins. Glycosidases have been shown to free the anthocyanidins from their sugar moieties, resulting in a destabilized aglycone which will spontaneously degrade to a colourless product (Eskin, 1979; Blom and Thomassen, 1985). Such enzymes are known as anthocyanases. Phenolases may react directly with anthocyanins to produce brown o-quinones but degradation is generally more vigorous when other phenolic compounds, which are better phenolase substrates than anthocyanins, are also present in the system (Pifferi et al., 1979; Markakis, 1982). Peng and Markakis (1963) proposed that nonanthocyanin phenolic compounds are first oxidized to o-

benzoquinones; the anthocyanins are then oxidized by o-benzoquinones to colourless products.

Anthocyanins may associate with certain other phenolic and non-phenolic compounds (copigments) to form highly stable complexes which produce an increase in absorbance and a bathochronmic shift (i.e. toward a longer wavelength) in the maximum visible wavelength. The formation of such complexes is known as copigmentation and may occur by covalent bonding of the copigment to the anthocyanin molecule, referred to as intramolecular copigmentation, or by noncovalent association, called intermolecular copigmentation (Osawa, 1982). Generally, copigments themselves do not contribute to colour. However, copigmentation is important for producing the striking colours of many flowers and fruits when, at the pH of the cell sap, they may be expected to have little or no colour (Asen et al., 1970, 1972).

Intramolecular copigments include the glycosyl groups of polyglycosylated anthocyanins and acyl groups of acylated anthocyanins (Osawa, 1982). These types of copigments are constituents of the anthocyanin molecule and function in stabilizing the coloured flavylium cation and quinoidal forms of the anthocyanin over a wide range of pH and other colour degradation conditions.

The most efficient intermolecular copigments are nonanthocyanin flavonoids (Brouillard, 1983), particularly flavonols such as rutin (Williams and Hrazdina, 1979), quercetin and kaempferol (Jurd and Asen,

1966; Asen et al., 1971a, b, 1975) and C-glycosylflavones such as swertisin (Asen et al., 1970). Other intermolecular copigments include alkaloids, amino acids and nucleotides (Asen et al., 1972; Mazza and Brouillard, 1987a).

The structure of the anthocyanin-copigment complex is controversial. Some workers favour a horizontally (end-to-end) bound complex between the quinoidal form of the anthocyanin and the copigment, stabilized by hydrogen bonding (Williams and Hrazdina, 1979; Somers and Evans, 1979; Chen and Hrazdina, 1981). Others support a vertically stacked (molecules in parallel arrangement) complex bound primarily by hydrophobic interactions between the aromatic rings of the pigment and copigment (Goto et al., 1979; Hoshino et al., 1980a, 1982; Brouillard, 1983). Sweeny et al. (1981) reported that flavonoid sulfonates can bind with anthocyanins by hydrophobic interactions and also through the attraction of the negative charge of the sulfonic acid groups to the flavylium cation.

The ability of anthocyanins to react with copigments is influenced by the physical and chemical properties of the environment in which they exist, as well as the types and amounts of reacting components themselves. Copigmentation has been reported to occur throughout the pH range 1 to 7 with the specific pH value affecting the degree of copigmentation (Asen et al., 1971a, 1972; Yazaki, 1976; Williams and Hrazdina, 1979). Mazza and Brouillard (1987c) observed a decrease in absorbance and a bathochromic shift with increasing temperature of solutions of an-

thocyanins copigmented with chlorogenic acid. Scheffeldt and Hrazdina (1978) found that the ability to form deeply coloured complexes with rutin varied with the type of anthocyanin. Other factors reported to influence copigmentation are the concentration of the anthocyanins and the molar ratio of copigments to anthocyanins (Asen et al., 1971b, 1972; Mazza and Brouillard, 1987c). An increase in either of these parameters produces an increase in copigmentation and colour intensification.

In concentrated aqueous solutions anthocyanins can self-associate to from highly coloured pigment-pigment complexes. Due to self-association, increasing the anthocyanin concentration produces an increase in the visible absorbance of the solution and depending on the type of anthocyanin, a hypsochromic (i.e. towards a lower wavelength) or bathochromic shift in the maximum absorbance wavelength (Asen et al., 1975; Hoshino et al., 1980b, 1981a). At high concentrations the increase in absorbance will be greater than that predicted by the Beer-Lambert law (a 'positive' deviation) (Brouillard, 1983). Scheffeldt and Hrazdina (1978) suggested that competition can take place between the copigmentation and self-association reactions.

Colour stabilization of anthocyanins may also occur by metal complexing, direct condensation with nonanthocyanin phenolic compounds and condensation with catechin and acetaldehyde. Metal ions such as Al^{3+}, Cu^{2+}, Fe^{2+} and Sn^{2+} can complex with anthocyanins possessing vicinal phenolic hydroxyls to produce a bathochromic shift in colour and a stabilization of the pigment (Starr and Francis, 1973; Coffey et al.,

1981; Kallio et al., 1986). Naturally occurring stable anthocyanin-metal complexes have been reported for tin (Salt and Thomas, 1957), copper (Somaatmadja et al., 1964) and aluminum (Jurd and Asen, 1966). As well, the presence of metal ions (Mg^{2+}, Fe^{2+}, K^{+}) has been noted in the structure of stable acylated anthocyanins (Takeda and Hayashi, 1977; Takeda, 1977). More recent reports, however, indicate that the presence of metal ions is not required to stabilize the colour of these metalloanthocyanins and although the metals provide some protection to the anthocyanins, these complexes are generally unstable and decompose with time (Hoshino et al., 1980a).

Anthocyanins may undergo direct condensation with various phenolic compounds. Jurd and Waiss (1965) demonstrated condensation products from synthetic flavylium salts with various phenolic compounds in aqueous solutions at pH 3 - 5 and by reaction with catechin, obtained a dimeric flavylium-flavene pigment (Jurd, 1967). All these reactions involved substitution in the 4-position of the heterocyclic ring and suggested that similar condensations may occur with anthocyanins. Concurrently, Somers (1966) reported that red wine tannins contain anthocyanin units in their structure. The term tannin is defined as those phenolic compounds of sufficiently high molecular weight (> 500) to form reasonably strong complexes with proteins and other polymers under suitable conditions of concentration and pH (Goldstein and Swain, 1963). The anthocyanidin pigments were easily released from the tannin structure by mild acid hydrolysis, suggesting that only weak chemical bonds were involved between the anthocyanins and main proanthocyanidin matrix. It

was also suggested that anthocyanins undergo direct condensation with various other phenolic compounds such as catechin (Somers, 1971). Such polymeric pigments are reddish-brown, with the actual tint depending on the ratio of anthocyanin to quinone moieties in the polymer (Ribereau-Gayon, 1982). Direct condensation of anthocyanins with other flavonoids stabilizes anthocyanin colour (Somers, 1971) and increases polymeric colour, browning, % colour due to tannin and possibly, turbidity (Wrolstad, 1976).

Condensation of anthocyanins with flavan 3-ols such as catechin may be induced by adding acetaldehyde. Timberlake and Bridle (1976, 1977) isolated a stable, highly coloured complex resulting from the interaction of anthocyanins, acetaldehyde and catechin-type compounds. The new compound was more violet and demonstrated a higher absorbance at 280 nm than the known anthocyanin. The increase in A_{280} was consistent with the presence of an equimolar amount of catechin which also showed a λmax at 280 nm. Formation of the new component was accompanied by loss of the anthocyanin and phenolic reactants. Although the exact structure has not been confirmed, the polymerized complex was believed to consist of anthocyanin and phenolic compounds linked by CH_3CH bridges. By analogy of the 7-hydroxy, 7-hydroxy-6-methyl and 7-hydroxy-8-methyl flavylium chlorides, they proposed the 6 and 8 positions of the A-ring as the reactive positions on the anthocyanin ring. Introduction of a methyl group into position 6 of the 7-hydroxy flavylium chloride did not alter the peak position (λmax) and had little effect on quinoidal base formation. Compared with the 7-(OH)-flavylium salt, however, 7-(OH)-8-

(CH_3)-flavylium chloride exhibited a bathochromic shift of 8 nm, was much more coloured at pH 2-4 and formed its quinoidal base at much lower pH. Also, addition of catechin and acetaldehyde to crude extracts of elderberry anthocyanin has been reported to enhance and stabilize colour (Timberlake, 1980; Timberlake and Bridle, 1980).

It may be expected that in fruit extracts and processed fruit products such as jam, jelly, syrup and juice, all reactants of the acetaldehyde-induced condensation of anthocyanins will be present. Catechin-type phenolic compounds and various other flavonoids occur in all plants and are produced by the same biosynthetic pathway as the anthocyanins (Figure 1) (Grisebach, 1982). Acetaldehyde is a naturally occurring plant metabolite which is usually found in fruit in trace amounts but begins to accumulate as fruits ripen and as a result of physiological disorders (Smagula and Bramlage, 1977; Paz et al., 1981). The exact biological function of acetaldehyde is not clear but it may contribute to the stimulation of ripening (Janes and Frenkel, 1978) and wound healing of the fruit after physiological damage (Smagula and Bramlage, 1977). In wine, acetaldehyde is formed by several mechanisms, principally by microbial action during fermentation and more slowly from ethanol by coupled oxidation of certain phenolic compounds (Wildenradt and Singleton, 1974).

2.2.6 Analytical Techniques for Anthocyanins

2.2.6.1 Extraction of Anthocyanins from Plant Material

Extraction is the first step in the determination of total as well as individual anthocyanins in any type of plant tissue. This is usually accomplished by quantitatively extracting the anthocyanins with an appropriate solvent (Fuleki and Francis, 1968a). Acidified methanol is the most effective and one of the most widely used solvents but acidified ethanol is only slightly less effective (Metivier et al., 1980). Hydrochloric acid is a common acidifying agent, although anthocyanins acylated with acetic acid undergo deacylation when exposed to mineral acids (Timberlake and Bridle, 1975). Organic acids, such as citric, acetic, tartaric and formic have also been reported to be efficient acidifying agents (Metivier et al., 1980). Fuleki and Francis (1968a) developed a method of anthocyanin extraction which has been widely applied. In this procedure, the anthocyanin containing sample is mechanically ground with the extracting solvent to disrupt the tissues and to increase the extraction of the pigment. The macerate is left in the dark at 4°C overnight to allow for equilibration of the pigment between the cell tissue and the solvent. The resulting mixture can then be diluted with extracting solvent and/or filtered until the final anthocyanin solution is clear enough for pigment measurement.

2.2.6.2 Quantitative Measurement of Anthocyanin

The total anthocyanin concentration is most often determined in the crude pigment extract by measuring the absorbance of the solution at a wavelength which is characteristic of the anthocyanins (510-550 nm) (Harborne, 1967; Markham and Mabry, 1975). There are three methods used to measure the absorbance of an anthocyanin containing solution; the single pH method, the pH differential method and the bleaching method. In the single pH method, the absorbance of the anthocyanin solution is measured at one pH where the anthocyanins are coloured (Fuleki and Francis, 1968a). The pH differential method is based on the fact that the absorptivity of anthocyanins is markedly dependent on pH (Sondheimer and Kertesz, 1948; Fuleki and Francis, 1968b). The absorbance of the solution is calculated as the difference between the readings at two different pH values - one pH at which the anthocyanins express colour and another at which they are colourless. This method corrects for any interfering absorbance due to anthocyanin degradation products, chlorophyll compounds and sugar-amino acid Maillard-type reaction products (Francis, 1982). The anthocyanin bleaching method is based on subtraction techniques in which the absorbance of the pigment solution is measured before and after a treatment which bleaches the colour of either the anthocyanins or the interfering compounds. Common bleaching agents used in this method are sodium sulfite and hydrogen peroxide (Swain and Hillis, 1959). An efficient sulfite bleaching technique for determining colour parameters of wines has been developed (Somers, 1971, 1972; Somers and Evans, 1974). This method (the Potassium Metabisulfite

Method) is advantageous because colour density, polymeric colour, % contribution by tannin, turbidity, browning and anthocyanin content all can be calculated from a few absorbance readings. Spayd et al. (1984) applied this procedure to determine the colour stability of various anthocyanin pigmented juices.

In addition to the spectrophotometric methods, tristimulus colourimetric measurements have also been used for the quantitation of anthocyanins in fruits and fruit products. Francis (1957) showed that the Hunter 'a' reading correlated very highly with anthocyanin content in fresh cranberries. Watada and Ab (1975) found high correlation between Hunter 'L' and the logarithm of the anthocyanin content of grapes. A significant relationship between hue angle and colour due to anthocyanins has been shown for strawberry preserves (Abers and Wrolstad, 1979) and wine (Ballinger et al., 1974; Bakker et al., 1986). Drake et al. (1982) obtained highly significant correlations for Agtron reflectance colour with anthocyanin content in both fresh and canned dark sweet cherries. Sapers et al. (1984), however, found that tristimulus reflectance measurements of highbush blue berries did not correlate with anthocyanin content nor anthocyanin pattern. Johnson et al. (1976) reported that Hunter 'b' and hue values are not good indicators of anthocyanin content in stored cranberry juice cocktail since pigment degradation products (browning compounds) obscure this relationship. On the other hand, they found that expanded L, a^* and b^* values developed previously by Eagerman et al. (1973a, b) were highly correlated with anthocyanin concentration.

Others have used Hunter colour parameters to describe visual colour deterioration in anthocyanin-containing products (Poei-Langston and Wrolstad, 1981; Spayd and Morris, 1981; Sistrunk and Gascoigne, 1983; Skrede et al., 1983; Skrede, 1985).

Recent developments in high performance liquid chromatography (HPLC) enable high resolution in separating mixtures of very similar phenolic compounds and provide the ability to analyze quantitatively for the individual anthocyanins in microgram quantities without extensive time or labour (Francis, 1982). Andersen (1987) calculated the total amount of anthocyanins in blueberries using HPLC alone.

2.2.6.3 Isolation and Identification of Anthocyanins

2.2.6.3.1 Preliminary purification of anthocyanin extracts

Crude anthocyanin extracts usually contain interfering compounds such as chlorophylls, sugars, amino acids, proanthocyanins (i.e. polymeric phenolic compounds which yield anthocyanins when heated in acid) and nonanthocyanin flavonoids (Francis, 1982). As a result, these pigment extracts often must be purified before individual anthocyanins can be separated and identified.

Anthocyanins can be purified by precipitation as lead salts by basic lead acetate, with subsequent removal of the precipitate by

centrifugation (Deibner and Bourzeix, 1963). Fuleki and Francis (1968c) found that precipitation by lead acetate was suitable for the preliminary purification of anthocyanins present in aqueous or alcoholic plant extracts since the most troublesome and plentiful impurities, the sugars, are soluble in this reagent. However, they reported that the method is not suitable for absolute purification of anthocyanins because nonanthocyanin flavonoids and tannins are also precipitated with the pigment.

Column chromatography has been widely applied for purifying crude anthocyanin extracts. Ion exchange chromatography on cationic resins, usually on Amberlite CG-50 (Fuleki and Francis, 1968c), is the most prevalent of these techniques (Chiriboga and Francis, 1973; Shrikhande and Francis, 1974; Attoe and von Elbe, 1981; Coffey et al., 1981; Karppa et al., 1984). The most common nonionic resin for the isolation and purification of anthocyanins is insoluble polyvinylpyrrolidone (Gallop, 1965; Fuleki and Francis, 1968c; Wrolstad and Putnam, 1969; Hrazdina, 1970; Torre and Barritt, 1977). Others have used alumina (Birkofer et al., 1966), cellulose powder (Vuataz et al., 1959), magnesol (Ice and Wender, 1952), sephadex (Somers, 1967), silica gel (Li and Wagenknecht, 1958), talc (Filippov et al., 1971) and various other polyamides (Chandler and Swain, 1959; Zapsalis and Francis, 1965; Fuleki and Francis, 1968c).

The classic column chromatographic techniques allow large quantities of anthocyanins to be isolated and purified. Although excellent

results have been obtained in certain cases, the technique is time consuming and not applicable to all plant materials (Seikel, 1962; Manley and Shubiak, 1975).

Ultrafiltration is a relatively new technique which has been applied for the purification and concentration of anthocyanins. Philip (1984) reported that a cellulose acetate ultrafiltration membrane with a molecular weight cutoff of 1,000 was suitable for purifying and concentrating grape skin anthocyanin extracts. He found that the membrane had unique properties which enable the separation of natural colourants from other dissolved solids. The procedure removed 75-90 % of the water and 50-60 % of the sugars in a single pass, thereby achieving a two-fold increase of pigment concentration relative to dissolved solids. Tannins, however, were not separated from the anthocyanins. Chung et al. (1986) reported that large, complex anthocyanins in crude pigment extracts were purified and concentrated by ultrafiltration through a membrane of molecular weight cutoff of 6,000. However, a long time period is required to achieve the desired concentration and mechanical abuses by recycling the retentate may damage some of the pigment.

2.2.6.3.2 Separation of individual anthocyanins

A variety of methods is available for the separation of complex mixtures of anthocyanins. Paper chromatography has been widely used, especially when only limited quantities of sample are available (Seikel, 1962). Numerous paper chromatographic techniques have been applied to

determine the individual anthocyanins and their relative proportions in fruits such as highbush blueberries (Ballinger et al., 1970), Montmorency cherries (Schaller and von Elbe, 1968), cranberries (Fuleki and Francis, 1968d), grapes (Yokotsuka et al., 1984), raspberries (Daravingas and Cain, 1965) and strawberries (Fuleki, 1969) as well as in model systems (Tinsley and Bockian, 1960; Adams, 1973). Paper chromatography is convenient for separating individual anthocyanins from complex mixtures at relatively low expense.

Thin layer chromatography (TLC) is a technique which is complementary to paper chromatography in that it provides a wider variety of media and detection reagents for the separation of anthocyanins (Markham, 1975). Adsorbents which have been used for anthocyanin analysis on TLC are cellulose (Wrolstad and Putnam, 1969; Torre and Barritt, 1977; Flora, 1978), silica gel and polyamide (Wrolstad, 1968). The TLC adsorbents have a high surface area which may enable faster analysis and better resolution than paper chromatography.

Paper electrophoresis has also been applied for the separation of anthocyanins. Markakis (1960) carried out fundamental paper electrophoretic studies of anthocyanins and confirmed their cationic nature. Schaller and von Elbe (1968) used this technique to separate three major pigments from Montmorency cherries and Cansfield and Francis (1970) used paper electrophoresis to separate anthocyanins from other phenolic components present in cranberry. In the past, this technique has had only limited application in anthocyanin isolation and separation,

largely because it offers little or no advantage over paper chromatography (Markham, 1975). Recently, however, paper electrophoresis has been found valuable in detecting the newly discovered malonated anthocyanins in their zwitterionic form (Cornuz et al., 1981; Bridle et al., 1984; Harborne and Boardley, 1985; Takeda et al., 1986).

Recent developments in gas-liquid chromatography (GLC) and high performance liquid chromatography (HPLC) show substantial benefits in the ability to separate complex mixtures of anthocyanins. Unfortunately, separation by GLC requires derivatization to provide volatility and this introduces problems in stability for anthocyanins (Francis, 1982). However, rapid qualitative separation and identification of anthocyanins has been obtained using GLC coupled with mass spectrometry (Bombardelli et al., 1976, 1977; Lanzarini et al.,1977).

Manley and Shubiak (1975) were the first to use HPLC for the separation of anthocyanins when they separated the three monoglucosides of malvidin, petunidin and peonidin. Since then, numerous other workers have applied HPLC to the separation of these pigments (Adamovics and Stermitz, 1976; Wilkinson et al., 1977; Williams et al., 1978; Wulf and Nagel, 1978; Camire and Clydesdale, 1979; Strack et al., 1980; Karppa et al., 1984; Mazza, 1986). Anthocyanins are separated on reversed phase HPLC columns, usually composed of an octasilane or octadecylsilane base (Akavia and Strack, 1980; Saag, 1982). Single mobile phases have included water-methanol-formic acid (74:16:10 v/v) (Karppa et al., 1984; Kallio et al., 1986) and methanol-acetic acid-water (37:10:53 v/v)

(Camire and Clydesdale, 1979). Two mobile phases such as 0.01 M trifluoroacetic acid (TFA) in water/0.01 M TFA in 40% (v/v) acetonitrile in water (Bishop and Nagel, 1984), 10% formic acid in water/10% formic acid in 50% methanol in water (Wulf and Nagel, 1978) and 5% formic acid in water/methanol (Mazza, 1986) have been applied in a gradient elution profile to obtain complete separation of very similar anthocyanins. Ultraviolet (UV) detectors are usually coupled with HPLC systems in order to allow identification of the separated pigments eluting off the column (Francis, 1982). The advantages of HPLC over other separation techniques for anthocyanins include short analysis time, high resolution, no derivatization, no risk of thermal decomposition, easy quantification and usually no initial purification of the crude pigment extract is required (Markham, 1975).

2.2.6.3.3 Identification of anthocyanins

The most common identification parameters for purified anthocyanins are: 1) relative retention factor (R_f) on paper chromatography, TLC and paper electrophoresis; 2) relative retention time (t_R) on GLC and HPLC columns; and 3) visible and UV absorbance spectra. Spectral characteristics such as shape of the spectrum, position of maxima, minima, inflections and shoulders are very useful for the elucidation of the chemical structure of a given anthocyanin (Timberlake and Bridle, 1975). Further structural information can be gained by the addition of specific reagents which can react with one or more functional groups on the anthocyanins to induce structurally significant shifts in the UV and

visible spectra (Harborne, 1958, 1967; Jurd, 1962; Mabry et al., 1970; Markham and Mabry, 1975).

Other forms of spectroscopy which have been used to a limited extent for the identification of anthocyanins are fluorescence (Lynn and Luh, 1964; Ristic and Baranac, 1967) and infrared (IR) spectroscopy (Ribereau-Gayon and Josien, 1960; Bendz et al., 1967). However, IR spectroscopy is of limited use for the study of anthocyanins in aqueous solutions since water strongly absorbs throughout the IR region. Resonance Raman spectroscopy has been proposed as a more viable alternative to the IR technique since water is a poor Raman scatterer (Brouillard, 1983).

Several new techniques have been applied for the identification of anthocyanin structures. Bombardelli et al. (1976, 1977) used electron-impact mass spectrometry (MS) to aid in determining the structures of anthocyanins eluting from a gas chromatograph. These workers reported that information about oxygenation patterns and the location of hydroxyl, methoxyl and acyloxyl groups on the A and B rings of the anthocyanidin skeleton can be obtained by this technique. However, the use of MS is hampered by the limited volatility of anthocyanins. As a result, these compounds must first be converted into volatile derivatives, usually by reaction with trimethylchlorosilane and hexamethyldisilane. More recently, Saito et al. (1983) and Takeda et al. (1986) avoided the derivatization step by using fast atom bombardment mass spectrometry (FAB-MS) to establish molecular weights and linkage modes

between the sugar and phenolic acid residues of complex acylated anthocyanins. Circular dichroism has been shown to be a sensitive probe in demonstrating the structure and association of anthocyanins (Goto et al., 1979; Hoshino et al., 1980b, 1981b) and has been applied to living plant tissue (Hoshino, 1986). Developments in nuclear magnetic resonance (NMR) spectroscopy have established this technique as an invaluable tool for anthocyanin structure analysis (Markham and Mabry, 1975; Timberlake and Bridle, 1975; Brouillard, 1983). It has been applied to elucidate structures of derivatized anthocyanidins (Bombardelli et al., 1976), complex acylated anthocyanins (Goto et al., 1982, 1983b) and condensed anthocyanins (Chen and Hrazdina, 1981; Hoshino et al., 1982; Bishop and Nagel, 1984).

Further confirmation of structure is often obtained by chemical treatment of the anthocyanins to systematically fragment the pigments. The constituent residues along with reference compounds can then be subjected to the physical methods of separation and identification mentioned above. Acid hydrolysis with hydrochloric acid is widely used to randomly cleave sugar residues from the anthocyanidin (Albach et al., 1963, 1965; Francis and Harborne, 1966; Mabry et al., 1970; Francis, 1982); peroxide hydrolysis is specific for sugars on the C-3 position (Francis and Harborne, 1966). Acyl substituents can be removed by mild alkaline hydrolysis (Albach et al., 1963). Demethylation occurs when anthocyanins are subjected to caustic alkaline conditions (Harborne, 1967) and reductive cleavage of the pyrilium ring can be obtained using sodium amalgam (Hurst and Harborne, 1967). The use of the enzyme

anthocyanase also gives an indication of anthocyanin structures (Harborne, 1967).

3. MATERIALS AND METHODS

3.1 ANTHOCYANINS AND OTHER CONSTITUENTS OF SASKATOON BERRIES

3.1.1 Saskatoon Berries

Samples from eight saskatoon berry cultivars (Beaverlodge, Honeywood, Northline, Parkhill, Porter, Regent, Smoky, and Success) showing a wide range of maturity were hand picked in mid-July of 1983 and 1984 from a plantation at the Agriculture Canada Research Station, Morden, MB. The berries were frozen within two hours after harvest and stored at -25°C ± 1°C. The 1983 berries were held in frozen storage for approximately ten months while the 1984 crop was stored for one to two months. From the two crops, five of the eight cultivars were separated by visual assessment into three levels of development according to colour: (1) red colour appearance over the total surface area of the berry; (2) purple with a red tinge over the total surface area of the berry; (3) dark purple colour over the total surface area of the berry. The frozen berries were sorted promptly under minimal light in order to decrease the chance of anthocyanin degradation. These samples (five cultivars of three maturity levels) were used to investigate the effect of maturity on anthocyanin content, anthocyanin degradation, pH, titratable acidity, total solids, soluble solids, soluble solids-titratable acidity ratio, total phenolic compounds, total phenolics-anthocyanin ratio, colour density, polymeric colour, browning,

percent colour due to tannin and Hunterlab parameters of the berries and the pigment extracts.

Limited quantities did not allow separation of all eight cultivars into the three levels of maturity. For cultivars which were not separated into ripeness levels, a random sample was taken and damaged or atypical berries were removed. In samples which had been separated into various stages of maturity, the results from the level two berries were used for comparison among all eight cultivars at the same level of ripeness. The reasoning behind this was that non-uniform ripening occurs on saskatoon bushes and the middle level of berry development would most likely be representative of the average level of maturity.

3.1.2 Extraction and Quantification of Anthocyanins

The anthocyanins were extracted with acidified ethanol by the method of Fuleki and Francis (1968a) as follows: 100 g of frozen berries were blended with 100 mL of 95% ethanol - 1.5 M HCl (85:15 v/v) in a Waring blender at full speed for three minutes. The homogenate was transferred to a beaker, covered and stored overnight at 4°C. The sample was then suction filtered through a Whatman No. 44 filter paper followed by three 100 mL washings and one washing with 75 mL of extracting solvent. The filtrate was poured into a 500 mL volumetric flask and brought to volume with further residue washings.

Total anthocyanin content was determined by the pH differential method of Fuleki and Francis (1968b) using pH 1.0 and 4.5 buffers (Appendix 1). Samples were diluted with pH 1.0 and 4.5 buffers and allowed to equilibrate in the dark for two hours before the visible absorbance spectrum was recorded on a Unicam 800 SP scanning spectrophotometer (Unicam Instrument Limited, Cambridge, England). Since the spectrum continually showed the anthocyanin peak at 510 nm, the difference in absorbance ($\triangle A$) was calculated from A_{510nm} pH 1.0-A_{510nm} pH 4.5. The anthocyanin content was expressed as mg cyanidin 3-galactoside per 100 g berries using $E^{1\%}_{1cm}510 = 765$ (Fuleki and Francis, 1968b). The extinction coefficient of cyanidin 3-galactoside (765) was chosen to express the anthocyanin pigment content because it is the major anthocyanin of saskatoon berries. A sample calculation is shown in Appendix 2.

Anthocyanin degradation index values were calculated from the ratio of anthocyanin content determined by the single pH method using the pH 1.0 sample and the anthocyanin content determined by the pH differential method (Fuleki and Francis, 1968b).

3.1.3 Determinatin of Solids, Acidity and Total Phenolic Compounds

The total solids content of the saskatoon berries was determined by the vacuum oven method; AOAC (1980) procedure 22.018. Soluble solids were expressed as percent sucrose and determined from thawed berry juice by the refractometer method, AOAC (1980) procedure 31.011, using a

Spencer 1362 refractometer (American Optical Company, Scientific Instrument Division, Buffalo, N.Y.).

The berries were prepared for analysis of titratable acidity, pH and total phenolic compounds by AOAC (1980) procedure 22.008. Samples (150 g) of saskatoon berries were macerated with 150 g of distilled water in a Waring Blender on high speed for three minutes. Distilled water (250 mL) was then added and the sample was boiled for one hour, replacing water lost to evaporation at 15 minute intervals. The resulting slurry was brought to a final volume of 1000 mL with distilled water and allowed to cool. After pre-filtration through two layers of fine cheese cloth, the sample was filtered through a Whatman No. 4 filter paper. Titratable acidity (percent malic acid) and pH were determined by the standard glass electrode method; AOAC (1980) procedure 22.061, using the pH meter function on a Radiometer Titrator type TTT1c (Radiometer, Copenhagen NV, Denmark). The total phenolic compounds content, expressed as percent tannic acid, was determined with the Folin-Denis reagent; AOAC (1980) procedures 9.098, 9.099 and 9.100, using a Bausch and Lomb Spectronic 20 spectrophotometer (Bausch and Lomb Inc. Rochester, N.Y.). A sample calculation for total phenolic content is given in Appendix 3.

3.1.4 Colour Evaluation

3.1.4.1 Spectrophotometric Colour Parameters

The saskatoon berry extracts prepared for measurement of total acidity, pH and total phenolic compounds (AOAC, 1980, procedure 22.008) were also used for spectrophotometric colour analysis by the potassium metabisulfite method (Somers, 1972; Wrolstad, 1976). Two hundred uL of 20 % potassium metabisulfite were added to 3.0 mL extract and 200 uL of distilled water were added to a second 3.0 mL control sample. The visible absorbance spectrum (350-700 nm) of each solution was obtained on a Unicam 800 SP scanning spectrophotometer (Unicam Instrument Limited, Cambridge, England) with distilled water in the reference cell. The total colour density (TCD) was determined for the control sample and calculated as TCD = $[(A_{520}-A_{700}) + (A_{420}-A_{700})]$ x dilution factor. Polymeric colour (PC) was determined by applying the same calculation to the bisulfite treated sample; PC = $[(A_{520}-A_{700}) + (A_{420}-A_{700})]$ x dilution factor. The percent colour due to tannin (%CDT), or nonmonomeric anthocyanin colour was calculated as %CDT = PC/TCD x 100. The absorbance of the control sample at 700 nm x dilution factor was used as a measure of turbidity. Browning was determined on the control sample and calculated as Browning = $(A_{420}-A_{700})$ x dilution factor.

3.1.4.2 Hunter Coordinates

Tristimulus colour measurements of 15 to 20 g samples of thawed berries and the acidified ethanol extracts were obtained on a Hunterlab model D25 Color Difference Meter (Hunter Associates Laboratory, Inc., McLean, VA). The instrument was standardized against a purple tile with calibrations L = 21.5, a = 21.8 and b = 6.6. The Hunter 'L', 'a' and 'b' values were measured and the hue angle (θ) was obtained by calculating $\tan^{-1}$ b/a (Little, 1976).

3.1.5 Statistical Analysis

The data were analyzed using a randomized block design with three replicates per cultivar sample for each year. Correlation coefficients and mean separation by Duncan's multiple range tests (5 % confidence level) were calculated using a VAX computer and ACTS statistical programs (Agrinet, Agriculture Canada, Ottawa, ON) following established statistical procedures (Federer, 1955; Steel and Torrie, 1980).

3.2 EFFECT OF ACETALDEHYDE AND CATECHIN ON ANTHOCYANINS FROM SASKATOON BERRIES

3.2.1 Preparation of Reaction Systems

The effect of acetaldehyde and catechin on anthocyanins was studied in an aqueous model system and in aqueous and alcoholic crude pigment

extracts from saskatoon berries. All anthocyanin solutions were prepared and analyzed in triplicates. Chemicals used in the pigment systems and their sources are shown in Table 4.

3.2.1.1 Cyanidin 3-glucoside Model System

Cyanidin 3-glucoside (2.4×10^{-3}g) was dissolved in 50 mL of sodium acetate-phosphoric acid pH 3.5 buffer. The resulting 1×10^{-4} M solution was divided into four equal volumes consisting of the pure pigment (control sample) and solutions containing, in addition to the pigment, acetaldehyde, catechin and acetaldehyde + catechin (Table 5).

3.2.3.2 Saskatoon Berry Aqueous Extract System

The crude anthocyanin extract was obtained from ripe saskatoon berries of the Honeywood cultivar. The berries were hand picked in the summer of 1985 from a plantation at the Agriculture Canada Research Station, Morden, MB; frozen within two hours after harvest and stored at -25 ± 1°C under light impervious conditions for approximately ten months before pigment extraction.

Prior to pigment extraction, the frozen berries were thawed at 4 ± 1°C in the dark for approximately 15 hours. The pigmented saskatoon berry juice was extracted by macerating 200 g berries in a model 6001 ACME juicerator (Acme Juicer Mfg. Co., Lemoyne, Pa). Approximately 90 mLs of juice was obtained. Additional pigment was extracted from the

Table 4. Chemical components in systems used to investigate the effects of acetaldehyde and catechin on anthocyanins of saskatoon berries

Component	Source
Acetaldehyde	Eastman Kodak Corp, Rochester, N.Y.
(+) D - Catechin	ICN Pharmaceuticals, Plainveiw, N.Y.
Cyanidin 3 - glucoside	Sarasynthese, Merignac, France
Rohament PC pectinase	Miles Laboratories, Victoria, Australia.
HPLC grade: Methanol	Fisher Scientific, Winnipeg, Manitoba
o-Phosphoric acid	Fisher Scientific, Winnipeg, Manitoba
Water	Fisher Scientific, Winnipeg, Manitoba
Reagent grade: Formic acid	Fisher Scientific, Winnipeg, Manitoba
Sodium acetate	Fisher Scientific, Winnipeg, Manitoba
Propionic acid	Fisher Scientific, Winnipeg, Manitoba

Table 5. Components of reaction systems used to determine the effect of acetaldehyde and catechin on anthocyanins from saskatoon berries.

Reaction System	Pigment concentration (cyanidin 3-glucoside) (M)	Solution number	Acetaldehyde concentration (M)	Acetaldehyde: pigment ratio	Catechin concentration (M)	Catechin: pigment ratio
Cyanidin 3-glucoside model	1.0×10^{-4}	1	0	0	0	0
		2	2.5×10^{-2}	250	0	0
		3	0	0	9.5×10^{-4}	9.5
		4	1.6×10^{-2}	160	9.5×10^{-4}	9.5
Aqueous extracts	1.2×10^{-4}[a]	1	0	0	0	0
		2	1.3×10^{-1}	1083	0	0
		3	1.3×10^{-2}	108	0	0
		4	0	0	6.5×10^{-4}	5.4
		5	0	0	1.6×10^{-4}	1.3
		6	1.3×10^{-1}	1083	1.6×10^{-4}	1.3
Alcohol extracts	1.3×10^{-3}[a]	1	0	0	0	0
		2	5.0×10^{-1}	385	0	0
		3	5.0×10^{-2}	30	0	0
		4	0	0	2.5×10^{-3}	2.0
		5	0	0	5.0×10^{-4}	0.4
		6	5.0×10^{-1}	385	4.0×10^{-4}	0.3

[a] Concentration estimated by pH differential method.

pulp with four washings of 100 mL of pH 3.5 aqueous buffer containing 0.01 % propionic acid (Appendix 5). The extract was placed in a 500 mL volumetric flask and brought to volume with further pulp washings. Rohament PC pectinase enzyme was added to the extract at a level of 0.02 % (w/v) in order to decrease the viscosity of the solution. After enzyme addition the extract was gently agitated for one hour at 21 ± 1°C in the dark. The solution was then suction filtered through a Whatman No. 44 filter paper and the filtrate was re-filtered in order to obtain a clear pigment extract. The extract was divided into six equal volumes consisting of the pure extract (control sample) and extracts containing acetaldehyde, catechin and acetaldehyde + catechin (Table 5).

3.2.1.3 Saskatoon Berry Alcohol Extract System

Honeywood saskatoon berries were picked in the summer of 1984 and subjected to the same harvest and storage conditions as those described for the berries used for the aqueous extract system (Section 3.2.1.2).

The pigments were extracted with a solvent consisting of methanol acidified to pH 3.5 with 88 % formic acid. Frozen saskatoon berries (200 g) were blended with 200 mL of the extraction solvent in a Waring blender at full speed for three minutes. After addition of 100 mL of solvent, the mixture was blended again at full speed for two minutes and then suction filtered through a Whatman No. 44 filter paper. The pH of the filtrate was measured and re-adjusted, if required, to 3.5 by adding a few drops of formic acid. The filtrate was poured into a 500 mL

volumetric flask and brought to volume with further residue washings; it was then divided into six equal volumes consisting of the pure extract (control sample) and extracts containing acetaldehyde, catechin and acetaldehyde + catechin (Table 5).

3.2.2 Analysis of Pigment Systems

After initial preparation of the reaction systems, the control samples were subjected to high performance liquid chromatography (HPLC) analysis. All samples were then stored in a model I-24 Conviron controlled environmental storage chamber (Controlled Environments, Winnipeg, MB) at 23 ± 1°C in the dark. The HPLC profiles, Hunter 'a' and 'b' values and UV and visible absorbance spectra were obtained periodically for all samples. However, Hunterlab coordinates were not obtained for the cyanidin 3-glucoside model systems since the sample volumes were low.

3.2.2.1 HPLC Analysis

The HPLC analysis was conducted on an LKB liquid chromatograph (LKB-Produkter AB, Bromma, Sweden) equipped with two LKB 2150 dual pumps, an LKB 2152 HPLC controller, an UltroPac prepared HPLC column (250 x 4 mm) of LiChrosorb RP-18 (10 um) and an LKB 2140 photodiode array spectral detector interfaced with an IBM Personal Computer (IBM Canada Limited, Winnipeg, MB) and a Canon A-1210 ink-jet printer. The samples were pre-filtered through a 0.45 um pore size MSI Cameo HPLC

nylon filter (Micron Separation, Inc., Honeoye Falls, N. Y.) and then injected onto the column through a Rheodyne 7125 injection valve (Rheodyne, Inc. Cotati, CA) with a 20 uL sample loop. The following solvent system and elution profiles were used for separation of the pigments: solvent A, formic acid-water (5:95 v/v); solvent B, methanol. Elution profile: 0-10 min, 17-22% B in A (linear gradient); 10-12 min, 22-27% B in A (linear gradient); 12-33 min, 27-37% B in A (linear gradient); 33-39 min, 37-55% B in A (linear gradient); 39-49 min, 55-59% B in A (linear gradient). The solvent flow rate was 0.90 mL/min and the column pressure 60-70 bar. All separations were performed at 22 ± 2°C and all solvents were filtered through a 0.45 um Millipore filter before use. Spectral detection was performed simultaneously at 190-370 nm and the data were evaluated using an IBM personal computer equipped with an LKB 2140-200 Wavescan Spectral Detector Program. The UV maximum of the anthocyanin peaks appeared at 280 nm. Peak areas and area % of the resulting chromatograms at 280 nm were calculated on the IBM computer equipped with a Nelson Analytical 3000 Series Chromatography Data Software System (Nelson Analytical, Inc., Cupertino, CA).

3.2.2.2 Spectral Analysis

The UV (250-400 nm) and visible (400-700 nm) absorbance spectra of all pigment samples were measured in a 1-cm square quartz cuvette using a Beckman DU-50 Spectrophotometer (Beckman Instruments, Inc., Irvine, CA) interfaced with an IBM personal computer equipped with a Beckman data capture software program. The maximum absorbance values and

wavelengths of maximum absorbance (λmax.) were recorded using the Peak Pick program of a Beckman DU-50 series Quant I Soft-Pak Module (Beckman Instruments, Inc., Irvine, CA). The spectrophotometer was blanked and samples were diluted with the respective reaction system with the pigment or pigment extract excluded.

3.2.2.3 Hunterlab Colourimeter Analysis

Hunter coordinates of the saskatoon berry pigment extract systems were measured on a Hunterlab tristimulus Colorimeter Model D25L-9 (Hunter Associates Laboratory, Inc., Reston, Virginia) calibrated against a white tile with Hunter coordinates 'L' = 90.67, 'a' = -0.81 and 'b' = 1.70. Twenty-five mLs of each sample were placed in a sample dish. The dish was covered with a white tile and the Hunter 'a' and 'b' values were measured three times with the sample dish being rotated approximately 45° before each reading. Hue angle (θ) was obtained by calculating $\tan^{-1}$ b/a. Readings of the three measurements were averaged and recorded. All measurements were performed in the dark.

3.2.2.4 Statistical Analysis

The data were analyzed using a factorial split-plot design. Means, correlation coefficients, split-plot analysis of variance, analysis of variance for linear and quadratic treatment effects, tests of hypotheses and estimates of the necessary parameters were calculated using a VAX computer and Statistical Analysis System programs (SAS Institute Inc.,

Box 8000, Cary, N.C., U.S.A.) following the General Linear Models Procedure. Standard error (S.E.) for the difference between any two means at a given time were calculated as follows (Steel and Torrie, 1980):

$$S.E. = \sqrt{2[(b - 1)E_b + E_a] / rb}$$

where S.E. = standard error of the treatments
b = number of analyses on a given sample during storage
r = number of replications
E_a = error mean square for error (a)
E_b = error mean square for error (b)

4. RESULTS AND DISCUSSION

4.1 FACTORS RELATED TO ANTHOCYANIN LEVELS IN SASKATOON BERRIES

4.1.1 Effect of Berry Ripeness

Physico-chemical parameters of the saskatoon berries picked in 1983 and 1984 and separated into three maturity levels are presented in Tables 6 to 9.

Anthocyanin content, anthocyanin degradation index (DI) values, total phenolic compounds content and anthocyanin-total phenolic compounds ratio of the ripening berries are shown in Table 6. The anthocyanin content ranged from 25.1 to 163.6 and 30.9 to 178.7 mg / 100 g of berries in the 1983 and 1984 crops, respectively. If the anthocyanin content of saskatoon berries is compared to that of other commercial berries, it can be considered high in comparison to cranberries which contain 35 to 100 mg / 100 g (Fuleki and Francis, 1968b; Deubert, 1978) but only moderate in comparison to blueberries (25 to 490 mg / 100 g; Ballinger et al., 1972). In all cultivars the anthocyanin content increased significantly with the level of berry ripeness. This suggests that saskatoon berries should be harvested at the dark purple stage in order to obtain processed products with maximum anthocyanin colour. Since saskatoon berries do not ripen simultaneously, the application of ripening agents prior to berry harvest may promote uniform ripening and significantly increase the

Table 6. Anthocyanin content, anthocyanin degradation index (DI), total phenolics content and anthocyanin-total phenolics (Anth/Ph) ratio of 1983 and 1984 saskatoon berry crops at 3 maturity levels[a].

Cultivar and maturity level		Anthocyanins (mg Cy.3-gal/ 100 g berries)		Anthocyanin DI		Total phenolic compounds (% tannic acid)		Anth/Ph ratio	
		1983	1984	1983	1984	1983	1984	1983	1984
Honeywood	1	57.2a[1]	57.2a	1.01a	0.99a	0.38a	0.38a	0.15a	0.15a
	2	103.5b	124.2b	1.01a	0.96a	0.42a	0.42b	0.25b	0.30b
	3	163.4c	178.7c	1.00a	0.98a	0.48b	0.52c	0.34c	0.34c
Regent	1	51.9a	30.9a	1.05b	1.09b	0.17a	0.17a	0.31a	0.18a
	2	76.8b	68.1b	0.97ab	1.02a	0.22b	0.18a	0.36a	0.37b
	3	120.9c	112.8c	0.95a	0.97a	0.24b	0.21b	0.50b	0.53c
Porter	1	58.8a	61.7a	1.06b	0.98a	0.38a	0.36a	0.16a	0.17a
	2	101.9b	113.8b	1.00a	0.96a	0.42b	0.39b	0.24b	0.30b
	3	163.6c	155.8c	0.96a	0.96a	0.45c	0.42c	0.36c	0.37c
Smoky[2]	1	25.1a	-	0.97a	-	0.24a	-	0.10a	-
	2	50.7b	-	1.00a	-	0.26a	-	0.20b	-
	3	69.5c	-	0.97a	-	0.30b	-	0.24c	-
Northline[3]	1	-	55.5a	-	0.98b	-	0.42a	-	0.13a
	2	-	114.8b	-	0.97b	-	0.40a	-	0.29b
	3	-	160.1c	-	0.94a	-	0.42a	-	0.38c

[1] For a given cultivar, means in the same column followed by a common letter are not statistically different at P 0.05 by Duncan's multiple range test.
[2] Limited quantities did not allow separation into 3 maturity levels in the 1984 crop.
[3] Limited quantities did not allow separation into 3 maturity levels in the 1983 crop.

colour quality of the processed products. Indeed, recent studies (Mahadeva, 1985) have indicated that the application of Ethrel may prove effective in promoting uniform ripening of saskatoon berries.

Anthocyanin degradation index (DI) values greater than 1.00 indicate the presence of degraded pigment or other browning compounds in the sample (Fuleki and Francis, 1968b). Within the limits of standard deviation, only the DI values of 1983 Honeywood level two, 1983 Porter level one and 1984 Regent level one berries implied any pigment degradation. However, only a slight degree of degradation was suggested since the margin by which the DI values exceeded 1.00 was extremely small; 1.01 ± 0.00, 1.06 ± 0.00 and 1.09 ± 0.03, respectively. Also, while half of the crops showed no marked distinction between the levels of maturity, the 1983 and 1984 Regent, 1983 Porter and 1984 Northline crops showed higher DI values in the red (level 1) berries than those at the dark purple stage (level 3). This suggests that the chemical parameters in red berries are less favourable for colour stability than in ripe berries. Also, at lower concentrations, anthocyanins may be less resistant to the pigment degradation conditions of harvest and storage. Similarly, Flora (1978) reported that grapes with high total anthocyanin contents possess greater colour stability than those containing lower pigment levels.

Red Regent berries had the lowest level of total phenolic compounds at 0.17 ± 0.01 % tannic acid in both 1983 and 1984 while dark purple (stage 3) Honeywood berries contained the highest amount at 0.48 ± 0.04

and 0.52 ± 0.01 % tannic acid for the 1983 and 1984 crops, respectively. With the exception of Northline, the levels of phenolic compounds increased with ripening in all cultivars tested. Pirie and Mullins (1977) also found an increase in the percentage of phenolic constituents with ripening in grape berry skins. Goldstein and Swain (1963) reported similar phenolic compounds-maturity relationships in other anthocyanin containing fruits such as peaches and plums while a decrease in the proportion of phenolic compounds with ripening was observed in anthocyanin deficient fruits such as bananas and persimmons. Anthocyanin production may be largely responsible for the observed increase in total phenolic compounds since the anthocyanin:total phenolic compounds ratio increased with maturity in all cultivars.

In the 1984 Northline berries the total phenolic compounds content was relatively constant at all stages of maturity (approximately 0.42 % tannic acid) implying that in this cultivar, the phenolic constituents had reached maximum level before the red colouration stage and maintained that level throughout ripening.

Prior to calculation of the anthocyanin-total phenolic compounds ratio, the anthocyanin content was converted to a grams per 100 grams of berries basis. Since total phenolic compounds were also expressed in this manner, an indication of the percentage of anthocyanins in the phenolic content could be obtained simply by multiplying the ratio by 100. These percentages are not listed in Table 6 but were found to range from 10 (1983 red Smoky) to 53 % (1984 dark purple Regent) and the

average for all cultivars combined was 28.0 ± 11.5 %. These values are similar to those reported by Singleton and Esau (1969) for red grape cultivars but substantially higher than data of Yokotsuka et al. (1984) for white grape skins.

Results for pH, titratable acidity, total solids and soluble solids of the ripening saskatoon berries are presented in Table 7. The values of these parameters for the Smoky level 2 berries are similar to those previously reported by others for the Smoky cultivar (Wodak and Wolfe, 1971; Mazza et al., 1978; Mazza, 1980a, 1982). The pH values showed differences among cultivars but remained essentially constant between the stages of ripeness considered. Similarly, titratable acidity displayed little change with ripening of the berries but marked differences among the cultivars. These relationships are in agreement with published data (Green, 1972) showing no changes in acidity during ripening of other soft fruits, although a decline in the percentage of titratable acidity during ripening of blueberries was reported by Woodruff et al. (1960). On the contrary, Sjulin and Robbins (1987) found that titratable acidity decreased and pH increased with increasing maturity of red raspberry.

The total solids content increased with ripening in all cultivars. The level of solids ranged from 16.8 % in red Regent to 27.0 % in dark purple Smoky berries in 1983 and from 20.4 % in red Porter to 25.1 % in dark purple Northline berries in 1984. In comparison to other berry fruits, the total solids content of saskatoon berries can be considered

Table 7: pH, titratable acidity, total solids, soluble solids and soluble solids-acidity ratio (SS/Ac) of 1983 and 1984 saskatoon berries at 3 maturity levels.

Cultivar and maturity level		pH		Titratable acidity (% malic acid)		Total solids (% wt.)		Soluble solids (% sucrose)		SS/Ac	
		1983	1984	1983	1984	1983	1984	1983	1984	1983	1984
Honeywood	1	3.8a[1]	3.8a	0.59a	0.56b	18.8a	21.7a	11.6a	13.7a	19.7a	24.5a
	2	3.8a	3.8a	0.58a	0.54ab	21.1b	22.6b	14.5b	15.8b	25.0a	29.3b
	3	3.8a	3.9a	0.54a	0.50a	25.6c	24.1c	18.7c	16.9b	34.7b	33.6c
Regent	1	4.4a	4.1a	0.26a	0.37c	16.8a	22.1a	9.6a	14.8a	36.7a	40.4a
	2	4.4a	4.1a	0.28a	0.33b	18.2b	21.8a	13.6b	16.0ab	49.5ab	47.9b
	3	4.4a	4.2b	0.29a	0.29a	20.8c	22.8b	14.8c	17.2b	52.8b	59.9c
Porter	1	3.8a	3.9a	0.54a	0.54a	19.8a	20.4a	13.0a	12.8a	24.1a	23.8a
	2	3.7a	3.9a	0.55a	0.51a	20.6b	21.1b	14.4b	15.3b	26.2ab	30.1b
	3	3.8a	4.0b	0.56a	0.49a	22.7c	21.9c	16.3c	16.6c	29.5b	34.0c
Smoky[2]	1	4.1a	-	0.38b	-	18.1a	-	11.1a	-	29.5a	-
	2	4.3b	-	0.32b	-	18.6a	-	13.3b	-	43.5a	-
	3	4.5c	-	0.25a	-	27.0b	-	16.3c	-	66.2b	-
Northline[3]	1	-	3.8a	-	0.59c	-	21.9a	-	14.9a	-	25.5a
	2	-	3.9b	-	0.50b	-	22.7b	-	16.0a	-	32.3b
	3	-	3.9b	-	0.45a	-	25.1c	-	16.1a	-	35.5b

[1] For a given cultivar, means in the same column followed by a common letter are not statistically different at P 0.05 by Duncan's multiple range test.
[2] Limited quantities did not allow separation into 3 maturity levels in the 1984 crop.
[3] Limited quantities did not allow separation into 3 maturity levels in the 1983 crop.

high; since the corresponding levels in ripe wild blackberry (Money and Christian, 1950), blueberry (Macara, 1931) and boysenberry (Rohrer and Luh, 1959) have been reported to be 19.1, 16.8, 13.7 and 13.0 %, respectively. Saskatoon berries have a high seed-pulp ratio (Mazza, 1982) and this could be a major reason for their relatively high total solids content.

Soluble solids also increased with ripening and varied among cultivars. Similar trends have been shown in blueberries (Woodruff et al.,1960) and grape berry skins (Pirie and Mullins, 1977) but a linear decrease in soluble solids was observed from inception to processing ripeness in red raspberries (Sjulin and Robbins, 1987). The increase in soluble solids between the first and last stage of saskatoon berry ripeness ranged from 8.1 % (1984 Northline) to 61.2 % (1983 Honeywood). Since high sugar levels are important for acceptable flavour (Green, 1972), berries to be used for manufacturing products such as jam, jelly, juice and wine should be harvested at the dark-purple colour stage.

The soluble solids-titratable acidity ratios (SS/A) increased with maturity (Table 7). This relationship agrees with results for blueberries (Woodruff et al., 1960), blackberries (Sapers et al., 1986b) and cranberries (Sapers and Hargrave, 1987) and suggests that, as in these other fruits, the SS/A ratio can serve as a practical measure of saskatoon berry ripeness. The ratios varied among cultivars, ranging from 19.7 to 66.2 in 1983 red Honeywood and 1983 dark purple Smoky berries, respectively. The average for all cultivars including the three

levels of maturity was 36.9. In general, the SS/A ratio of saskatoon berries is similar to that reported for blueberries (SS/A = 31.0; Woodruff et al.,1960) and considerably higher than those of various other berry fruits (SS/A < 10; Green, 1972). The major factor influencing the SS/A ratio was the soluble solids content since it exhibited marked change, whereas titratable acidity displayed relatively small variation with ripening.

Results of colour parameters (i.e. colour density, browning, polymeric colour and % colour due to tannin) obtained by the potassium metabisulfite method are shown in Table 8. The colour density increased with maturity in most of the berry samples. This was expected since colour density is a measure of colour due to anthocyanins as well as coloured tannins and browning compounds (Somers, 1971; Somers and Evans, 1974), all of which also increased with ripeness. In most of the berry samples the colour density was approximately twice as large as the corresponding browning values. This suggests that compounds arising from enzymic and nonenzymic browning could be responsible for up to 50 % of the initial colour of processed water based saskatoon berry products such as jam and jelly. Visual perception and assessment of brown colour, however, may be more dependent on the total anthocyanin content rather than the relative contribution of browning compounds (Spayd and Morris, 1981). Skrede et al. (1983) reported that 75 % of the initial anthocyanin content was deteriorated before blackcurrent syrup was judged as unacceptable with respect to brown colour by a sensory panel.

Table 8. Colour density, browning, polymeric colour and percent colour due to tannin of 1983 and 1984 saskatoon berries at 3 maturity levels.

Cultivar and maturity level		Colour density (absorbance/g)		Browning (absorbance/g)		Polymeric colour (absorbance/g)		% tannin	
		1983	1984	1983	1984	1983	1984	1983	1984
Honeywood	1	4.47a[1]	4.78a	2.18a	2.58a	1.54a	1.73a	33.2a	36.4b
	2	6.98a	8.45b	3.94a	4.00ab	1.93a	2.27ab	33.5a	27.4ab
	3	9.38a	12.65c	4.40a	4.47b	2.71a	2.80b	27.1a	22.5a
Regent	1	2.98a	2.87a	2.07a	1.93a	2.29a	2.40a	77.1a	81.4b
	2	5.43b	3.56a	3.51b	2.33a	3.98a	2.82a	72.7a	78.2b
	3	6.38b	4.94a	4.07b	3.07a	4.09a	2.76a	63.9a	56.4a
Porter	1	4.02a	6.62a	1.91a	3.36a	0.96a	2.78a	23.4c	41.4a
	2	6.76b	7.52a	2.78b	3.65a	1.40b	2.24a	20.5b	32.6a
	3	10.07c	9.09a	4.05c	4.32a	1.76c	2.13a	17.4a	25.4a
Smoky[2]	1	1.89a	-	1.20a	-	0.91a	-	48.4a	-
	2	3.27b	-	1.96b	-	1.60b	-	48.6a	-
	3	5.45c	-	3.34c	-	2.87c	-	52.7a	-
Northline[3]	1	-	3.96a	-	2.09a	-	1.36a	-	34.8a
	2	-	5.96ab	-	2.96b	-	1.91b	-	33.0a
	3	-	7.40b	-	3.69b	-	2.42b	-	34.5a

[1] For a given cultivar, means in the same column followed by a common letter are not statistically different at $P < 0.05$ by Duncan's multiple range test.
[2] Limited quantities did not allow separation into 3 maturity levels in the 1984 crop.
[3] Limited quantities did not allow separation into 3 maturity levels in the 1983 crop.

Polymeric colour is a measure of colour due to polymerized anthocyanins, nonanthocyanin phenolic compounds and browning compounds (Somers, 1971). The mean values of polymeric colour increased with ripening of the saskatoon berries in most of the samples but were not always statistically different. This indicates that polymerization of phenolic compounds was not affected by ripeness of the berries.

The percent contribution of tannins determines the percentage of colour due to polymerized tannins, including non-monomeric anthocyanins (Wrolstad, 1976). Percentages ranged from 17.4 in dark purple Porter to 77.1 % in red Regent berries in 1983 and from 22.5 in dark purple Honeywood to 82.4 % in red Regent in 1984. In most samples there was no significant difference in tannin contribution between the levels of berry maturity. However, values for the aqueous extracts may be higher than for the whole berries since the *in vivo* environment probably favours greater pigment colour expression due to colour enhancing factors such as copigmentation (Asen *et al.*,1971a, b, 1972; Chen and Hrazdina, 1981), self-association (Asen *et al.*, 1972; Hoshino *et al.*, 1981a) and localized cellular regions of low pH (Yasuda, 1967; Asen *et al.*, 1971). This applies to all parameters of colour measurement by the potassium metabisulfite method and implies that they may be practical for characterizing expression in water based saskatoon products such as jams, jellies and juices but may not be reliable for explaining colour expression in the whole berry.

Hunterlab colourimeter values of the acidified ethanol saskatoon berry extracts are shown in Table 9. The Hunter 'L' measures lightness and varies from 100 for perfect white to zero for black, approximately as the eye would evaluate it. Hunter 'a' measures redness when positive and greenness when negative and 'b' measures yellowness when positive and blueness when negative. The hue angle (θ) identifies the hue (i.e. attribute by which a colour is identified as red, yellow, green, etc.) (Little, 1976). In most samples, the Hunter values did not distinguish between solutions of anthocyanins which differed significantly in concentration. This indicates that Hunter colour parameters cannot be used as reliable predictors of pigment content in wines, juices and other highly coloured extracts of saskatoon berries. The lack of relationship between concentration of anthocyanins and Hunter colour parameters may be due to a decrease in the luminosity of the sample which, in turn, causes the photocell of the colourimeter to be less sensitive (Eagerman et al., 1973a ,b).

The Hunter 'L', 'a', 'b' and hue angle values of the saskatoon berries are shown in Table 10. All Hunter parameters decreased with increasing berry maturity. The 'L' values ranged from 13.1 in 1983 Honeywood level three to 21.0 in 1984 Honeywood level one berries. With the exception of the 1984 Honeywood sample, all Hunter parameters were significantly different between the red and dark purple maturity levels. However, 'L' values for light purple (second level) berries were not statistically different from those at the dark purple stage. The 'a' values varied from 5.6 in 1983 dark purple Honeywood to 11.4 in 1984 red

Table 9. Hunterlab colourimeter parameters of 1983 and 1984 saskatoon berries at 3 maturity levels.

Cultivar and maturity level		L		a		b		Hue angle (θ)	
		1983	1984	1983	1984	1983	1984	1983	1984
Honeywood	1	15.9b[1]	21.0a	10.6c	11.3c	3.6b	3.1c	18.5a	15.1b
	2	13.6a	19.2a	6.7b	8.4b	2.0a	2.1b	16.6a	13.9ab
	3	13.1a	20.0a	5.6c	7.4a	1.6a	1.6a	16.4a	12.2a
Regent	1	15.6b	16.5b	8.0b	9.3b	2.2b	3.1b	15.5a	18.5a
	2	14.0a	14.9a	6.5a	6.8b	1.8ab	2.0a	15.1a	16.2a
	3	13.8a	14.5a	5.7a	5.7a	1.5a	1.8a	14.8a	17.2a
Porter	1	14.9b	16.6b	10.5c	10.2b	3.2b	3.0b	17.1a	16.4b
	2	14.1a	15.7ab	8.1b	7.7ab	2.4a	2.1a	16.8a	15.1a
	3	13.3a	15.3a	6.6a	6.9a	1.7a	1.8a	14.8a	14.4a
Smoky[2]	1	16.7b	-	11.0b	-	3.4c	-	17.3b	-
	2	15.4a	-	8.0a	-	2.5b	-	17.3b	-
	3	14.7a	-	6.8a	-	1.7a	-	14.0a	-
Northline[3]	1	-	16.5b	-	11.4b	-	3.5c	-	17.2b
	2	-	15.0a	-	8.2a	-	2.4b	-	16.6ab
	3	-	15.1a	-	7.1a	-	2.0a	-	15.4a

[1] For a given cultivar, means in the same column followed by a common letter are not statistically different at $P < 0.05$ by Duncan's multiple range test.
[2] Limited quantities did not allow separation into 3 maturity levels in the 1984 crop.
[3] Limited quantities did not allow separation into 3 maturity levels in the 1983 crop.

Table 10. Hunterlab colourimeter parameters of alcoholic extracts from 1983 and 1984 saskatoon berries at 3 maturity levels.

Cultivar and maturity level		L		a		b		Hue angle (θ)	
		1983	1984	1983	1984	1983	1984	1983	1984
Honeywood	1	10.9a[1]	11.8a	5.1c	6.4a	1.7c	2.3a	18.8b	19.4a
	2	10.8a	11.8a	4.8b	6.2a	1.5b	2.1a	17.5a	19.0a
	3	10.7a	11.8a	4.1a	6.2a	1.2a	2.2a	16.5a	19.3a
Regent	1	10.7a	11.0a	4.5b	4.3a	1.4a	1.5a	17.7a	18.7a
	2	10.6a	10.9a	4.1a	4.2a	1.4a	1.3a	18.6a	17.0a
	3	10.7a	10.8a	4.2ab	4.2a	1.4a	1.3a	18.0a	17.3a
Porter	1	10.6a	10.8a	4.4a	4.2a	1.4a	1.5b	17.2b	19.2b
	2	10.8a	10.7a	4.6a	3.8a	1.3a	1.2a	15.8a	17.8ab
	3	10.6a	10.7a	4.1a	3.7a	1.1a	1.2a	15.4a	17.5a
Smoky[2]	1	10.7a	-	4.8b	-	1.5a	-	17.6a	-
	2	10.6a	-	3.8a	-	1.4a	-	19.7a	-
	3	10.6a	-	4.1ab	-	1.5a	-	19.7a	-
Northline[3]	1	-	10.6b	-	3.9a	-	1.2b	-	17.6a
	2	-	10.6b	-	3.4a	-	1.1a	-	18.2a
	3	-	10.5a	-	3.5a	-	1.2ab	-	18.8a

[1] For a given cultivar, means in the same column followed by a common letter are not statistically different at $P < 0.05$ by Duncan's multiple range test.
[2] Limited quantities did not allow separation into 3 maturity levels in the 1984 crop.
[3] Limited quantities did not allow separation into 3 maturity levels in the 1983 crop.

Northline berries while 'b' values ranged from 1.5 in 1983 dark purple Regent to 3.6 in 1983 red Honeywood berries. Both the 'a' and 'b' values showed differences between red and dark purple berries for all samples and distinctly differentiated the middle stage of ripeness in three of the samples. This implies that 'a' and 'b' values are more sensitive to colour of saskatoon berries and are the most reliable Hunter parameters for predicting ripeness. These results are in close agreement with those reported for various other fruits. Large differences in 'L', 'a' and 'b' values have been found between light and dark whole cranberries with the best indicator of ripeness being the 'a' value (Francis, 1957; Sapers et al., 1983). Ingalsbe et al., (1965) reported that visual colour differences showed significant negative correlation with Hunter 'a' and 'b' values in dark sweet cherries and with 'a' values in purple plums.

The hue angle ranged from 12.2 in 1984 dark purple Honeywood berries to 18.5 in 1983 red Honeywood and 1984 red Regent berries and was the least sensitive Hunter parameter for discriminating between the levels of berry maturity. Francis (1957) also reported that hue angle showed no relationship with ripening in cranberries.

4.1.2 Effect of Cultivar on Levels of Anthocyanins and Other Chemical and Physical Parameters

Levels of anthocyanins of the eight saskatoon berry cultivars analyzed at the intermediate stage of maturity or randomly sampled are

shown in Table 11. In both years, Honeywood, Northline and Porter had the highest level of anthocyanins while the remaining cultivars contained significantly lower quantities of these pigments. This implies that the total anthocyanin content can vary with the saskatoon berry cultivar and products manufactured from Honeywood, Northline or Porter berries, may be more intensely coloured. Sapers et al. (1986a) reported similar pigment-cultivar relationships in cranberries.

In order to account for the variation in anthocyanin content among cultivars of saskatoon berry, the physico-chemical properties of berries of all eight cultivars were compared. The cumulative DI value for the 1983 cultivars was 23.91 (average = 1.00; standard deviation = 0.02) while the comparative value for the 1984 cultivars was 23.28 (average = 0.97; standard deviation = 0.01). This corresponds to a DI difference of only 2.63 % between the 1983 and 1984 crops and implies that the longer storage period of the 1983 berries had little effect on the degradation of anthocyanins. Consequently, the 1983 and 1984 data for the eight saskatoon berry cultivars analyzed at the average level of ripeness (i.e. intermediate colour stage or a randomn sample) were combined and used to calculate the relationships between all variables. Results of the chemical and physical analyses for level two berries of cultivars separated into 3 stages of maturity were shown in Tables 6 to 10 and results for cultivars which were not separated into ripeness levels are tabulated in Appendices 5 and 6.

Table 11. Anthocyanin content of 1983 and 1984 Honeywood, Northline, Porter, Parkhill, Success, Regent, Beaverlodge and Smoky saskatoon berries.

Cultivar	Anthocyanin content[1] (mg Cy.3-galactoside/100 g berries) 1983	1984	Combined 1983 and 1984 data
Honeywood	103.5a	124.2a	113.8a
Northline	107.1a	114.8a	111.0ab
Porter	101.9a	113.8a	107.8b
Parkhill	86.9b	66.5cd	76.7c
Success	81.2bc	63.2d	72.2cd
Regent	76.8c	68.1cd	72.4cd
Beaverlodge	56.1d	76.8bc	66.5d
Smoky	50.7d	86.2b	68.4a

[1] Values within crop year column followed by a common letter are not statistically different at $P < 0.05$ by Duncan's multiple range test.

The relationships of total anthocyanin content with the various chemical and physical parameters of level two or randomly sampled berries are illustrated in Figures 5 to 8. The observed relationships were not influenced by maturity, since all samples were analyzed at the same level of ripeness according to colour. Degradation index, total solids, soluble solids, anthocyanin-total phenolic compound content ratios and Hunter parameters of the berries and berry extracts are not shown since they produced no identifiable trend with anthocyanins. The lack of relationship indicates that these parameters cannot be utilized as measures of prediction for pigment content among the cultivars.

The relationship between anthocyanin content and pH of the berries is shown in Figure 5. As can be observed, pigment level decreased as the pH increased. In accordance with this result, the three cultivars containing the largest quantities of anthocyanins (Honeywood, Northline and Porter) had pH values less than 4.0 (Table 7) while the remaining cultivars showed higher pH levels and lower anthocyanin contents. These results suggest that the quantity of anthocyanins in the berries has some dependency on the pH of the cultivar. Furthermore, cultivars which were high in titratable acidity contained higher concentrations of anthocyanins. Consequently, anthocyanin content increased linearly with titratable acidity (Figure 6), suggesting that higher levels of berry acidity (about 0.48 to 0.65 % malic acid) may also be more favourable for the synthesis of these pigments. Similarly, it has been reported that soil conditions which produce high acidity in grapes also produce high anthocyanin levels (Peynaud and Ribereau-Gayon, 1971). However,

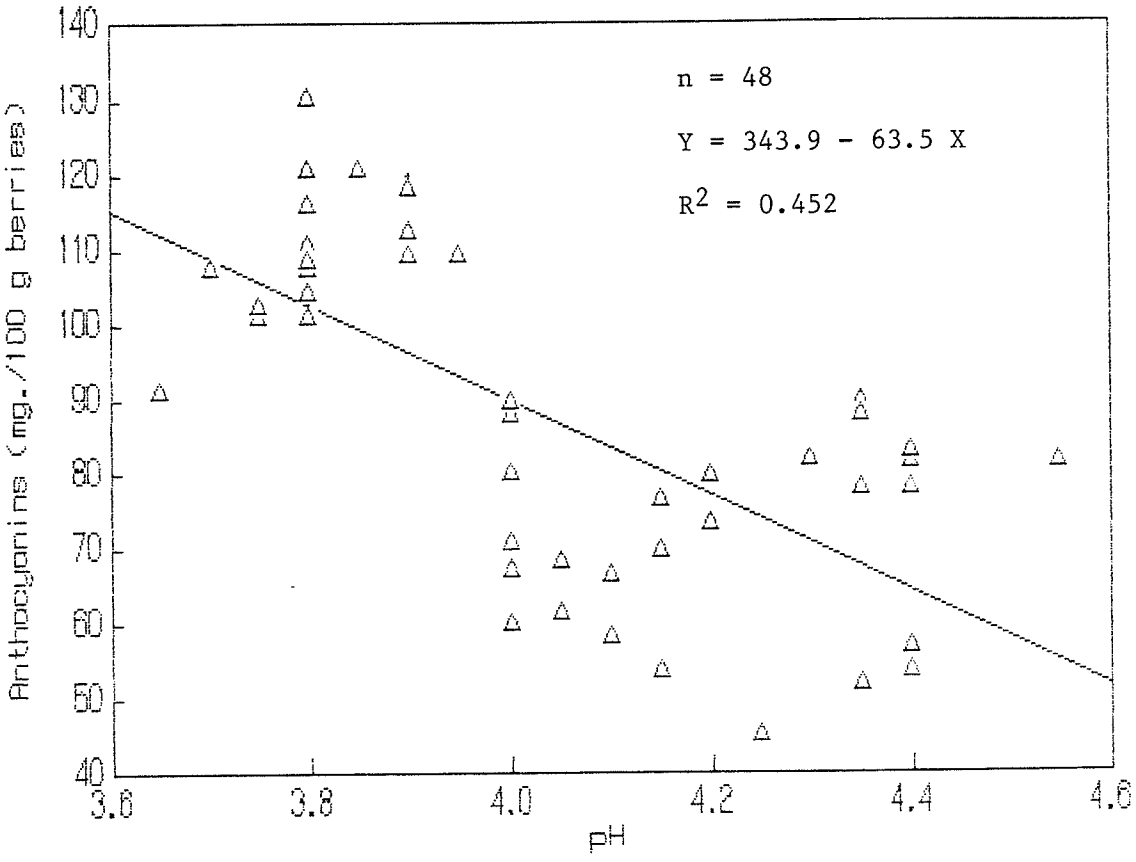

Figure 5. Relationship between anthocyanin content and pH of saskatoon berries.

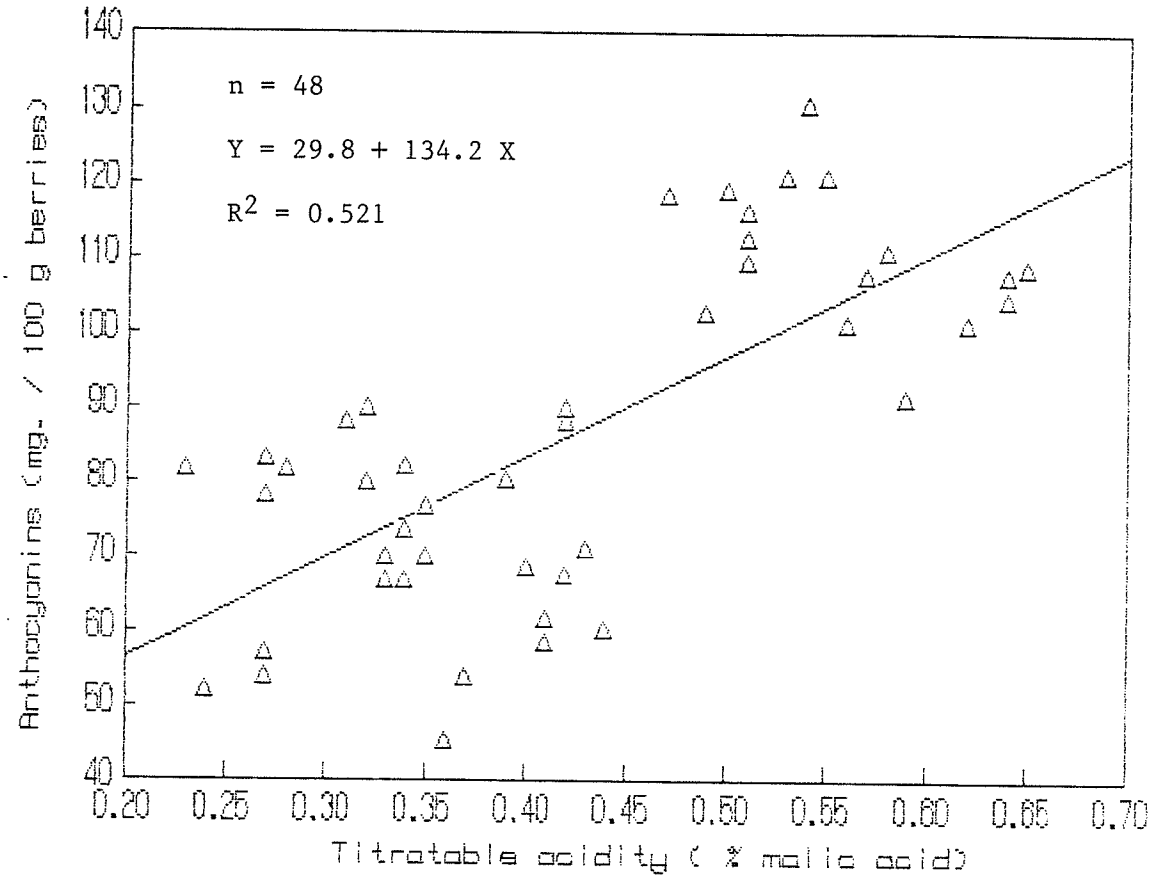

Figure 6. Relationship between anthocyanin content and titratable acidity in saskatoon berries.

titratable acidity and pH levels among various cultivars of blueberry (Ballinger et al., 1972; Kushman and Ballinger, 1975) and clones of black grape (Ballinger et al., 1974) have not shown any significant relationship with anthocyanin content.

Anthocyanin content increased with total phenolic content (Figure 7). As can be seen, cultivars which contained a total phenolic compounds content greater than 0.35 % also contained higher levels of anthocyanins. This relationship suggests that higher levels of anthocyanins result from an increased availability of precursors for synthetic purposes. Another possibility is that the total phenolic content increased only as a result of increased anthocyanin production (Griesbach, 1982; Slinkard and Singleton, 1984). Others (Pirie and Mullins, 1977; Wicks and Kliewer, 1983), have also reported higher anthocyanin levels in grape cultivars which are high in phenolic constituents.

The anthocyanin content decreased linearly with increasing SS/A ratios (Figure 8) and cultivars with SS/A ratios less than 32 contained higher levels of anthocyanins. Since the SS/A ratio can serve to indicate ripeness (Woodruff et al., 1960; Sapers et al., 1986a, b), any differences in the maturity level of the cultivars may have actually been responsible for this relationship. Results from the maturity level study, however, indicated a distinct increase in anthocyanins and SS/A ratios with ripening (Tables 6 and 7). Therefore, increasing SS/A ratios would have resulted in higher anthocyanin contents if variation in

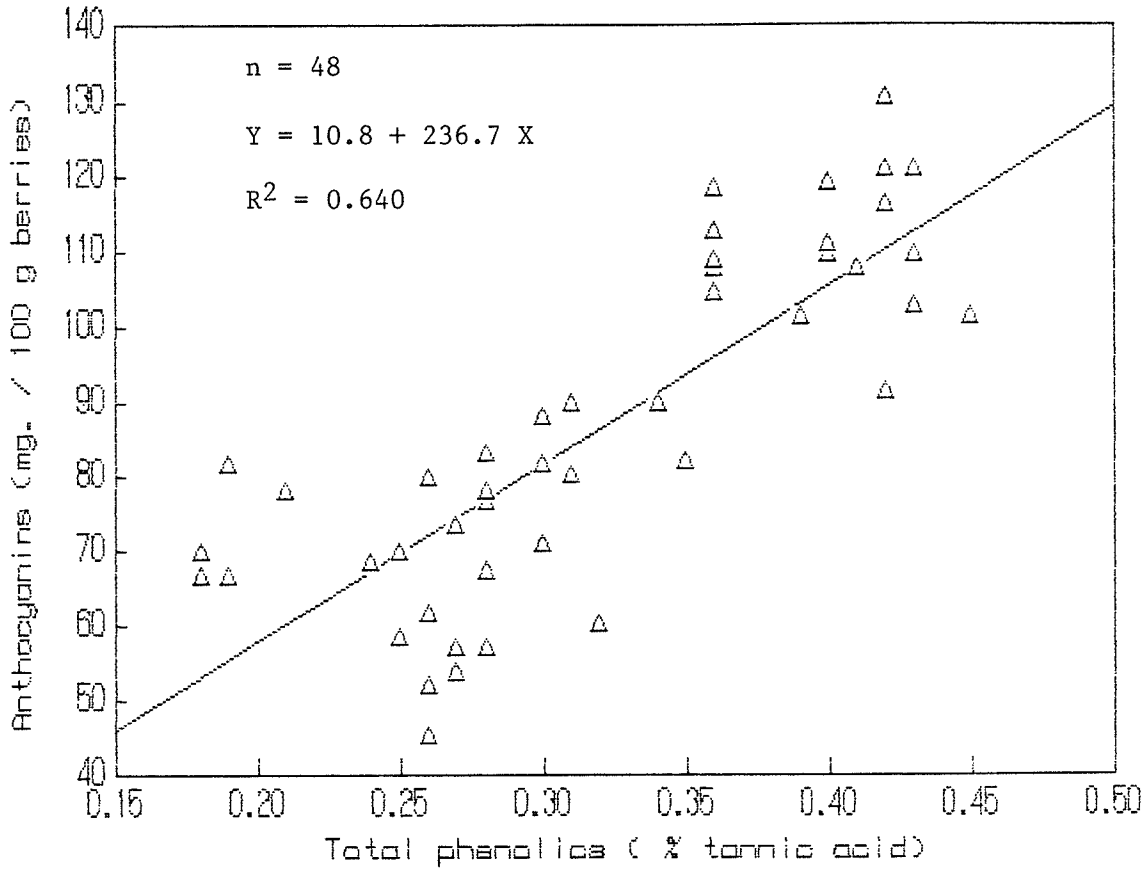

Figure 7. Relationship between anthocyanin content and total phenolic content in saskatoon berries.

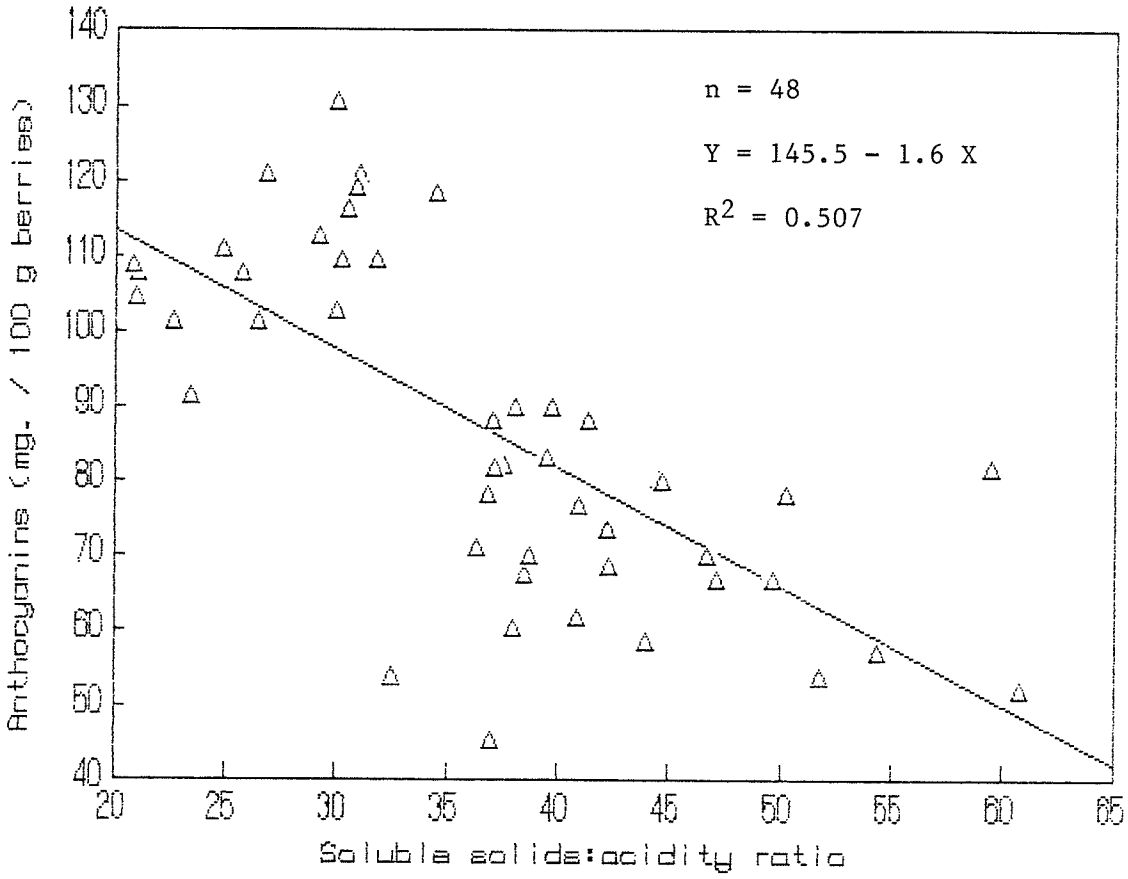

Figure 8. Relationship between anthocyanin content and soluble solids-titratable acidity ratio in saskatoon berries.

maturity of the cultivars was a factor. Also, titratable acidity was the parameter largely responsible for this relationship since soluble solids showed no identifiable trend with anthocyanins. The soluble solids content has shown no correlation with anthocyanins in other fruits such as grapes (Wicks and Kliewer, 1983) and cranberries (Sapers et al., 1986a).

Correlation coefficients among the quality parameters of the 1983 and 1984 saskatoon berry crops and the combined 1983 and 1984 data are presented in Tables 12, 13 and 14, respectively. In agreement with the observed trends in Figures 5 to 8, highly significant correlations ($P < 0.001$) were found between anthocyanin content, pH, titratable acidity, SS/A ratios and total phenolic compounds in both the 1983 and 1984 crops and in the combined data. The signs of the coefficients indicate that high levels of anthocyanins are associated with high total phenolic compounds and acidity and low pH and SS/A ratios. These conditions correspond with factors which have been reported to stabilize anthocyanins, specifically, high acidity, low sugar content (Markakis, 1982; Daravingas and Cain, 1968) and high phenolics content (Simard et al., 1982). This suggests that favourable parameters for increasing the stability of anthocyanins in vitro also provides more favourable conditions for their occurrence in vivo. It is also suggested that the sugar-acid ratio plays a larger role than the sugar content in the influence of anthocyanin production and stability.

The correlation coefficients between total solids and anthocyanin content were low but significant ($P < 0.05$) in both the 1983 ($r = 0.465$)

Table 12a. Significant correlation coefficients among quality parameters of Beaverlodge, Honeywood, Northline, Parkhill, Porter, Regent, Smoky and Success 1983 saskatoon berry crops[a].

	Extract hue	Extract b	Extract a	Extract L	Berry hue	Berry b	Berry a	Berry L	% tannin	Polymeric colour
Anthocyanins	NS	-	-	-	-	-	-	-	-0.636^{1}	-
Anthocyanin DI	-	-	-	-	-	-	-	-	-	-
Total Phenolics	-	-	-	-	-	-	-	-	-0.799^{1}	-0.440^{3}
Anth/Ph	-	-	-	-	-	-	-	-	-	-
pH	-	-	-	-	-0.456^{3}	-	-	-	0.874^{1}	0.586^{2}
Titratable acidity	-	-	-	-	0.511^{2}	-	-	-0.369^{4}	-0.820^{1}	-0.549^{2}
Total solids	-0.369^{4}	-	0.350^{4}	-	0.671^{1}	-	-0.384^{4}	-0.635^{1}	-0.600^{2}	-0.612^{1}
Soluble solids	-	-	-	-	0.560^{2}	-	-	-0.578^{2}	-0.378^{4}	-0.548^{2}
SS/Ac	-	-	-	-	-	-	-	-	0.722^{1}	-
Colour density	-	-	-	-	-	-	-	-	-0.445^{3}	-
Browning	-	-	-	-	-	-	-	-	-	0.712^{1}
Polymeric colour	-	-	-	-	-0.478^{3}	-	-	-	0.755^{1}	
% tannin	-	-	-	-	-0.388^{4}	-	-	-		
Berry L	0.527^{2}	-0.773^{1}	-0.856^{1}	-0.818^{1}	-0.573^{2}	0.686^{1}	0.898^{1}			
Berry a	0.368^{4}	-0.810^{1}	-0.851^{1}	-0.862^{1}	-0.356^{4}	0.900^{1}				
Berry b	-	-0.709^{1}	-0.742^{1}	-0.769^{1}	-					
Berry hue	-	0.358^{4}	0.391^{3}	0.359^{4}						
Extract L	-0.589^{2}	0.819^{1}	0.938^{1}							
Extract a	-0.553^{2}	0.934^{1}								
Extract b	-									

[a] N = 24
NS Not significant
1, 2, 3 & 4 Significant at 0.001, 0.01, 0.05 and 0.10 level, respectively.

Table 12b. Significant correlation coefficients among quality parameters of Beaverlodge, Honeywood, Northline, Parkhill, Porter, Regent, Smoky and Success 1983 saskatoon berry crops[a].

	Browning	Colour density	SS/Ac	Soluble solids	Total solids	Titratable acidity	pH	Anth/Ph	Total phenolics	Anth DI
Anthocyanins	-0.384^{4}	0.691^{1}	-0.740^{1}	-	0.465^{3}	0.760^{1}	-0.711^{1}	0.443^{3}	0.728^{1}	-
Anthocyanin DI	$-^{NS}$	-	-	-	0.354^{4}	-	-	-0.626^{1}	-	
Total phenolics	-	0.607^{2}	-0.791^{1}	-	0.675^{1}	0.821^{1}	-0.872^{1}	-		
Anth/Ph	-	-	-	-	-	-	-			
pH	-	-0.504^{2}	0.855^{1}	-0.435^{3}	-0.695^{1}	-0.958^{1}				
Titratable acidity	-	0.464^{3}	-0.895^{1}	0.358^{4}	0.688^{1}					
Total solids	-	-	-0.416^{3}	0.778^{1}						
Soluble solids	-	-	-							
SS/Ac	-	-0.564^{2}								
Colour density	0.604^{2}									

[a] N = 24
NS Not significant
1, 2, 3 & 4 Significant at 0.001, 0.01, 0.05 and 0.10 level, respectively.

Table 13a. Significant correlation coefficients among quality parameters of Beaverlodge, Honeywood, Northline, Parkhill, Porter, Regent, Smoky and Success 1984 saskatoon berry crop[a].

	Extract hue	Extract b	Extract a	Extract L	Berry hue	Berry b	Berry a	Berry L	% tannin	Polymeric colour
Anthocyanins	NS	-	-	-	-	-	-	-	-0.865^{1}	-0.464^{3}
Anthocyanin DI	-0.403^{3}	-0.434^{3}	-0.359^{4}	-0.395^{3}	-	-	-0.362^{4}	-	0.365^{4}	-
Total Phenolics	-	-	-	-	-	-	-	-	-0.824^{1}	-
Anth/Ph	-	-0.371^{4}	-0.385^{4}	-	-	-0.526^{2}	-0.655^{1}	-	-	-0.430^{3}
pH	-	-	-	-	-	-	-	-0.339^{4}	0.624^{1}	-
Titratable acidity	-	-	-	-	-	-	-	0.394^{3}	-0.655^{1}	-
Total solids	-	-	-	-	-	-	-	-	-	-
Soluble solids	-	-	-	-	-	0.421^{3}	0.436^{3}	-	-	-
SS/Ac	-	-	-	-	-	-	-	-	0.683^{1}	-
Colour density	-	-	-	-	-	-	-	0.380^{4}	-0.437^{3}	-
Browning	-	-	-	-	-	-	0.398^{3}	0.386^{4}	-	0.658^{1}
Polymeric colour	-	-	-	-	-	0.417^{3}	0.426^{3}	-	0.676^{1}	
% tannin	-	-	-	-	-	-	-	-		
Berry L	-	0.563^{2}	0.564^{2}	0.653^{1}	-0.573^{2}	-	0.480^{3}			
Berry a	0.337^{4}	-	-	-	-	0.858^{1}				
Berry b	-	-	-	-	0.610^{1}					
Berry hue	-	-0.351^{4}	-0.421^{3}	0.494^{3}						
Extract L	-	0.914^{1}	0.953^{1}							
Extract a	-	0.954^{1}								
Extract b	0.482^{3}									

[a] N = 24
NS Not significant
1, 2, 3 & 4 Significant at 0.001, 0.01, 0.05 and 0.10 level, respectively.

Table 13b. Significant correlation coefficients among quality parameters of Beaverlodge, Honeywood, Northline, Parkhill, Porter, Regent, Smoky and Success 1984 saskatoon berry crops[a].

	Browning	Colour density	SS/Ac	Soluble solids	Total solids	Titratable acidity	pH	Anth/Ph	Total phenolics	Anth DI
Anthocyanins	NS	0.533^{2}	-0.847^{1}	-	0.443^{3}	0.823^{1}	-0.826^{1}	-	0.874^{1}	-
Anthocyanin DI	-	-	0.382^{4}	-	-	-	-	0.519^{2}	-0.410^{3}	
Total phenolics	0.508^{2}	0.726^{1}	-0.929^{1}	-	0.422^{3}	0.920^{1}	-0.866^{1}	-		
Anth/Ph	-0.510^{2}	-0.354^{4}	-	-	-	-	-			
pH	-0.500^{2}	-0.671^{1}	0.873^{1}	-	-0.656^{1}	-0.948^{1}				
Titratable acidity	0.590^{2}	0.766^{1}	-0.950^{1}	-	0.526^{2}					
Total solids	0.364^{4}	0.403^{3}	-0.426^{3}	-						
Soluble solids	-	-	-							
SS/Ac	-0.557^{2}	-0.737^{1}								
Colour density	0.901^{1}									

[a] N = 24
NS Not significant
1, 2, 3 & 4 Significant at 0.001, 0.01, 0.05 and 0.10 level, respectively.

Table 14a. Significant correlation coefficients among quality parameters of Beaverlodge, Honeywood, Northline, Parkhill, Porter, Regent, Smoky and Success saskatoon berry 1983 and 1984 combined data[a].

	Extract hue	Extract b	Extract a	Extract L	Berry hue	Berry b	Berry a	Berry L	% tannin	Polymeric colour
Anthocyanins	NS	-	-	-	-	-	-	-	-0.743^{1}	-0.343^{3}
Anthocyanin DI	-0.430^{2}	-	-	-	0.342^{3}	-0.240^{4}	-0.393^{2}	-0.469^{1}	-	-
Total Phenolics	-	-	-	-	-	-	-	-	-0.811^{1}	-0.314^{3}
Anth/Ph	-	-	-	-	-0.264^{4}	-	-	-	-	-
pH	-	-	-	-	-	-	-	-	0.691^{1}	0.388^{2}
Titratable acidity	-	-	-	-	0.251^{4}	-	-	-	-0.710^{1}	-0.359^{2}
Total solids	-	-	-	-	-	0.249^{4}	-	-	-0.253^{4}	-
Soluble solids	-	-	-	-	-	0.295^{3}	0.250^{4}	0.297^{3}	-	-
SS/Ac	-	-	-	-	-	-	-	-	0.675^{1}	-
Colour density	-	-	-	-	-	-	-	-	-0.441^{2}	0.263^{4}
Browning	-	-	-	-	-	-	-	-	-	0.774^{1}
Polymeric colour	-	-	-	-	-	-	-	-	0.712^{1}	
% tannin	-	-	-	-	-	-	-	-		
Berry L	0.506^{1}	-	-0.398^{2}	-0.564^{1}	-0.603^{1}	0.569^{1}	0.823^{1}			
Berry a	0.448^{2}	-0.366^{3}	-0.560^{1}	-0.708^{1}	-0.304^{3}	0.897^{1}				
Berry b	0.390^{2}	-0.380^{2}	-0.536^{1}	-0.644^{1}	-					
Berry hue	-	-	-	0.258^{4}						
Extract L	-0.474^{1}	0.706^{1}	0.892^{1}							
Extract a	-0.341^{3}	0.913^{1}								
Extract b	-									

[a] N = 48
NS Not significant
1, 2, 3 & 4 Significant at 0.001, 0.01, 0.05 and 0.10 level, respectively.

Table 14b. Significant correlation coefficients among quality parameters of Beaverlodge, Honeywood, Northline, Parkhill, Porter, Regent, Smoky and Success saskatoon berry 1983 and 1984 combined data[a].

	Browning	Colour density	SS/Ac	Soluble solids	Total solids	Titratable acidity	pH	Anth/Ph	Total phenolics	Anth DI
Anthocyanins	0.287^{3}	0.583^{1}	-0.712^{1}	-	0.385^{2}	0.722^{1}	-0.672^{1}	0.332^{3}	0.800^{1}	-
Anthocyanin DI	NS	-	-	-0.327^{3}	-0.245^{4}	-	-	-	-	
Total phenolics	0.370^{2}	0.675^{1}	-0.787^{1}	-	0.357^{3}	0.782^{1}	-0.743^{1}	-0.279^{4}		
Anth/Ph	-	-	-	-	-	0.254^{4}	-			
pH	-	-0.454^{2}	0.807^{1}	-0.436^{2}	-0.656^{1}	-0.941^{1}				
Titratable acidity	-	0.511^{1}	-0.894^{1}	0.266^{4}	0.527^{1}					
Total solids	-	-	-0.252^{4}	0.827^{1}						
Soluble solids	-	-	0.392^{2}							
SS/Ac	-0.340^{3}	-0.582^{1}								
Colour density	0.774^{1}									

[a] N = 48
NS Not significant
1, 2, 3 & 4 Significant at 0.001, 0.01, 0.05 and 0.10 level, respectively.

and 1984 ($r = 0.443$) crops as well as the combined 1983-84 data ($r = 0.385$; $P < 0.01$). However, no identifiable trend was evident in the graphical analysis of these two parameters. Therefore, total solids content is limited as an indicator of anthocyanin content.

Levels of anthocyanins were higher in berries which showed higher colour density (Figure 9). The correlation coefficient between these two parameters was significant ($P < 0.01$) for the 1984 crop ($r = 0.533$) and highly significant ($P < 0.001$) for the 1983 crop ($r = 0.691$) and the combined 1983-84 data ($r = 0.583$). This relationship is reasonable since the anthocyanins are important contributors to colour density (Somers, 1971).

Graphical analysis (not shown) of polymeric colour and browning of the aqueous berry extracts showed no identifiable trends with anthocyanin content. Differences in correlation coefficients of these two parameters with anthocyanins among the two crop years and the combined data were evident. Loss of anthocyanin colour due to polymerization and degradation contributes to polymeric colour and browning (Somers, 1971; Somers and Evans, 1974; Wrolstad, 1976). Therefore, the lack of relationship of anthocyanin content with polymeric colour and browning can be partly attributed to the fact that very little pigment degradation occurred in the berry samples. The lack of degradation was further evidenced by the nonsignificant correlation between anthocyanin content and the degradation index.

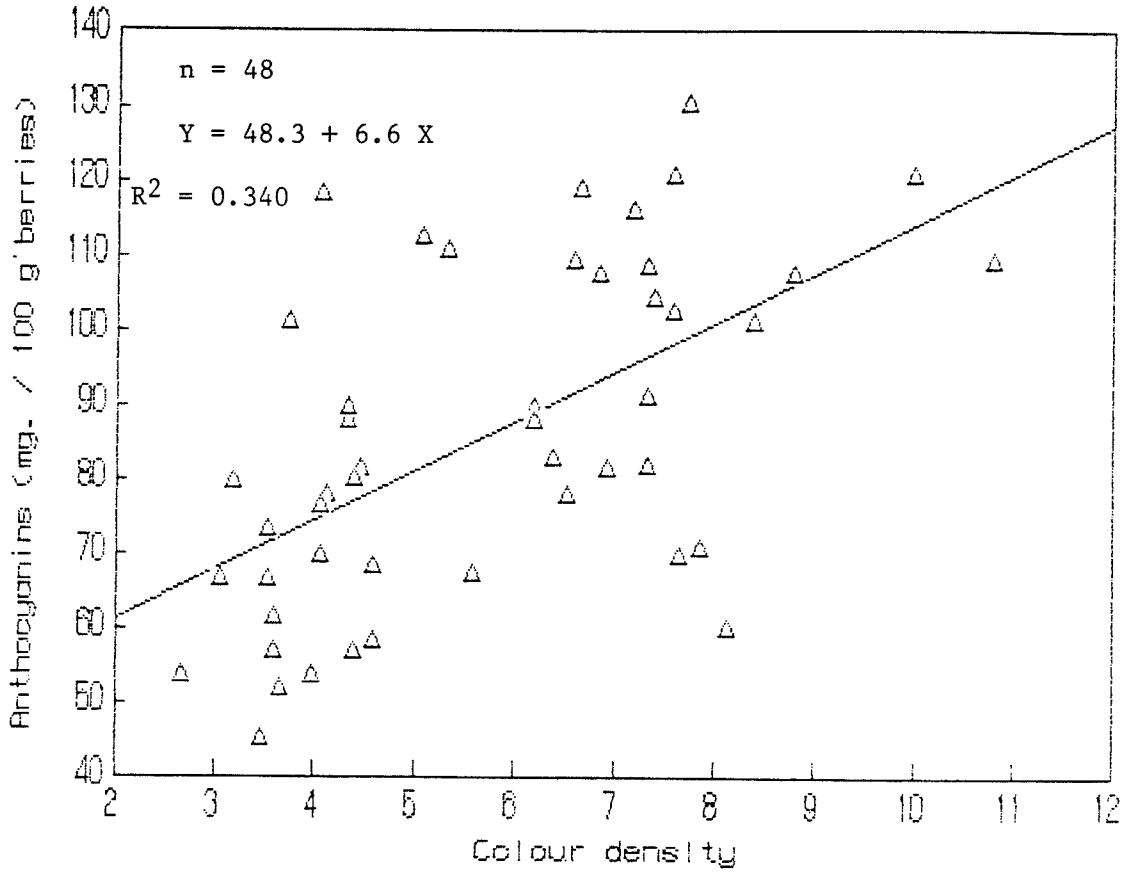

Figure 9. Relationship between anthocyanin content and colour density in saskatoon berries.

As expected, the percentage of colour due to tannin was higher in berries of cultivars which contained lower levels of anthocyanins (Figure 10). The correlation coefficient between these two variables was highly significant ($P < 0.001$) in the 1983 and 1984 crops and the combined two year data ($r = -0.636$, -0.865 and -0.743, respectively). This relationship could not be due to anthocyanin degradation/polymerization, since the percentage of colour due to tannin showed no significant correlation with anthocyanin DI values. Thus, it must be due to polymerization of nonanthocyanin phenolic compounds to form tannins.

4.2 EFFECT OF ACETALDEHYDE AND CATECHIN ON SOLUTIONS OF ANTHOCYANINS OF SASKATOON BERRIES

4.2.1 Cyanidin 3-glucoside Aqueous Model System

The UV and visible absorbance spectra of all samples after initial preparation are shown in Figure 11. The visible spectra of all samples were initially identical. On storage, the spectra of the control, pigment + acetaldehyde and pigment + catechin samples remained essentially constant, whereas the sample containing both acetaldehyde and catechin displayed a bathochromic shift of about 16 nm, from 511 to 528 nm, in the λmax (Figure 12) and about a 40 % increase in absorbance (Figure 13). Statistical analysis for the effect of reactants and time on the visible λmax and absorbance at λmax are shown in Appendix 7 and 8, respectively. Timberlake and Bridle (1976, 1977) reported a similar effect of acetaldehyde in anthocyanin model systems containing catechin-

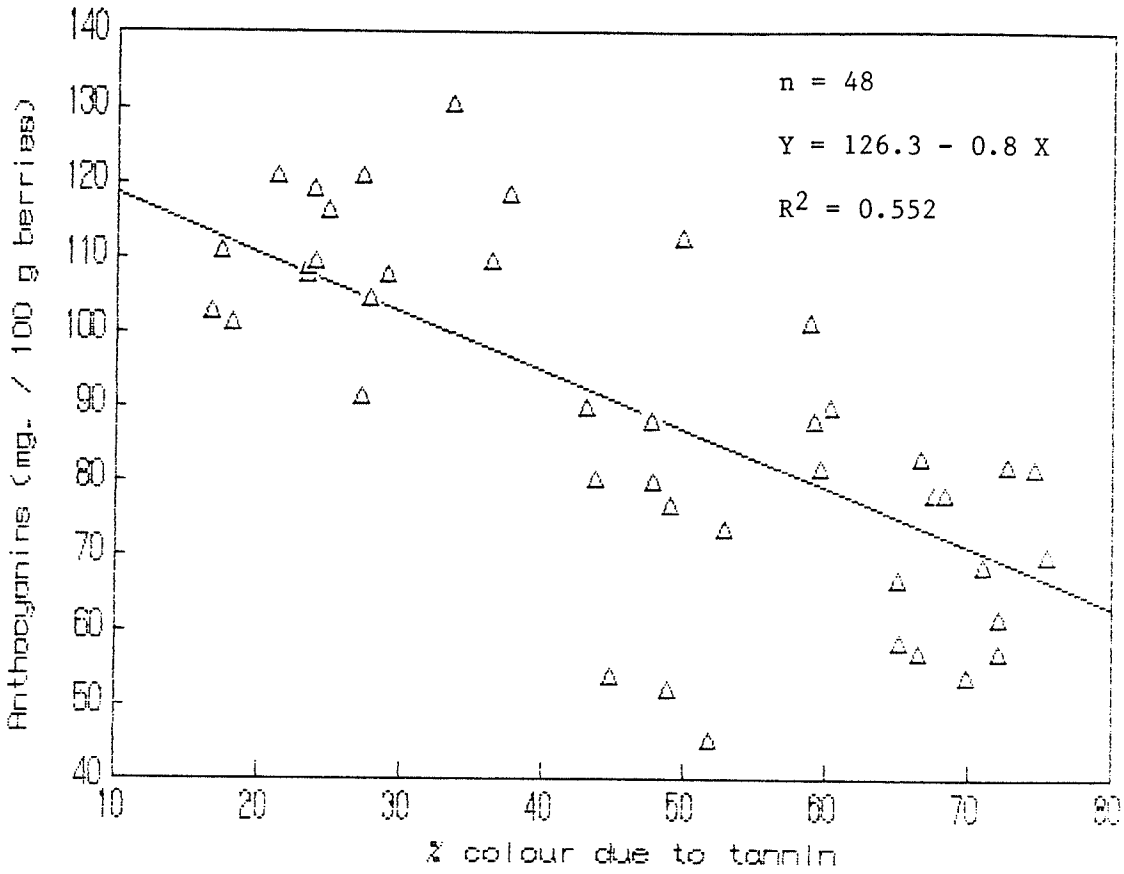

Figure 10. Relationship between anthocyanin content and percent colour due to tannin in saskatoon berries.

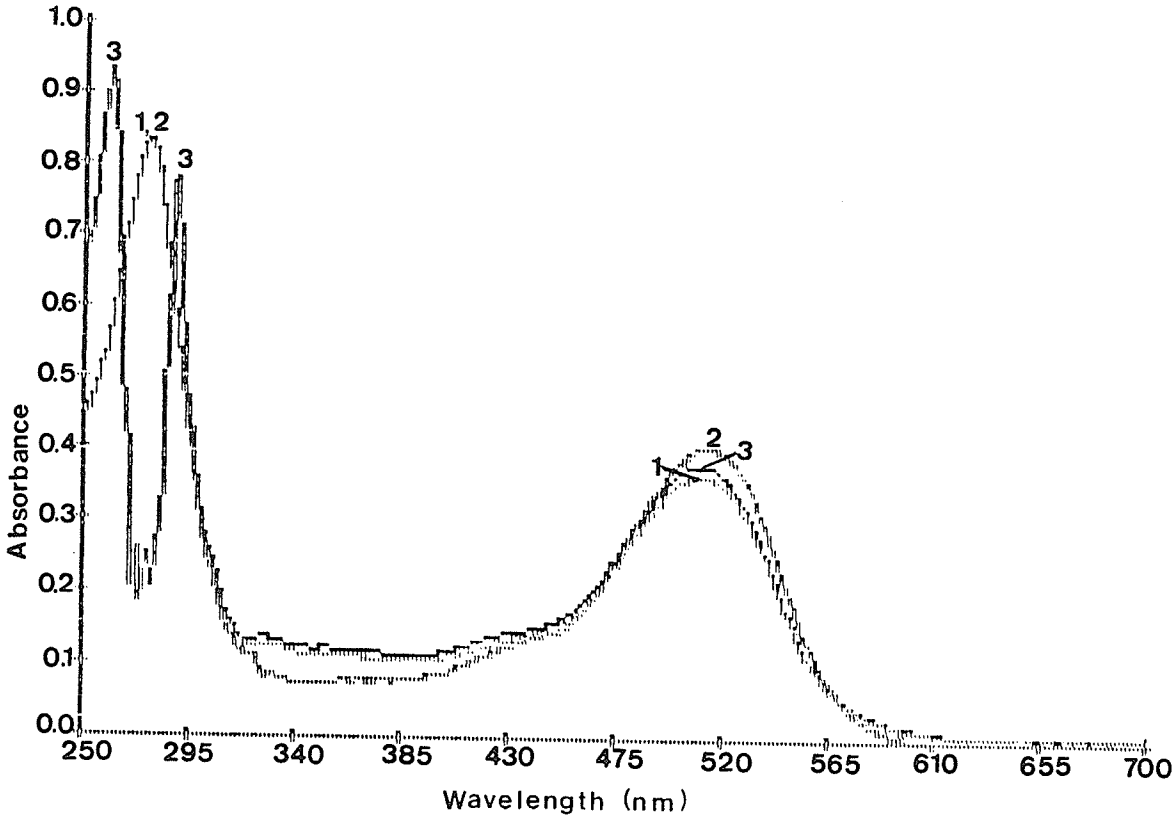

Figure 11. UV/visible spectrum of cyanidin 3-glucoside in aqueous sodium acetate-o-phosphoric acid buffer, pH = 3.5. All spectra were obtained immediately after preparation. Control (pure) pigment solution (1) and solutions containing acetaldehyde (2) and catechin (3).

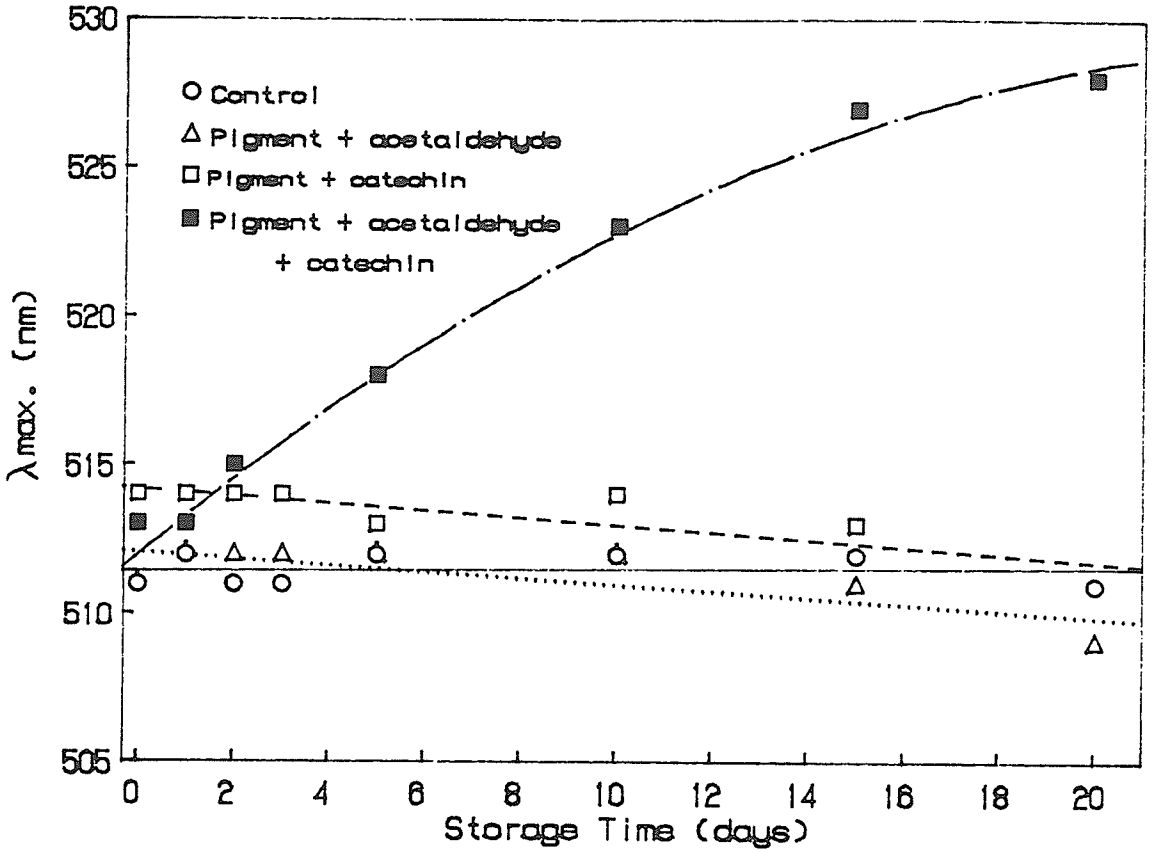

Figure 12. Effect of acetaldehyde and catechin on visible λmax of cyanidin 3-glucoside model system stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 2.1 nm.

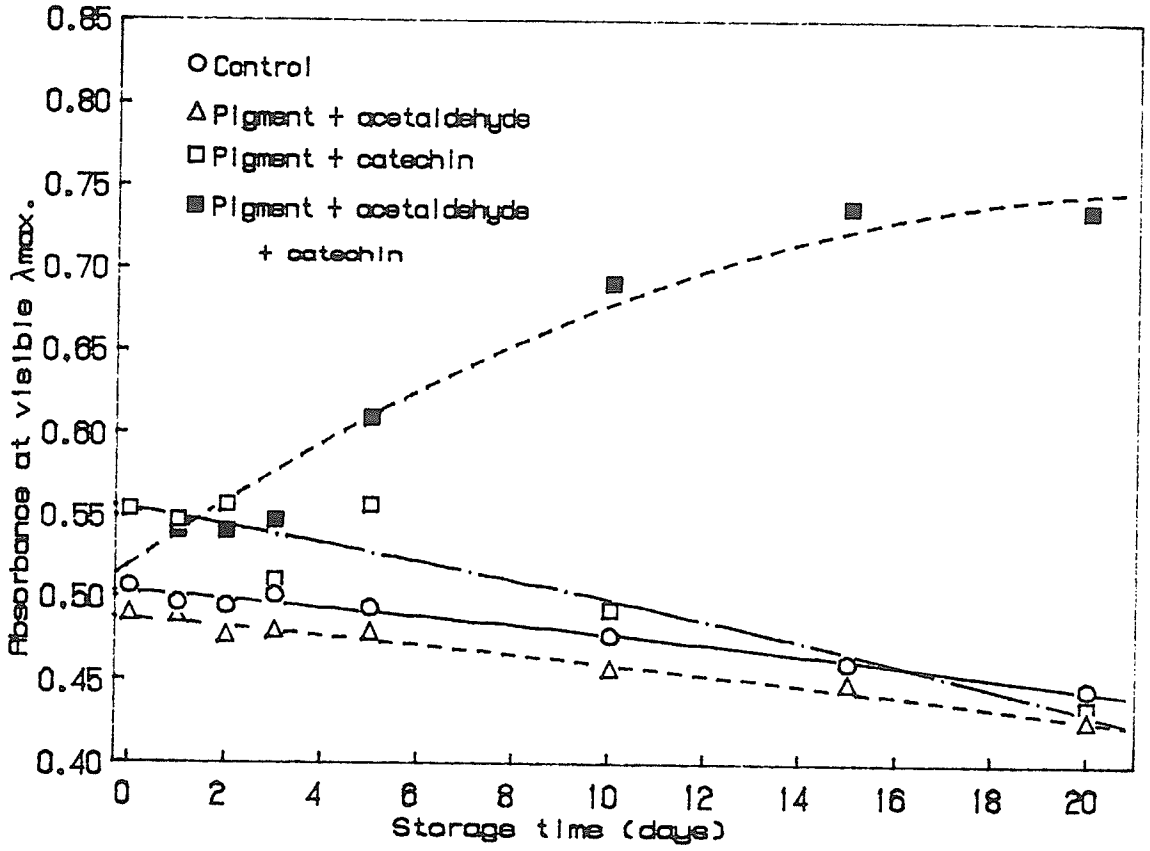

Figure 13. Effect of acetaldehyde and catechin on absorbance at visible λmax of cyanidin 3-glucoside model system stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 0.03.

type phenolic compounds. Colour augmentation was attributed to the formation, by Baeyer-type condensations, of highly coloured intermediates containing anthocyanin and catechin linked by CH_3CH bridges. The mechanism they proposed is initiated by the generation of an acetaldehyde carbonium ion, capable of reacting with the active positions (6 and 8) of the catechin-type phenolic compound. However, under acidic conditions, it is more probable that the carbonyl oxygen becomes protonated and carries a positive charge on the acetaldehyde cation (Morrison and Boyd, 1977). The carbonyl carbon then readily undergoes nucleophilic attack by position 8 of catechin (Hillis and Urbach, 1959). The proposed reaction (Timberlake and Bridle, 1976) of acetaldehyde with catechin and cyanidin 3-glucoside, including modification of the initiating step, is shown in Figure 14. The acetaldehyde-catechin complex forms a reactive carbonium ion which has the property of combining with the anthocyanin flavylium ion, probably at position 8. Formation of the quinoidal form occurs more readily as a result of the substitution. This accounts for the subsequent increase in colour and violet shift. Further condensation onto the polymer could occur via the reactive 6 positions. The 6 and 8 positions of the A-ring have been proposed as the reactive positions on the anthocyanin, with position 8 being the most reactive, by analogy with the behaviour of the simple flavylium salts (Bendz et al., 1967; Timberlake and Bridle, 1976). However, Dournel (1985) reported that CH_3CH bridges occur with the same probability between the C_8/C_8, C_8/C_6 and C_6/C_6 positions of anthocyanin and catechin.

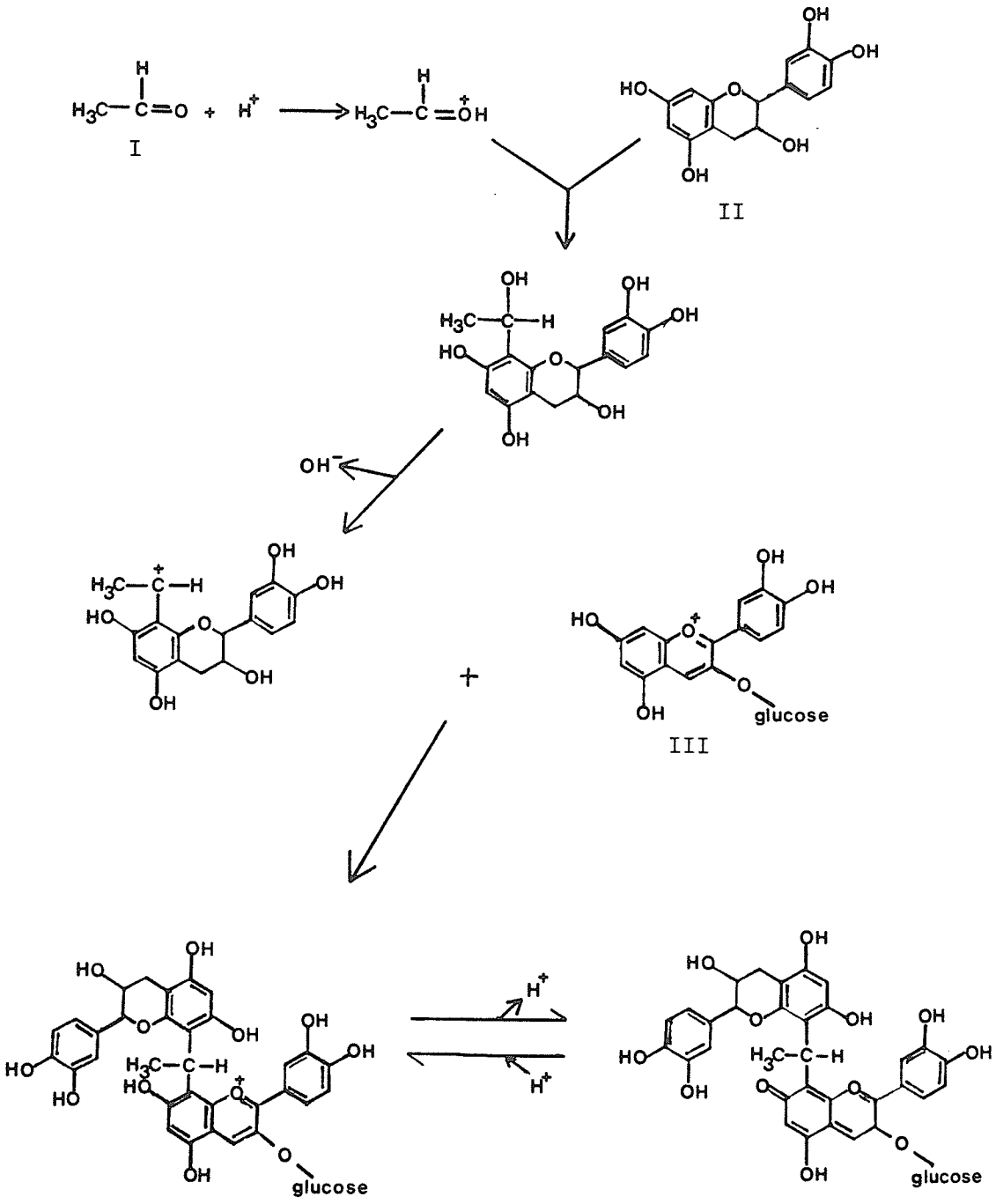

Figure 14. Reaction of acetaldehyde (I) with catechin (II) and cyanidin 3-glucoside (III). (Adapted from Timberlake and Bridle, 1976.)

A similar but slower reaction mechanism was reported for acetaldehyde and anthocyanin alone (Timberlake and Bridle, 1976). This effect, however, was not observed in the cyanidin 3-glucoside + acetaldehyde sample during the 20 day study.

It is also known (Hillis and Urbach, 1959) that catechin itself will condense with aldehydes by a similar mechanism as described for anthocyanin-acetaldehyde-catechin condensation. Therefore, there may be some self-condensation of catechin with acetaldehyde as well. Since the sample used as a spectrophotometric reference also contained acetaldehyde and catechin, evidence of this reaction would not appear on the spectrum.

The HPLC profiles of the control, pigment + acetaldehyde and pigment + catechin solutions did not change throughout the study. Chromatograms of the pigment solution containing both acetaldehyde and catechin, however, showed the appearance of six new peaks and a decrease in the peak areas of catechin and cyanidin 3-glucoside (Figure 15). The HPLC peak areas of catechin and cyanidin 3-glucoside and the cumulative area of the new unidentified peaks of the pigment sample containing both acetaldehyde and catechin during storage are plotted in Figure 16. The cyanidin 3-glucoside and catechin peaks decreased by 80 and 20 %, respectively, while the new peak area increased at a rate similar to the disappearance of the reactants. Similarly, the cumulative area of the new peaks showed highly significant ($P < 0.001$) negative correlation coefficients with the peak areas of cyanidin 3-glucoside ($r = -0.552$)

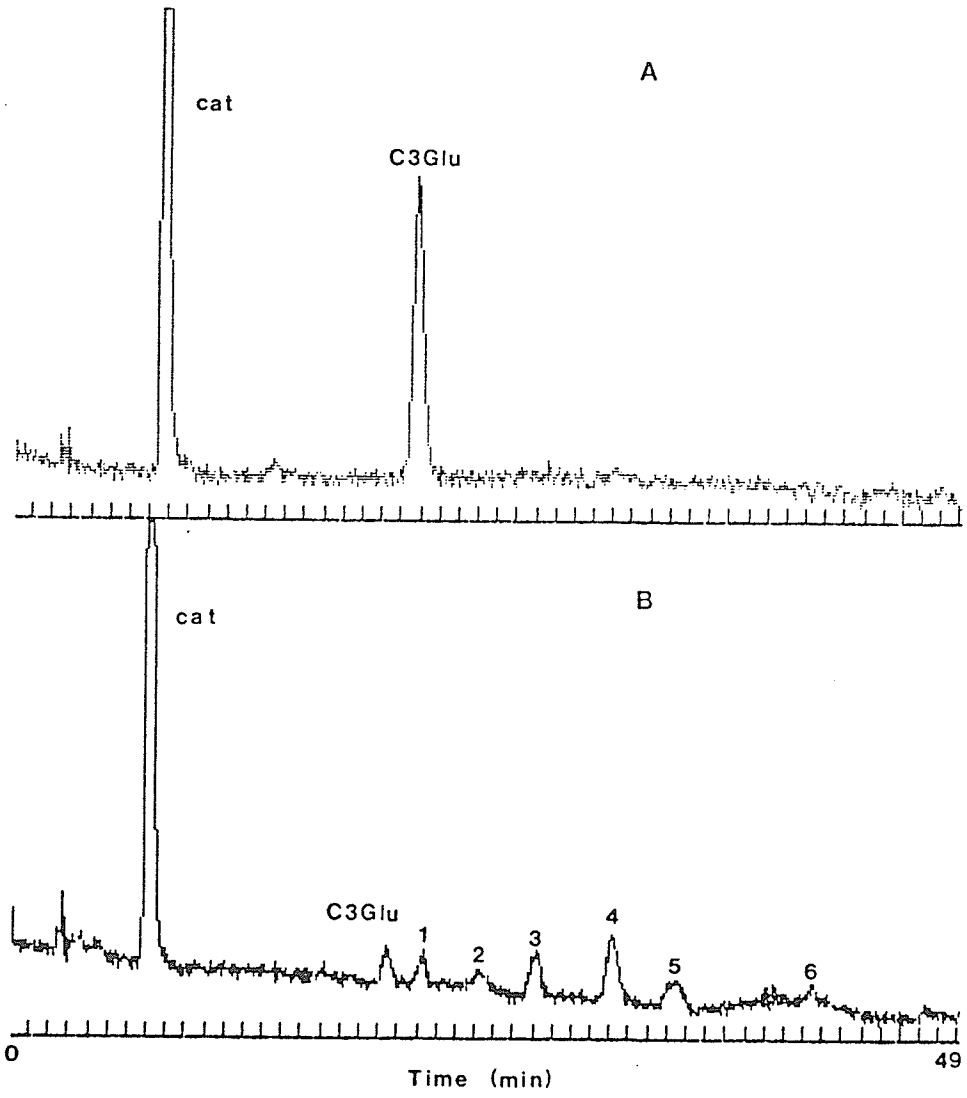

Figure 15. The HPLC of cyanidin 3-glucoside model system containing acetaldehyde and catechin after initial preparation (A) and 20 days at 23 ± 1°C in the dark (B). Peaks: catechin (cat), cyanidin 3-glucoside (C3G) and unidentified new peaks (1-6).

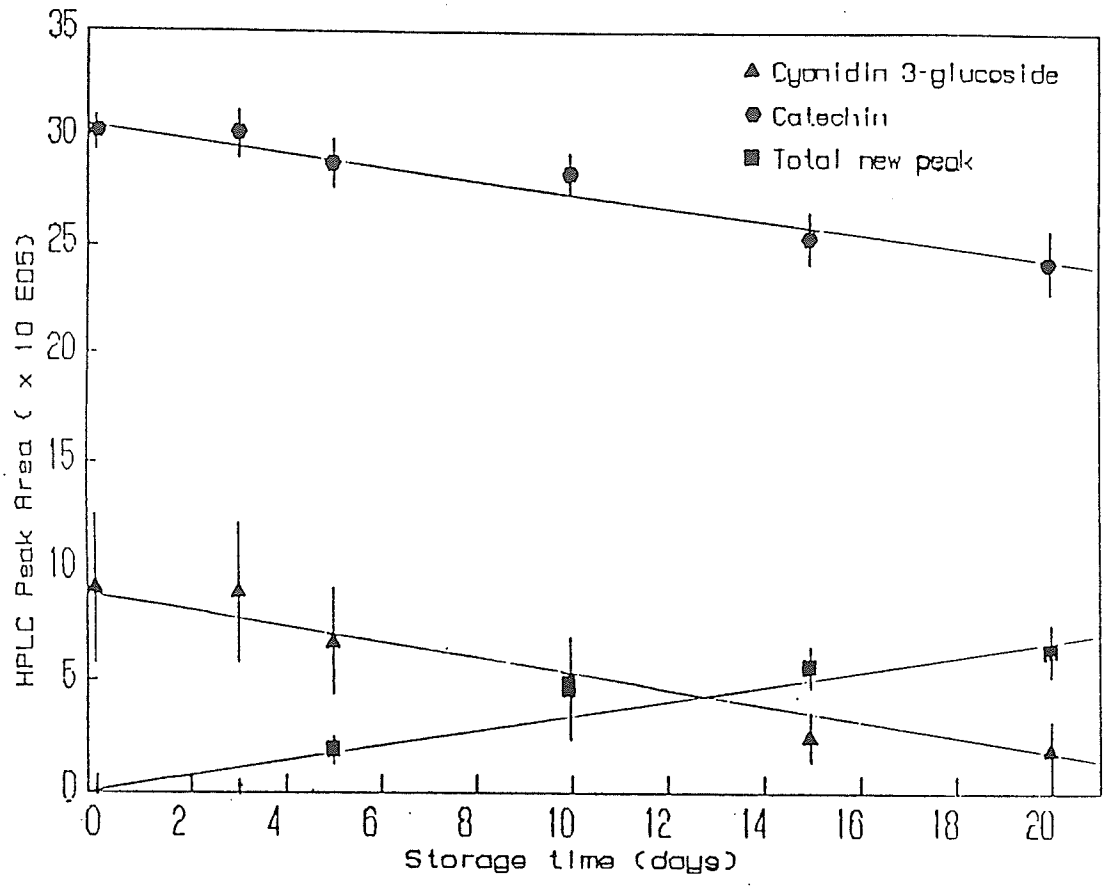

Figure 16. The HPLC peak area of cyanidin 3-glucoside, catechin and total new unidentified peaks in cyanidin 3-glucoside aqueous model system at 23 ± 1°C in the dark. Vertical bars represent standard error of each mean.

and catechin (r = -0.713) throughout the study. These results further demonstrate that cyanidin 3-glucoside, catechin and acetaldehyde reacted to form six new components which appeared on the HPLC profile. The new compounds probably consisted of intermediates of the acetaldehyde-induced condensation of catechin and cyanidin 3-glucoside, acetaldehyde-bridged polymers of catechin and other anthocyanin and/or catechin reaction products.

Plots of the six new HPLC peak areas as a function of time are shown in Figure 17 and the significance of the linear and quadratic effect of storage time on each peak is shown in Appendix 9. The peaks are numbered in ascending order according to the time of elution from the HPLC column. The assigned peak number does not necessarily indicate the molecular size and complexity, since the overall polarity of the molecule and substitution of the A-ring of flavonoid compounds are the major factors which determine separation and elution time on the RP-HPLC column (Strack et al., 1980; van de Casteele et al., 1983). As can be seen, peaks 1, 3, 4 and 5 first occurred on day three while peaks 2 and 6 appeared on day 15. Peaks 3 and 4 were approximately twice as large as the others. The UV spectrum, as measured by the photodiode array detector, showed a λmax of 280 nm for all six new peaks (Table 15). This wavelength is characteristic of anthocyanins (Markham and Mabry, 1975), catechin (Mazza, 1986) and acetaldehyde (Appendix 10), inferring that one or more of these compounds were components in the unidentified HPLC fractions. Similarly, Timberlake and Bridle (1976, 1977) found a λmax of 280 nm for a new component formed from the reaction of various

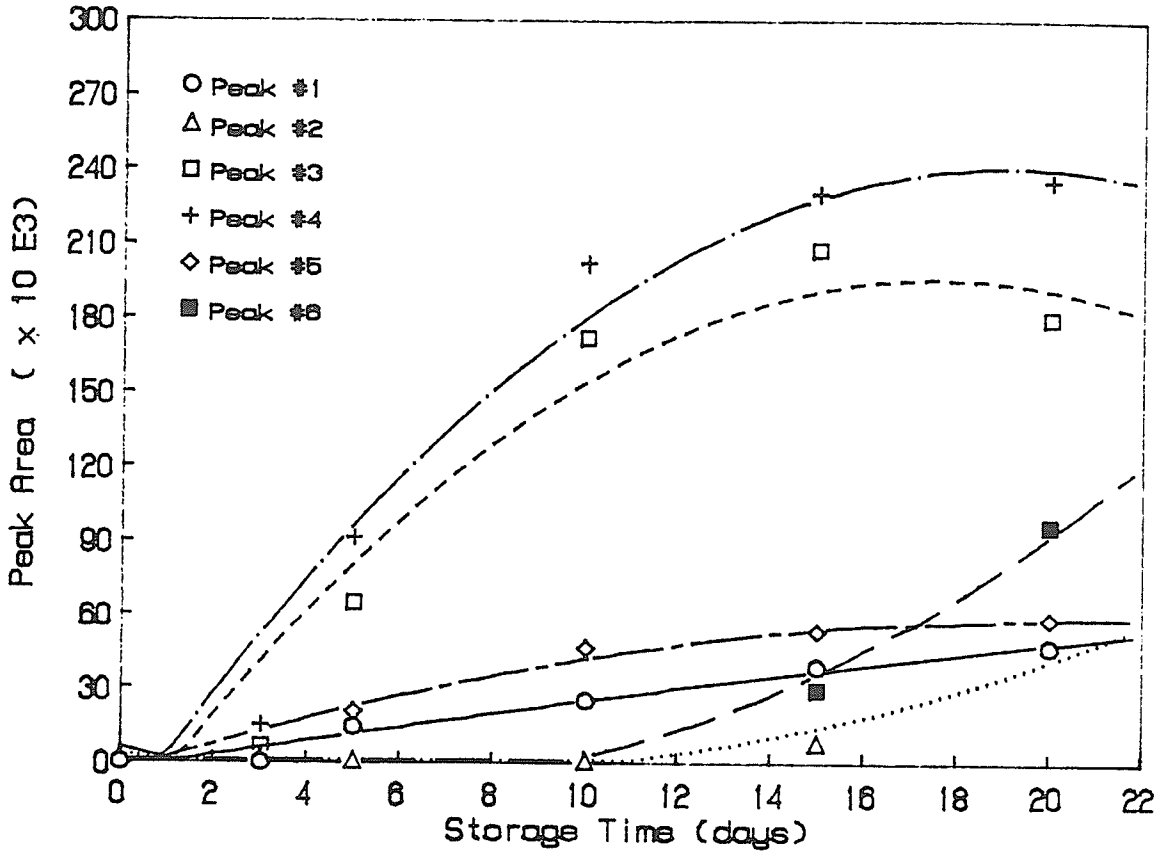

Figure 17. Area of new unidentified HPLC peaks in cyanidin 3-glucoside model system containing acetaldehyde and catechin stored at 23 ± 1°C in the dark.

Table 15: Retention times (t_R) and UV maxima(λmax) of new HPLC peaks.

Pigment System	Peak	t_R (min)	λmax(nm)
Cyanidin 3-glucoside model system	1	21.8	280
	2	24.8	280
	3	28.0	280
	4	32.6	280
	5	36.0	280
	6	41.8	280
Aqueous extract	1	39-42	280
Alcohol extract	1	39-42	280

anthocyanins with catechin and acetaldehyde. Using preparative paper chromatography they isolated only one new compound which appeared violet in colour. The six new fractions eluting from the HPLC column were colourless, even after concentrating approximately 10 mL to dryness using a Buchi Rotavapor R110 flash evaporator (Brinkmann Instruments, Inc., Westbury, N.Y.) at 25°C. Interestingly, after approximately six months of storage a violet precipitate formed in the sample containing both acetaldehyde and catechin. Much larger volumes, more highly concentrated pigment samples and the use of an HPLC preparatory column would be required to obtain sufficient quantities of the new components for further analytical and characterization studies. The clear separation of six new peaks by RP-HPLC from only 20 L of sample demonstrates the superior separatory power of this analytical technique in comparison to other methods for separation of these phenolic compounds.

The UV spectrum of the control and pigment + acetaldehyde samples showed a peak at 280 nm and remained essentially constant during storage. The presence of catechin shifted the λmax from 280 to 290 nm and also resulted in the formation of a second peak at 260 nm. This indicates that a fast reaction occurred between catechin and cyanidin 3-glucoside. The reaction, however, did not affect colour of the solution because the visible spectrum of the pigment + catechin sample was identical to that of the control. It is well known (Harborne, 1967; Markham and Mabry, 1975) that the ultraviolet absorbance maximum for anthocyanins is at 270 - 280 nm and this is due to absorbance of the A-

ring (Jurd, 1962) of the flavone structure. Also, absorbance of UV and visible light by flavonoid compounds is dependent on the dispersal of electrons of the molecules. The more loosely held the electrons, the less energy is required to produce a resonating effect and thus, the λmax shifts to a longer wavelength (Swain, 1976). The shift in λmax from 280 to 290 nm suggests that electrons of the anthocyanin A-ring became less tightly bound. This may be due to sharing of electrons between the anthocyanin A-ring and an aromatic ring of catechin. Such interaction was weak since HPLC analyses revealed that the chromatograms of the pure (control) pigment, pigment + acetaldehyde and pigment + catechin samples were essentially identical and did not change during storage (data not shown). This indicates that any catechin-anthocyanin products formed in samples containing catechin were unstable and dissociated when subjected to the low pH conditions on the HPLC column. Various forms of direct catechin-anthocyanin interactions have been reported previously (Jurd, 1967; Somers, 1971; Bishop and Nagel, 1984) but the shift in UV λmax was not observed in any of those studies. Therefore, further evidence on the structure of the reaction product is required before a mechanism of catechin-anthocyanin direct complexation can be postulated.

On storage, the pigment solution containing acetaldehyde and catechin also displayed browning, as evidenced by the increase in absorbance in the 400 - 460 nm region (Figure 18). This effect was not seen in any of the other samples (Figure 19; Appendix 11) and indicates that reaction of acetaldehyde, anthocyanin and catechin not only shifts the colour to violet but also increases browning. The only noticeable

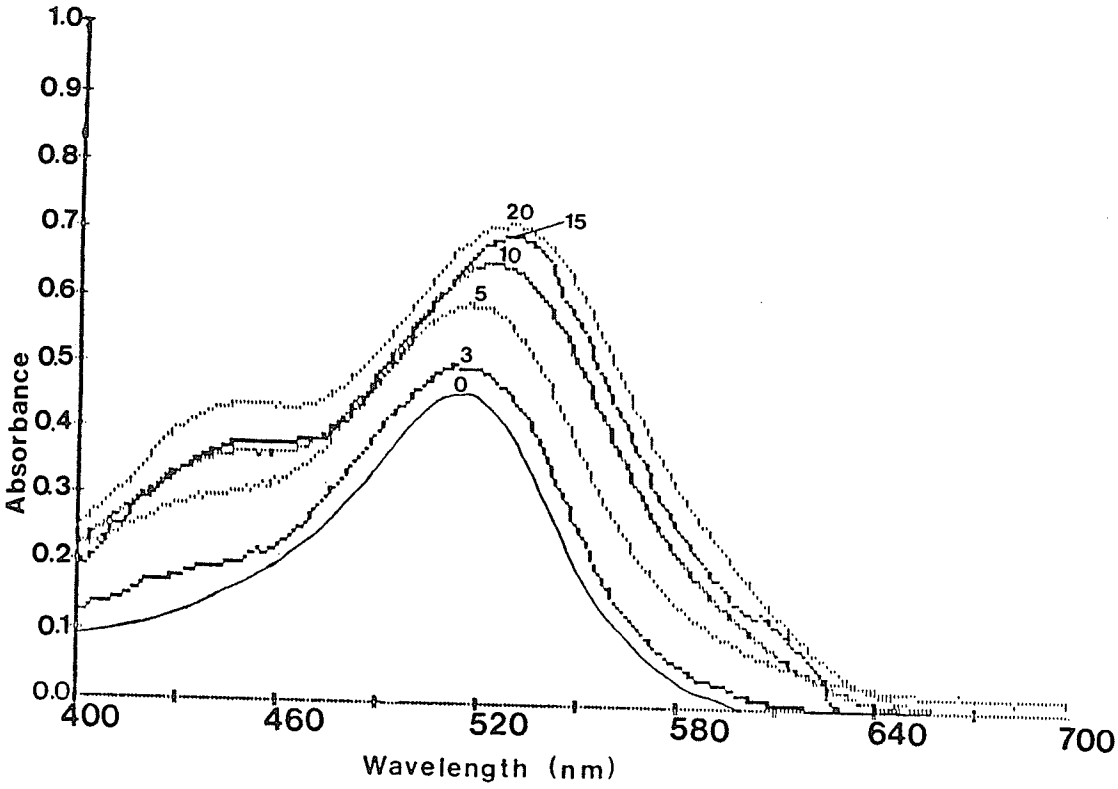

Figure 18. Colour augmentation of cyanidin 3-glucoside, acetaldehyde and (+)-catechin solution after 0, 3, 5, 10, 15 and 20 20 days at 23 ± 1°C in the dark.

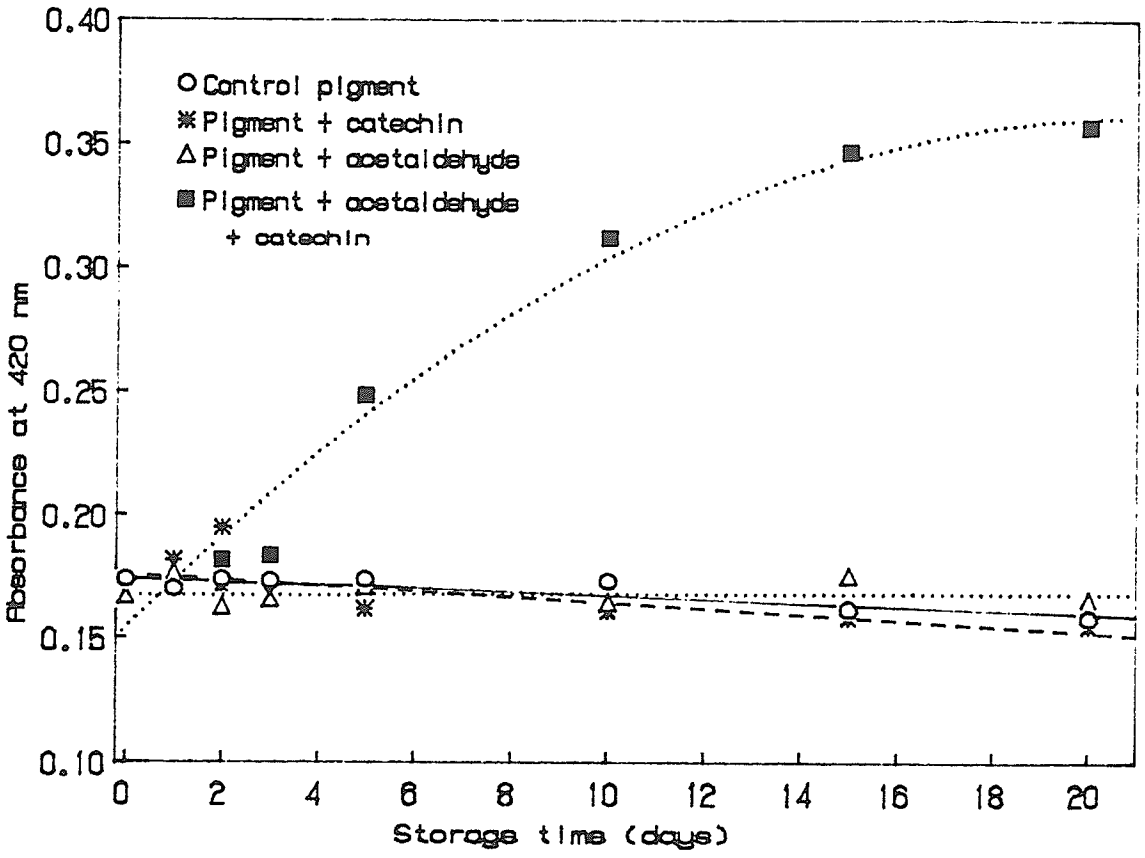

Figure 19. Effect of acetaldehyde and catechin on A_{420} of cyanidin 3-glucoside model system, pH = 3.5, stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 0.02.

visual effect, however, was the shift in colour of the pigment sample from orange-red to violet. As the size of the anthocyanin-acetaldehyde-catechin polymer increases, interaction among the phenolic hydroxyl groups may also increase. This may allow for easier oxidation of the phenolic hydroxyls to quinones, thus accounting for the browning effect. Such reactions are similar to those of highly condensed tannins (Somers, 1971). An increased absorbance in the browning region of the spectrum under similar reaction conditions was also observed by Timberlake and Bridle (1977) but no explanation was given.

Acetaldehyde is known to accumulate both during ripening and as a result of physiological disorders of fruits (Smagula and Bramlage, 1977; Pesis and Ben-Arie, 1984). Its role in these situations has not been fully explained, however, these results suggest that one effect may be to facilitate browning in old and injured fruit tissue.

The increase in the $A_{440}/A_{\lambda max}$ ratio as a result of increased absorbance in the 400 - 460 nm region of the spectrum may be significant in the interpretation of the structure of the new colour intensified compounds. The $A_{440}/A_{\lambda max}$ is a constant ratio for a given anthocyanin pigment. For cyanidin 3-glucoside the ratio has been reported to be 24 % (Harborne, 1958; Francis, 1962). This was the case initially; however, after 20 days of storage, the $A_{440}/A_{\lambda max}$ had increased to 65 % (Figure 18). The absorbance at 440 nm appears to be influenced by the degree of dispersal of electrons around the A-ring of the anthocyanidin, since substitution of a free hydroxyl group at

position C-5 with a destabilizing, electron withdrawing glycosyl group, reportedly decreases absorbance in this region (Harborne, 1958, 1967; Francis, 1982). Considering this fact, a further increase in absorbance at 440 nm may be due to stabilization of the A-ring of cyanidin as a result of π-π interactions with the A-ring of catechin. Such interaction would create an electron-rich environment around the anthocyanin A-ring and increase the absorbance intensity at 440 nm. This hypothesis supports a vertically stacked (i.e. juxtapositional) arrangement of the catechin and anthocyanin molecules. In such a structure, in addition to CH_3CH bridges and π-π interactions, further stabilization of the complex may result from H-bonding (Sweeney et al., 1981).

4.2.2 Saskatoon Berry Aqueous Extract

Typical UV and visible spectra of the saskatoon berry aqueous extracts after initial preparation and 20 days of storage are shown in Figure 20. The UV spectrum of all extracts showed no noticeable differences and displayed peaks at 280 and 320 nm. The peak in the 280 nm region is characteristic of anthocyanins (Markham and Mabry, 1975) and the peak at 320 nm may be caused by chlorogenic acid, since it displays a λmax near this wavelength and is a major constituent in saskatoon berry extracts (Mazza, 1986). In contrast to the cyanidin 3-glucoside model system (Figure 11), catechin did not alter the UV spectrum. It is possible that interaction between catechin and the anthocyanins did in fact occur but the reaction was not evident in the spectrum due to a masking effect of various other extract phenolic

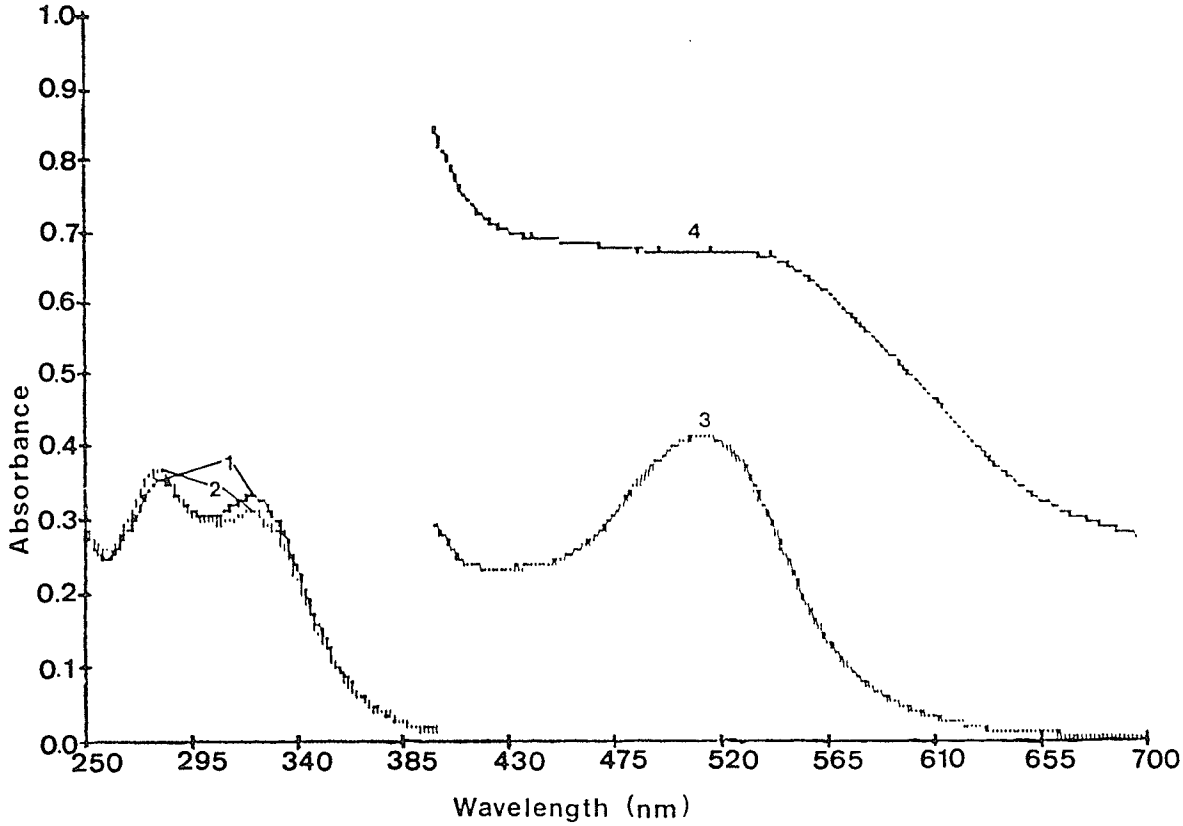

Figure 20. Effect of acetaldehyde and catechin on UV and visible spectra of saskatoon berry aqueous extracts after initial preparation and 20 days at 23 ± 1°C in the dark. The UV spectra of control and extracts containing acetaldehyde on day 1 and 20 (1); extracts containing catechin, day 1 and 20 (2); visible spectra of all extracts on day 1 (3); extracts containing acetaldehyde on day 20 (4).

components (Mazza, 1986) which also absorb strongly at 280 nm. However, the lack of pigment-catechin interaction may also be attributed to interactions of the pigments with various other flavonoids (Mazza, 1986) present in the extract. Such associations may be similar to the unstable but coloured copigment complexes proposed as being present in grape juice (Somers and Evans, 1979), stabilized by charge interactions and by hydrogen bonding of the anthocyanins to related phenolic compounds (Figure 21). Recently, Maccarone et al. (1987) have also reported that copigmentation of anthocyanins with tannins protect the pigments from reaction with other constituents in blood orange juice.

The visible spectra of all extracts initially were not different (Figure 20) and were similar to the spectra of the cyanidin 3-glucoside model systems. Changes in the λmax and absorbance at λmax during storage are shown quantitatively in Figures 22 and 23, respectively and statistical analysis is provided in Appendices 12 and 13, respectively. As can be seen, all samples showed an initial λmax near 512 nm. Berry extracts which did not contain acetaldehyde displayed a hypsochromic shift of about one to two nm and approximately a 32 % decrease in absorbance. These results indicate a decrease in the concentration of the cyanidin 3-monoglycoside pigments, probably due to oxidative degradation or polymerization to colourless products (Daravingas and Cain, 1965; Clydesdale et al., 1978; Markakis, 1982).

The berry extract containing 1.26×10^{-2} M acetaldehyde showed a bathochromic shift of 4 nm and a slight decrease in absorbance during

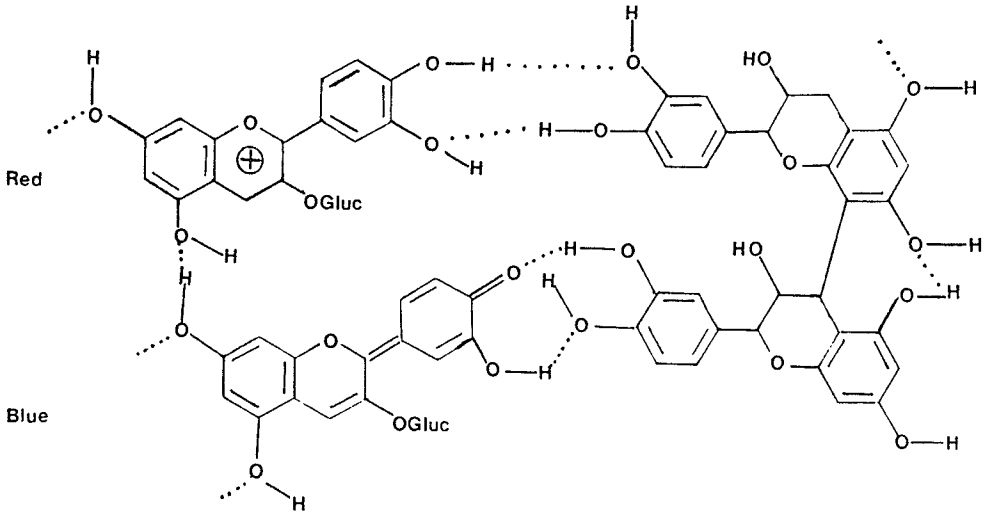

Figure 21. Anthocyanin-related flavonoids complex stabilized by charge interactions and hydrogen bonding. (From: Somers and Evans, 1979)

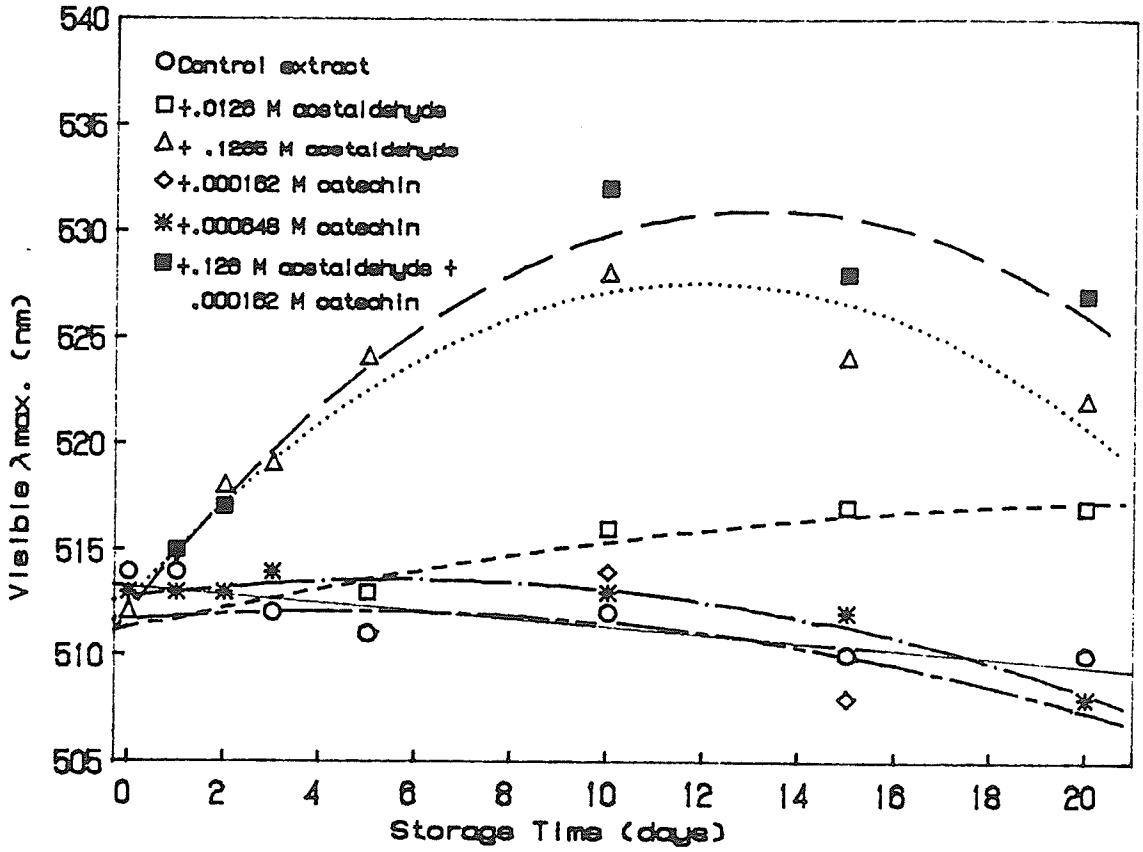

Figure 22. Effect of acetaldehyde and catechin on visible λmax of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 1.6.

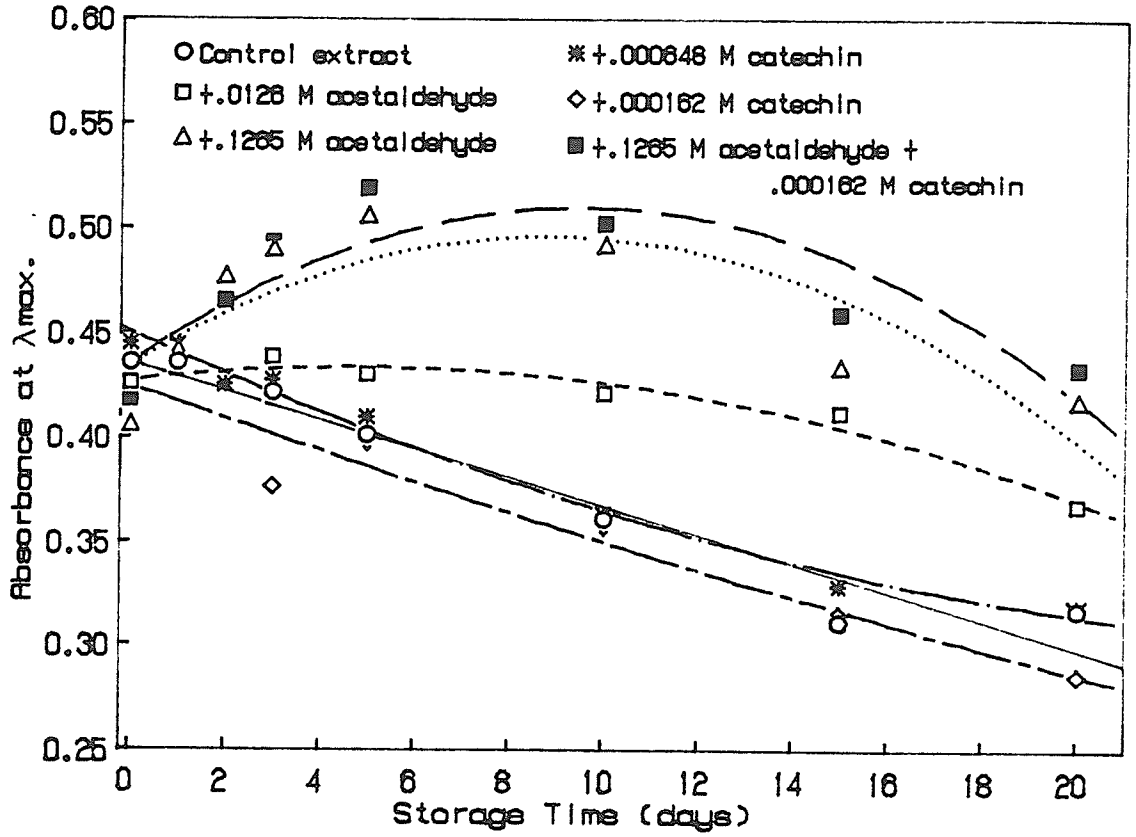

Figure 23. Effect of acetaldehyde and catechin on absorbance at visible λmax of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 0.02.

storage. The spectrum of the extract containing both acetaldehyde and catechin was not significantly different from that of the extract containing only acetaldehyde at the same concentration. This result implies that other colourless flavonoids present in the berry extracts were also reactants in the acetaldehyde-induced colour intensification reaction and the presence of added catechin was not required for the reaction to occur. A maximum bathochromic shift of 13 to 14 nm occurred after about 13 days of storage in extracts which contained a higher level of acetaldehyde (extract + 1.26×10^{-1} M acetaldehyde and extract + 1.26×10^{-1} M acetaldehyde + 1.6×10^{-4} M catechin) followed by a 4 nm decrease in the magnitude of the shift by the end of the study (Figure 22). The absorbance of these samples reached a maximum increase of approximately 16 % on day ten and then decreased during the next ten days to the initial absorbance level (Figure 23). However, from day ten until the end of the study, the absorbance values showed an increase of 33 % in the extract containing 1.26×10^{-2} M acetaldehyde and of 43 % in samples containing 1.26×10^{-1} M acetaldehyde in comparison to the control extract on the same day. Thus, the presence of acetaldehyde in the saskatoon berry extracts caused colour intensification of the anthocyanins and the rate and extent of intensification increased with acetaldehyde concentration. Others have reported similar findings on the reaction rate in model pigment solutions when the concentration of acetaldehyde (Baranowski and Nagel, 1983) and catechin (Timberlake and Bridle, 1977) was altered. In the presence of 1.26×10^{-1} M acetaldehyde, the subsequent decrease in colour after 10 days of storage can be explained by the eventual precipitation of polymers too large to

remain in solution. This was evidenced by the increase in absorbance at 700 nm, a measure of turbidity (Wrolstad, 1976), in solutions which contained the higher concentration of acetaldehyde (Figures 20 and 24; Appendix 14). Furthermore, it is known that quinoid structures are not very soluble (Timberlake and Bridle, 1977) and this may have contributed to the decreased solubility of the polymer. This precipitation effect has been reported previously in model systems of acetaldehyde, catechin and other anthocyanins (Timberlake and Bridle, 1977; Baranowski and Nagel, 1983). The initial augmentation of colour can be attributed to the formation of anthocyanin-acetaldehyde-colourless flavonoid polymers and the subsequent decrease to the precipitation of the polymers. In the extract containing 1.26×10^{-2} M acetaldehyde, the level of acetaldehyde was too low to cause precipitation and thus a decrease in absorbance of the extract.

In Figure 25, the absorbance at 420 nm (calculated as $A_{420} - A_{700}$), a measure of browning (Wrolstad, 1976), is presented. Statistical analysis of the effects of the reactants is given in Appendix 15. The A_{420} remained essentially constant in extracts which did not contain acetaldehyde but showed a 22 % increase in the extract containing 1.26×10^{-2} M acetaldehyde and a 100 % increase in samples which contained 1.26×10^{-1} M acetaldehyde. The presence of catechin had no added effect on browning of the samples. Browning in the presence of acetaldehyde may be due largely to polymers of various flavonoids, both anthocyanin and nonanthocyanin types, linked through CH_3CH bridges (Timberlake and Bridle, 1976).

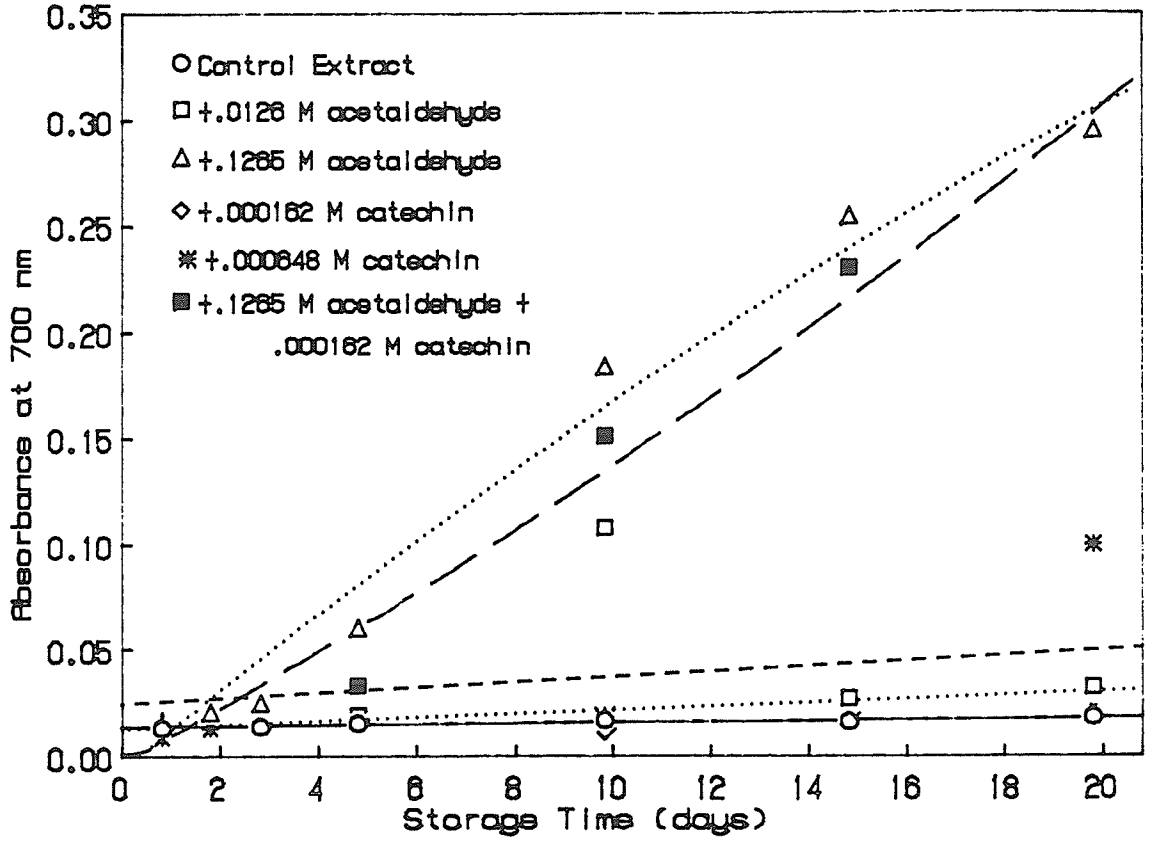

Figure 24. Effect of acetaldehyde and catechin on absorbance at 700 nm of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 0.03.

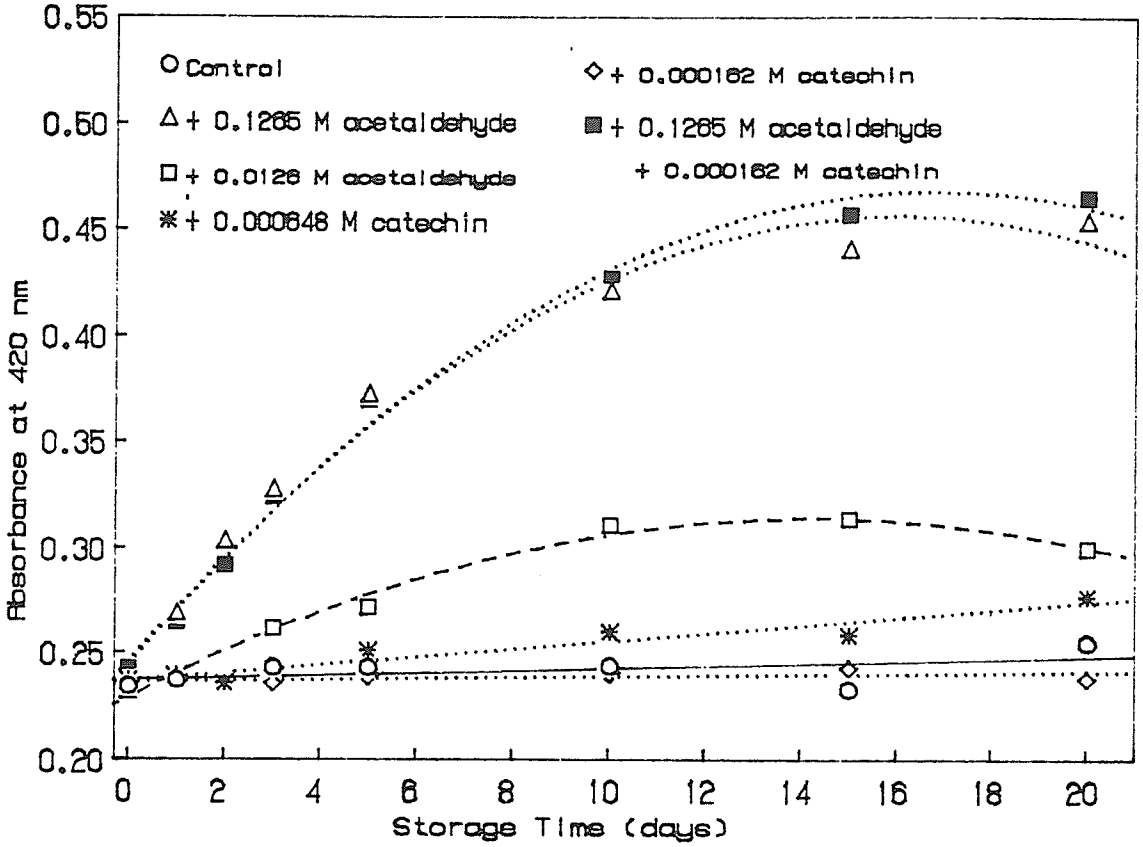

Figure 25. Effect of acetaldehyde and catechin on absorbance at 420 nm of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 0.01.

The colour of extracts which did not contain acetaldehyde (control and extracts + catechin) shifted to a lighter shade of red. This effect was evident by the change in hue angle (θ) of these samples, which increased from 22 initially to about 29 on day 20 (Figure 26; Appendix 16). The presence of acetaldehyde shifted the colour of the berry extracts to a deeper, violet shade. A decrease from 23 to about 21 occurred in the hue angle of the extract + 1.26×10^{-2} M acetaldehyde during the study while that of extracts containing 1.26×10^{-1} M acetaldehyde showed a decrease from 22 to 12 by day 12 and then increased to 14 by day 20. These results agree with those from the spectral analyses indicating that the presence of acetaldehyde caused an intensification of colour in the saskatoon berry extracts. Although, as shown previously (section 4.1.1), hue angle is not a reliable predictor of anthocyanin concentration, it is clearly an accurate measurement for a shift in colour due to acetaldehyde-induced colour intensification of the pigments. This indicates that the hue angle is largely influenced by the wavelength of absorbance and is relatively insensitive to absorbance intensity of crude anthocyanin extracts. These findings agree with those of Bakker et al. (1986) which have also shown that hue angle is a reliable discriminating parameter for expressing the shade of colour in anthocyanin pigment systems such as wine.

The HPLC profiles of the control and extracts containing only catechin did not change during storage whereas profiles of extracts containing acetaldehyde exhibited a noticeable loss in the peak areas of cyanidin 3-galactoside, cyanidin 3-glucoside and catechin and the

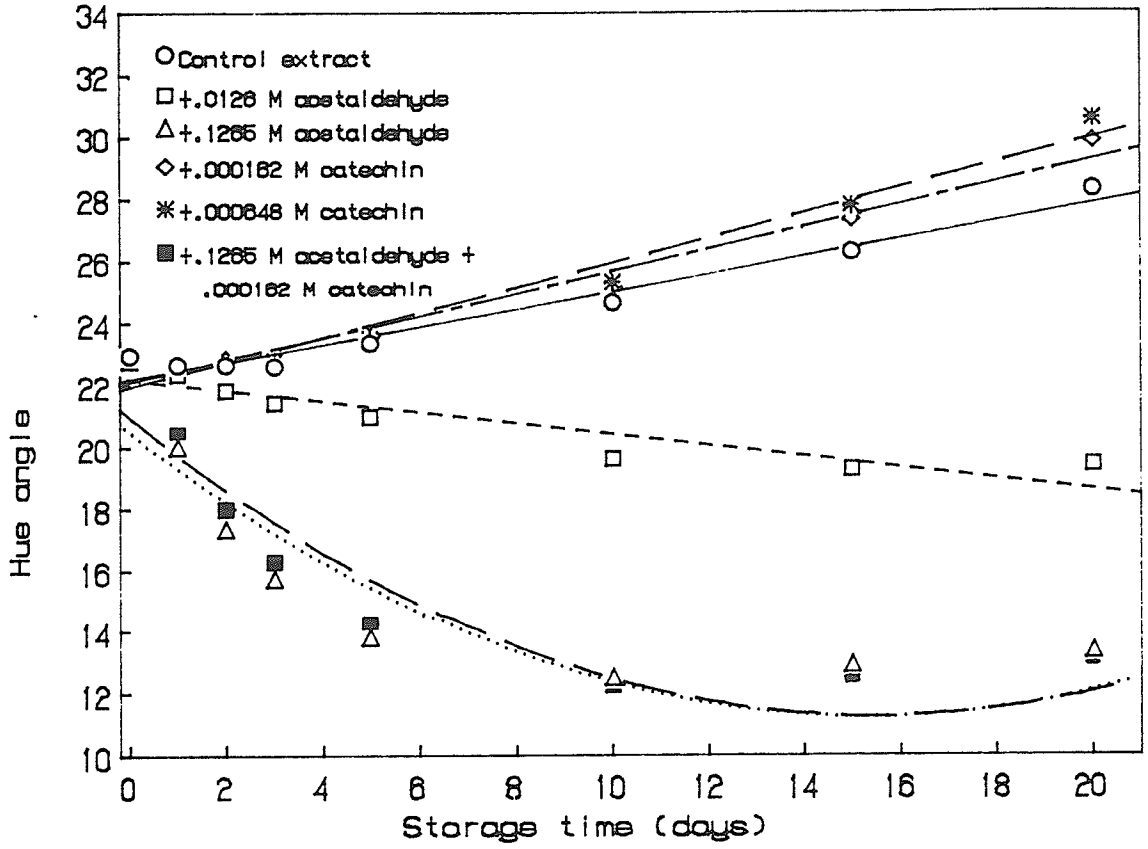

Figure 26. Effect of acetaldehyde and catechin on hue angle of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 0.6.

appearance of a new unidentified peak near 40 minutes (Figure 27). Appearance of the new component on the chromatogram corresponded closely to the decrease of the two anthocyanin peaks and catechin (Figures 28 and 29). As well, development of the peak coincided with the observed shifts in λmax (Figure 22) and absorbance intensity (Figure 23) of the visible spectrum. The compounds represented by the new HPLC peak may have been responsible for intensifying the colour of the extracts and some of the components of this peak were probably the polymerized product of the acetaldehyde-induced colour augmentation reaction (Figure 14). In agreement with this, the precipitate which occurred in the extracts was violet in colour. Timberlake and Bridle (1977) isolated a single, violet coloured component from reaction of anthocyanins with catechin-type phenolic compounds and acetaldehyde.

Examination of the chromatograms of the extracts containing acetaldehyde indicates that the newly formed compounds were different than those formed in the cyanidin 3-glucoside model system containing both acetaldehyde and catechin. Six distinct peaks occurred throughout the latter half of the chromatogram of the model system (Figure 15) whereas a large, not well defined peak was observed near the end of the HPLC profile of the berry extracts containing acetaldehyde (Figure 27). However, similar to the new peaks in the model system, the new extract component showed a λmax at 280 nm (Table 15). As noted previously, this wavelength is characteristic of all the proposed reactants; acetaldehyde, anthocyanin and catechin as well as various other possible phenolic reactants in the crude extract (Mazza, 1986). Furthermore, four

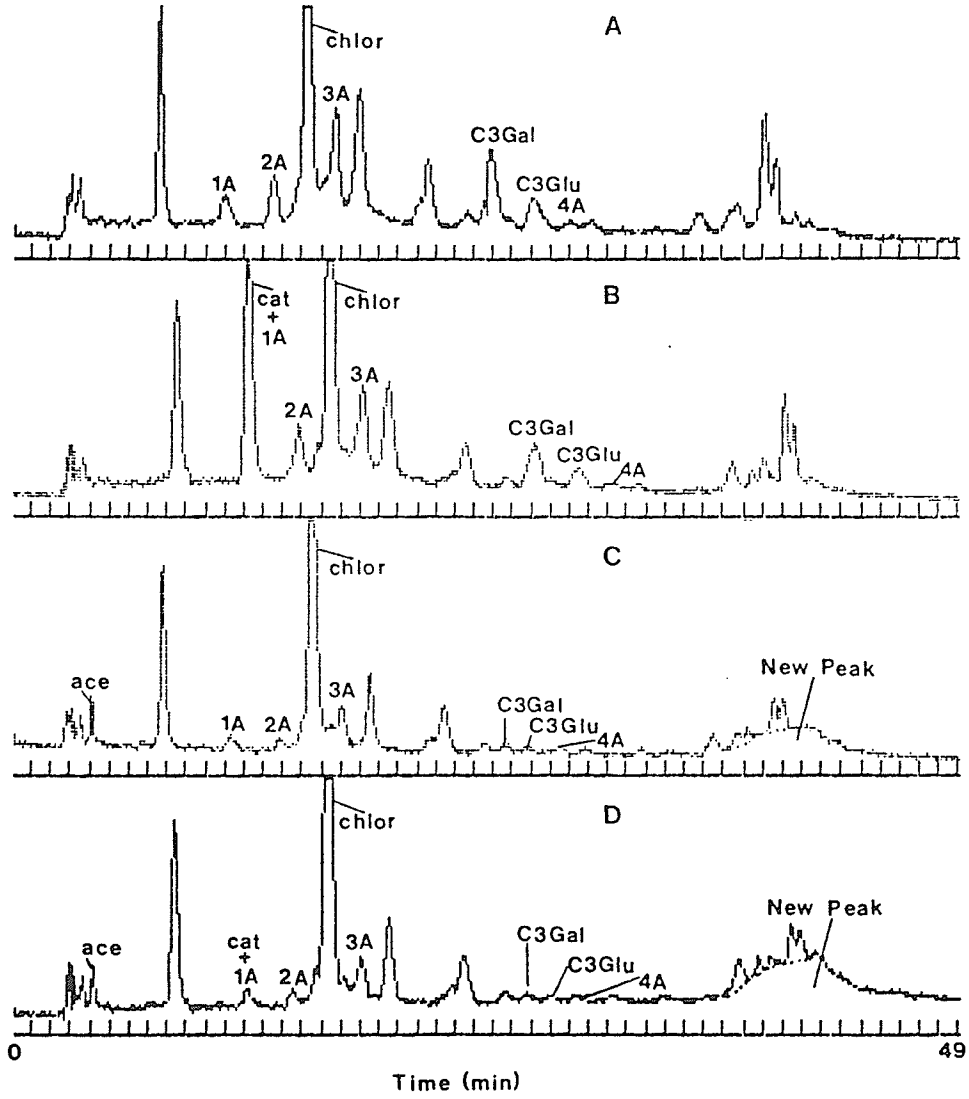

Figure 27. The HPLC of saskatoon berry extracts on LiChrosorb RP-18. Control extract (A) and extracts containing catechin (B), acetaldehyde (C) and acetaldehyde + catechin (D). Peaks: ace = acetaldehyde; cat = catechin; chlor = chlorogenic acid; C3Gal = cyanidin 3-galactoside; and C3Glu = cyanidin 3-glucoside. Peaks 1A, 2A, 3A and 4A were tentatively identified previously (Mazza, 1986).

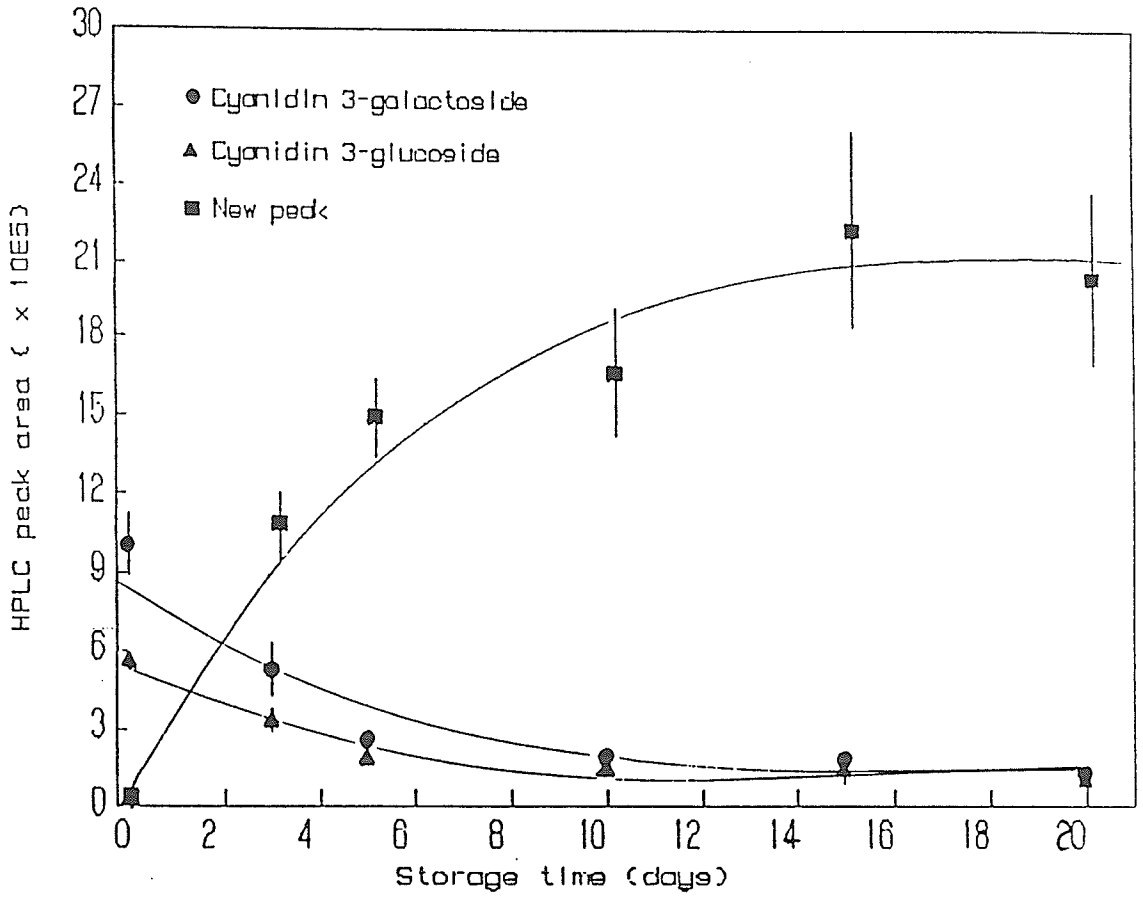

Figure 28. The HPLC peak area of cyanidin 3-galactoside, cyanidin 3-glucoside and the new unidentified peak in saskatoon berry aqueous extract containing 1.26×10^{-1} M acetaldehyde stored at 23 ± 1°C in the dark. Vertical bars represent standard error of the mean.

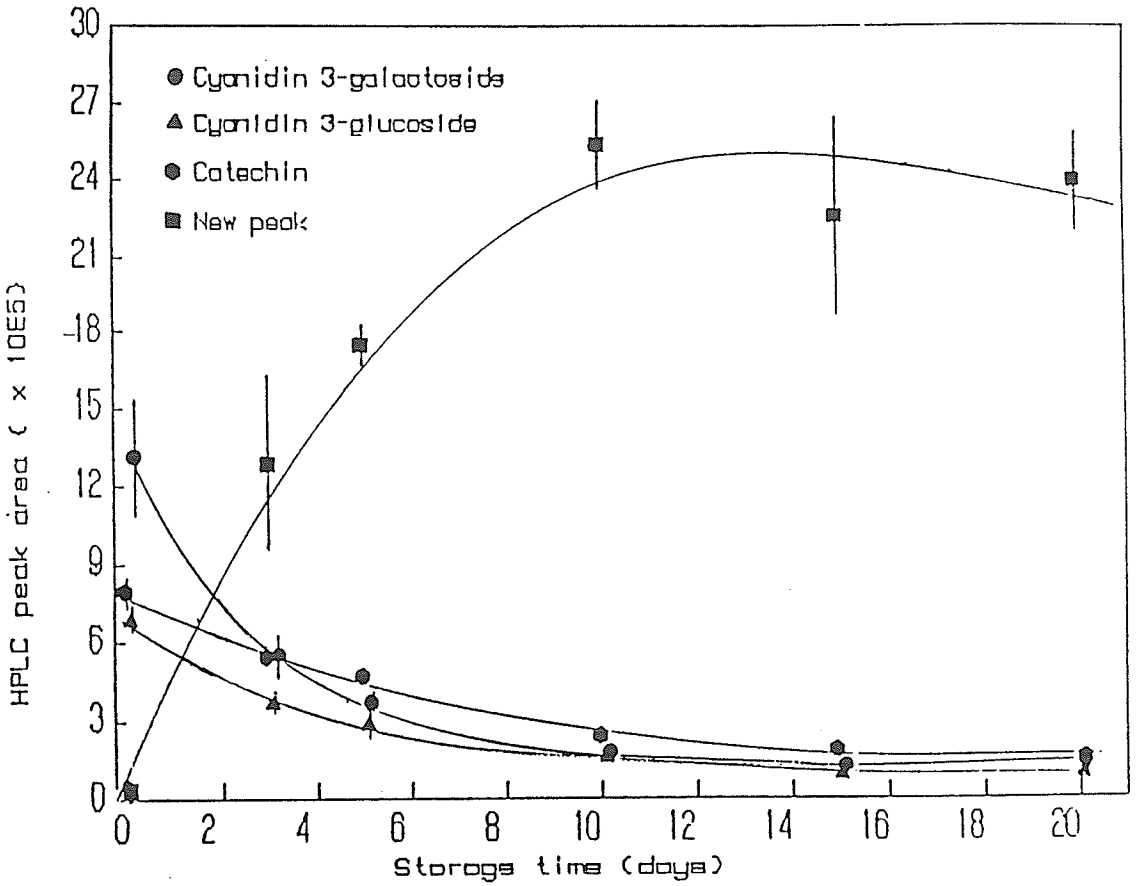

Figure 29. The HPLC peak area of cyanidin 3-galactoside, cyanidin 3-glucoside, catechin and the new unidentified peak in saskatoon berry aqueous extracts containing 1.26×10^{-1} M acetaldehyde and 1.62×10^{-4} M catechin. Vertical bars represent standard error of the mean.

other regular extract peaks besides the two known pigment peaks decreased during storage (Figure 27). These peaks were designated as 1A, 2A, 3A and 4A according to their elution time in ascending order. The UV maximum of peaks 1A and 2A, as measured by photodiode array detector was 279 and 285 nm, respectively. The identity of these two peaks, however, is not known. Peak 3A corresponds to peak 3 on a similar saskatoon berry HPLC profile characterized previously by Mazza (1986). The peak showed λmax values at 280, 328 and 525 nm and was believed to consist of an unidentified anthocyanin present as a mixture with (-)-epicatechin or an isomer of chlorogenic acid. Peak 4A coincides with peak 9 on the chromatogram reported by Mazza (1986). The λmax values for this peak were 280 and 528 nm and the compound was tentatively identified as cyanidin 3-xyloside. The tentative identities of these peaks as anthocyanins and their structural and spectral characteristics implies that they were reactants in the acetaldehyde-induced colour intensification reaction. As well, (-)-epicatechin and various other more complex phenolic compounds are known reactants in this colour augmentation mechanism (Timberlake and Bridle, 1976, 1977).

Catechin may be involved in the newly formed complexes since its peak area decreased in the presence of acetaldehyde (Figure 29) but remained constant when acetaldehyde was not included in the extracts (data not shown). The new peak formed at the same rate and had the same UV spectral and chromatographic characteristics, both in the presence and absence of added catechin. This further suggests that other extract constituents in addition to catechin were included in the new HPLC

component. Such reactants may be the compounds represented by peaks 1A, 2A, 3A and 4A as well as other phenolic compounds which do not absorb at 280 nm, so that they did not appear on the HPLC profile. Thus, a variety of anthocyanin and nonanthocyanin phenolic constituents may be included in the acetaldehyde-bridged polymers. Timberlake and Bridle (1976) found that the rate of reaction of anthocyanins with acetaldehyde and other phenolic compounds increases with complexity of the nonanthocyanin phenolic reactant. It is probable that some catechin-type and larger noncatechin phenolic compounds in the extracts, possibly peaks 1A, 2A and 3A were more reactive than catechin in the polymerization reaction. These dimers may have been relatively nonpolar so that they eluted near the end of the HPLC elution gradient. This would account for the absence of intermediate peaks throughout the chromatogram. Subsequent condensation of catechin and other phenolic compounds onto the already large, nonpolar polymers may then have occurred.

The peak area of acetaldehyde did not change with storage time but as in the model system, this peak was small and difficult to integrate precisely.

In fruit and aqueous fruit products such as jam, jelly and juice, the reactions of anthocyanins will depend upon the nature and relative concentrations of the components present. On long term storage these pigments will undergo polymerization with other phenolic compounds either by direct condensation or by acetaldehyde-induced condensation. The products of the direct condensation mechanism will impart a reddish-

brown colour (Somers, 1971) whereas the acetaldehyde-induced reaction produces a more intensely coloured pigment. Thus, in regard to colour, it is more favourable for the phenolic compound-acetaldehyde-anthocyanin reaction to predominate in stored saskatoon berry fruit and processed products. The rate and extent of the acetaldehyde-induced condensation increases with the acetaldehyde concentration. The level of acetaldehyde in saskatoon berries is not known but in apple peels and Bartlett pears it has been reported to be 1.4×10^{-3} M and 5×10^{-3} M, respectively (Smagula and Bramlage, 1977). The optimum levels of acetaldehyde for colour intensification of saskatoon berry products remain to be investigated. Previous studies have indicated that post-harvest application of acetaldehyde vapours to other fruits enhanced sensory quality attributes (Paz et al., 1981). Similarly, the addition of acetaldehyde to post-harvest saskatoon berry fruit may prove to significantly increase colour quality of the fruit and processed products.

4.2.3 Saskatoon Berry Acidified Methanol Extract

The UV and visible spectra of the saskatoon berry methanol extracts at the beginning and end of the study period are shown in Figure 30. As can be seen, these spectra are similar to those of the corresponding aqueous extract samples (Figure 20) although some deviation exists between the peak wavelengths. Variation in λmax between different solvent systems is typical for anthocyanins (Wrolstad, 1976). The UV spectra of all methanol extracts displayed peaks characteristic of

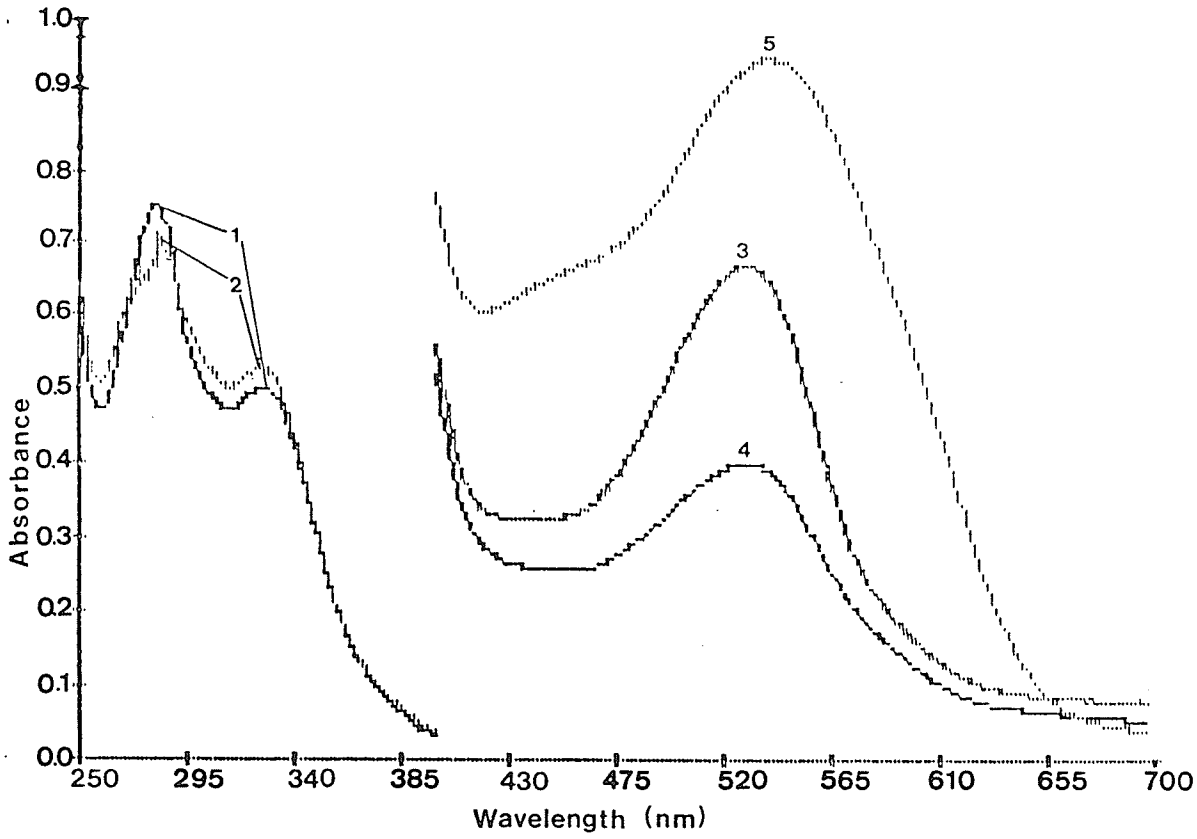

Figure 30. Effect of acetaldehyde and catechin on UV and visible spectra of saskatoon berry methanol extracts after initial preparation and 30 days at 23 ± 1°C in the dark. Control and extracts containing acetaldehyde, day 1 and 30 (1); extracts containing catechin, day 1 and 30 (2); control and extracts containing catechin and acetaldehyde, day 1 (3); control and extracts containing catechin, day 30 (4); extracts containing acetaldehyde, day 30 (5).

chlorogenic acid at 325 nm (Mazza, 1986) and anthocyanins and other flavonoids at 280 nm (Harborne, 1967; Markham and Mabry, 1975).

The effect of the added reactants on the visible λmax and absorbance at λmax of the saskatoon berry alcoholic extracts are shown in Figures 31 and 32, respectively and statistical analysis is given in Appendices 17 and 18, respectively. Initially, the λmax of all extracts occurred at 529 nm. The λmax of the control decreased slightly (about 1 nm) and the absorbance intensity decreased by about 35 % during storage. These results indicate a decrease in concentration of coloured structure which may have been magnified by a slight shift in pH of the extracts from 3.5 to 4.0 (Appendix 19). The increase in pH, which occurred in all methanol extracts, was probably caused by loss of volatile formic acid during storage and may have led to a shift in the equilibrium of anthocyanin structure from the coloured flavylium ion to primarily the colourless carbinol pseudobase form and to a lesser extent, the noncoloured chalcone and coloured quinoidal base forms (Brouillard, 1982; Mazza and Brouillard, 1987b). The net effect would be a loss of anthocyanin colour. Since the pH of all extracts at a given time were equivalent, however, the properties of the spectrum at any given time provide an accurate comparison of the effects of the reactants in each system. Catechin alone did not interact with the pigments since the UV and visible spectra of these samples were identical to those of the control. As well, the HPLC profiles of the control and extract + 2.5 x 10^{-3} M catechin were essentially identical (Figure 33a and b) and did not change with storage time. The lack of noticeable reaction between

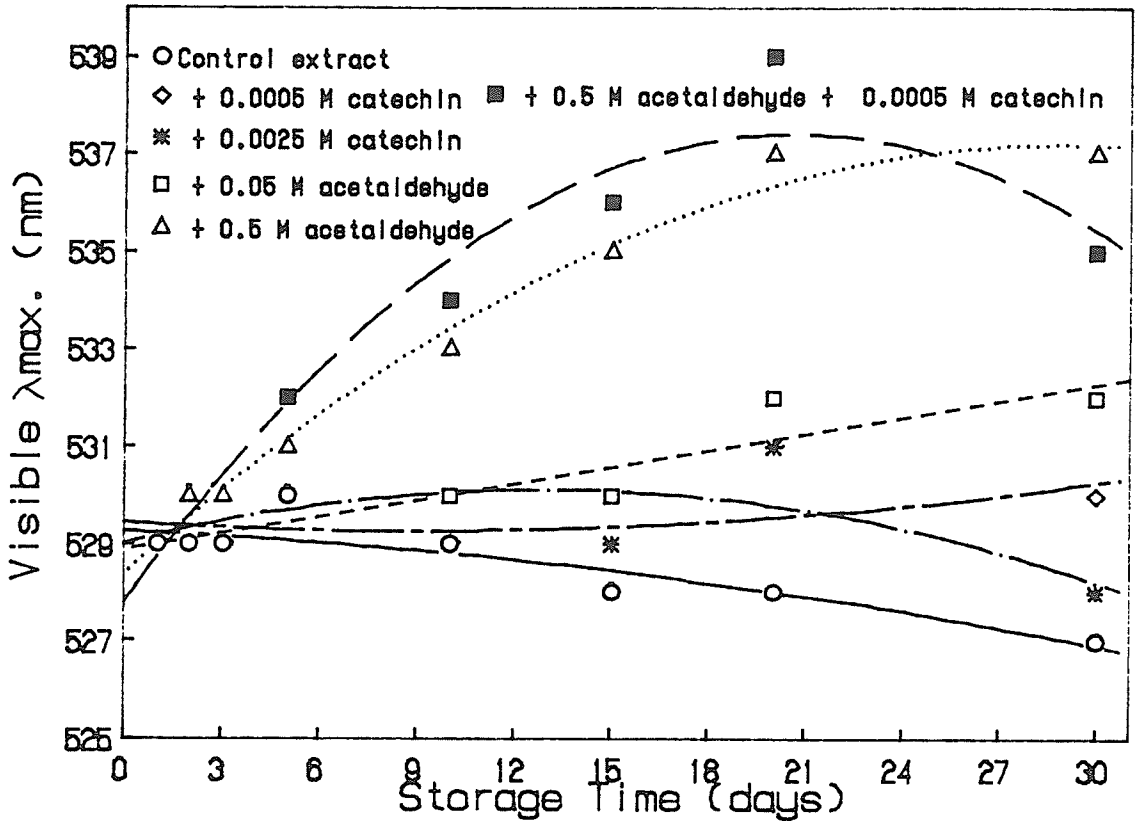

Figure 31. Effect of acetaldehyde and catechin on visible λmax of saskatoon berry alcoholic extracts stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 1.4 nm.

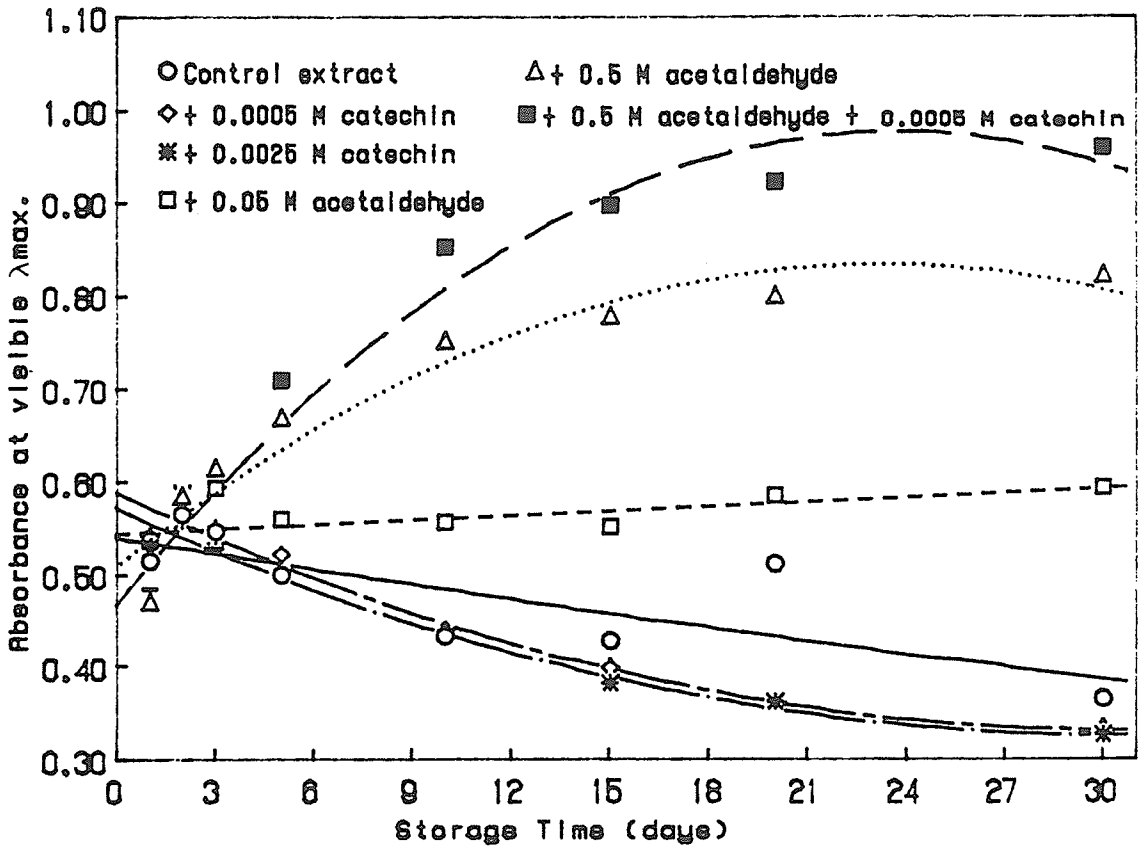

Figure 32. Effect of acetaldehyde and catechin on absorbance at visible λmax of saskatoon berry alcoholic extracts stored at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 0.06.

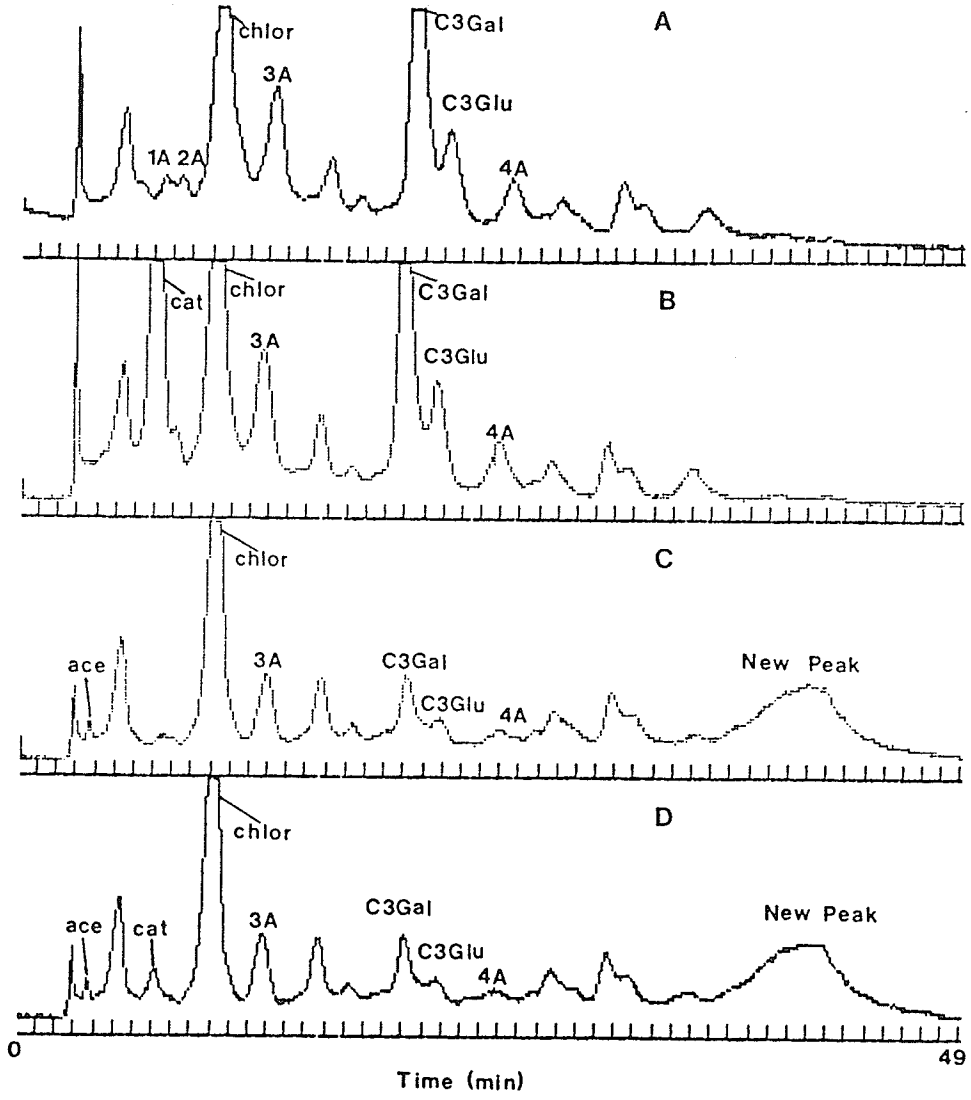

Figure 33. The HPLC profiles of saskatoon berry alcoholic extracts on LiChrosorb RP-18. Control extract (A) and extracts containing catechin (B), acetaldehyde (C) and acetaldehyde + catechin (D) all after 30 days at 23 ± 1°C in the dark. Peaks: ace = acetaldehyde; cat = catechin; chlor = chlorogenic acid; C3Gal = cyanidin 3-galactoside; and C3Glu = cyanidin 3-glucoside. Peaks 1A, 2A, 3A and 4A were tentatively identified previously (Mazza, 1986).

catechin and anthocyanins indicates that other extract constituents may have interacted with the pigments and/or catechin. Such associations may be similar to anthocyanin-flavonoid complexes stabilized by charge interactions and by hydrogen bonding (Figure 21).

On storage, a bathochromic shift of approximately 7 nm occurred in the visible spectrum of methanol extracts which contained 5×10^{-1} M acetaldehyde and about 2 nm in the extract containing 5×10^{-2} M acetaldehyde (Figure 31). Samples which contained 5×10^{-1} M acetaldehyde (i.e. extract + 5×10^{-1} M acetaldehyde and extract + 5×10^{-1} M acetaldehyde + 5×10^{-4} M catechin) showed an increase in absorbance during storage while the absorbance of the extract + 5×10^{-2} M acetaldehyde remained constant (Figure 32). After 30 days the absorbance values showed an increase of 57 % in the extract containing 5×10^{-2} M acetaldehyde and of more than 100 % in samples containing 5×10^{-1} M acetaldehyde in comparison to the control extract on the same day. The increase in pH may have caused some loss of colour and thus, diminishing in part, the effect of the colour intensification. However, the newly formed colour augmented compounds may be more stable than monomeric anthocyanins to higher pH conditions (Timberlake 1980; Bakker and Timberlake, 1986). The absorbance increase and bathochromic shift in the visible band of samples containing acetaldehyde were also seen in the saskatoon berry aqueous extracts and the cyanidin 3-glucoside model system.

Further evidence of reaction in extracts containing acetaldehyde was demonstrated in the HPLC profiles of the extract + 5 x 10^{-1} M acetaldehyde and extract + 5 x 10^{-1} M acetaldehyde + 5 x 10^{-4} M catechin. The chromatograms of these samples showed noticeable losses in the peak areas of the reactants acetaldehyde, catechin and cyanidin 3-galactoside and 3-glucoside and the appearance of a new unidentified peak near 40 minutes (Figures 33c and d, 34 and 35). As in the aqueous extracts, peaks 1A, 2A, 3A and 4A decreased during storage, indicating that other extract constituents were reactants in the acetaldehyde-induced polymerization. The new component occurring on the HPLC chromatogram showed a λmax at 280 nm, similar to those of the aqueous solutions and cyanidin 3-glucoside model system (Table 15). This UV λmax is also consistent with that of the unidentified compound isolated by Timberlake and Bridle (1976, 1977) from a similar reaction system. Fractions of the new peak were collected and concentrated as described previously but as in the aqueous model and berry extract systems, the components did not display colour. Interestingly and in accordance with the colour augmentation theory, after approximately six months a violet precipitate appeared in the extracts containing acetaldehyde.

It has been reported previously (Mazza and Brouillard, 1987c) that anthocyanins undergo copigmentation with chlorogenic acid almost instantly. The peak due to chlorogenic acid (Mazza, 1986) was distinctly smaller in methanol extracts which contained acetaldehyde but did not decrease further on storage (Figure 36; Appendix 20). This suggests that chlorogenic acid was a constituent of the new component and further

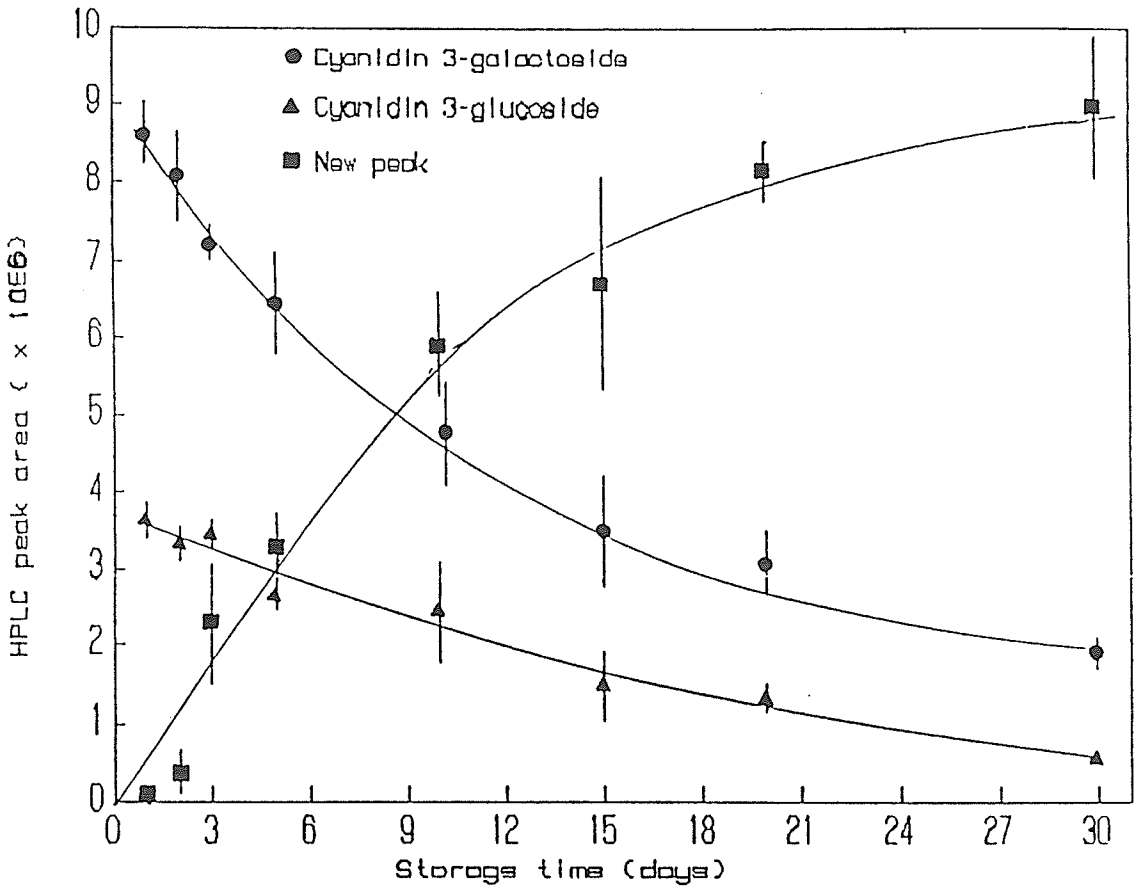

Figure 34. The HPLC peak area of cyanidin 3-galactoside, cyanidin 3-glucoside and the new unidentified peak in saskatoon berry alcoholic extracts containing 5×10^{-1} M acetaldehyde stored at 23 ± 1°C in the dark. Vertical bars represent standard error for each mean.

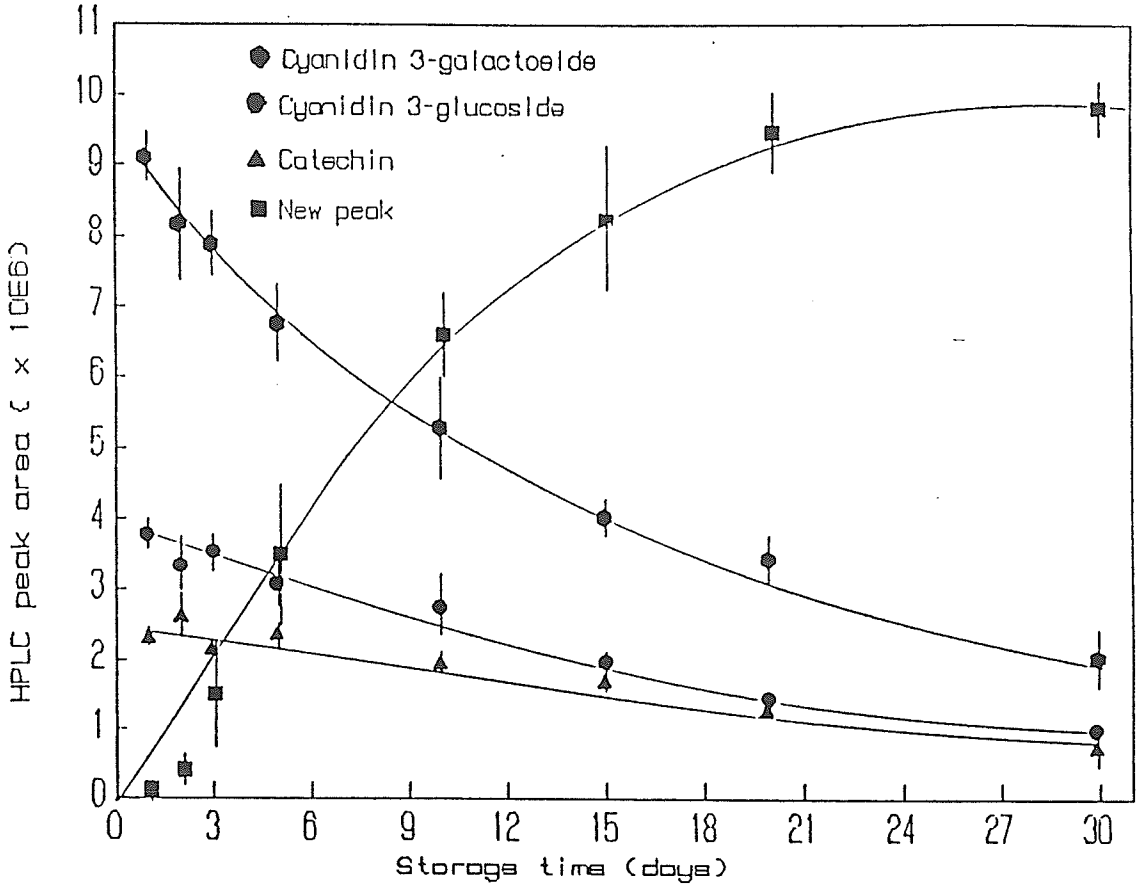

Figure 35. The HPLC peak area of cyanidin 3-galactoside, cyanidin 3-glucoside, catechin and the new unidentified peak in saskatoon berry alcoholic extracts containing 5×10^{-1} M acetaldehyde and 5×10^{-4} M catechin stored at 23 ± 1°C in the dark. Vertical bars represent standard error of each mean.

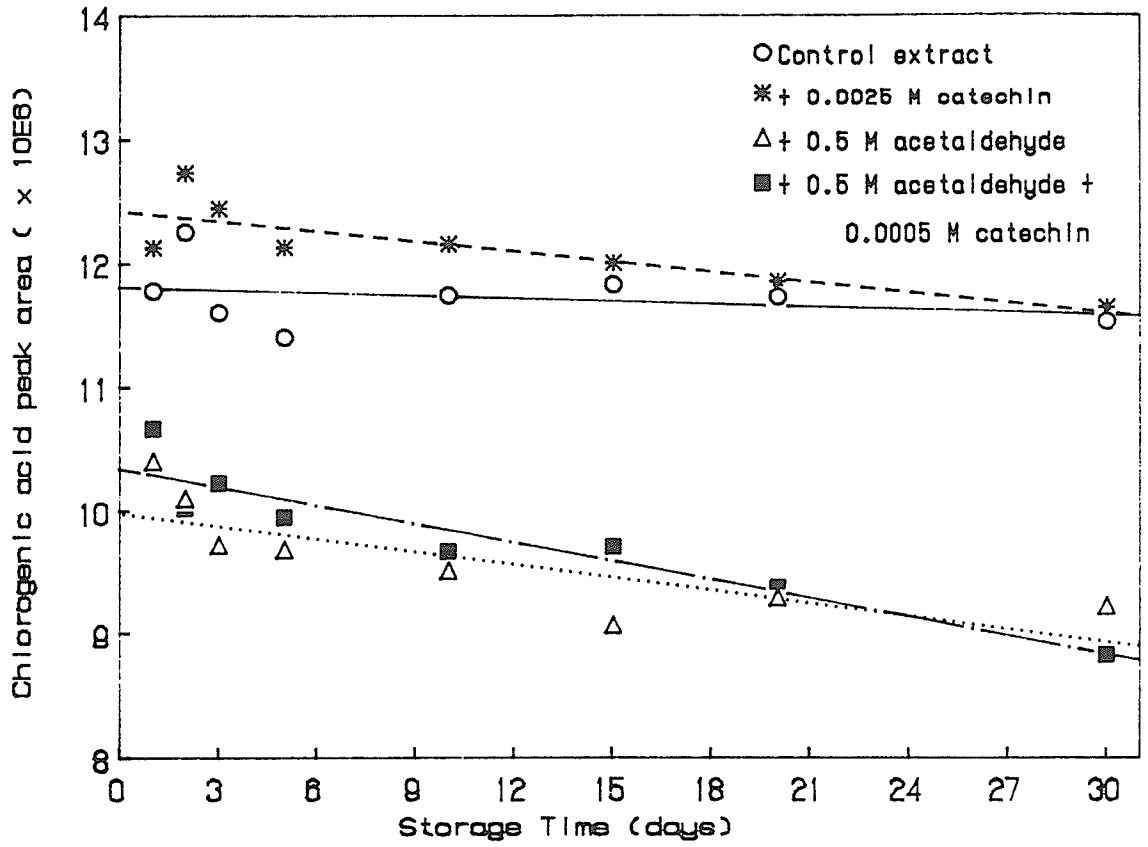

Figure 36. Effect of acetaldehyde and catechin on the HPLC peak area of chlorogenic acid in saskatoon berry alcoholic extracts stored at 23 ± 1°C in the dark. S.E. between any two treatment means at a given time ± 0.4 x 10^6.

supports the hypothesis that other extract phenolic components are more reactive than catechin with acetaldehyde. Anthocyanins and other phenolic compounds may further polymerize onto the chlorogenic acid-acetaldehyde complex.

Precipitation did not occur in any of the methanol extracts, as evidenced by the lack of absorbance at 700 nm (Figure 30). This is in contrast to the aqueous berry extracts which displayed precipitation in colour intensified samples containing higher levels of acetaldehyde (Figure 24). The lack of turbidity in the colour enhanced alcohol extracts can be attributed to the greater solubility of augmented polymers in alcohol, since methanol is a stronger solvent than water for phenolic compounds (Metivier et al., 1980). Also, the acetaldehyde/anthocyanin molar ratio for the alcohol extracts was approximately 64 % lower than that of the aqueous extracts (Table 5).

Browning occurred in methanolic extracts which contained acetaldehyde, as shown by an increase in absorbance at 420 nm (Figure 37; Appendix 21). This effect was also seen in the cyanidin 3-glucoside solution containing both acetaldehyde and catechin (Figure 18) and the aqueous extracts containing acetaldehyde (Figure 25) and has been explained previously (section 4.2.2).

On storage, the hue angle (θ) remained constant in the control methanol extract and extracts containing only catechin (Figure 38; Appendix 22). As for the aqueous berry extracts, the presence of

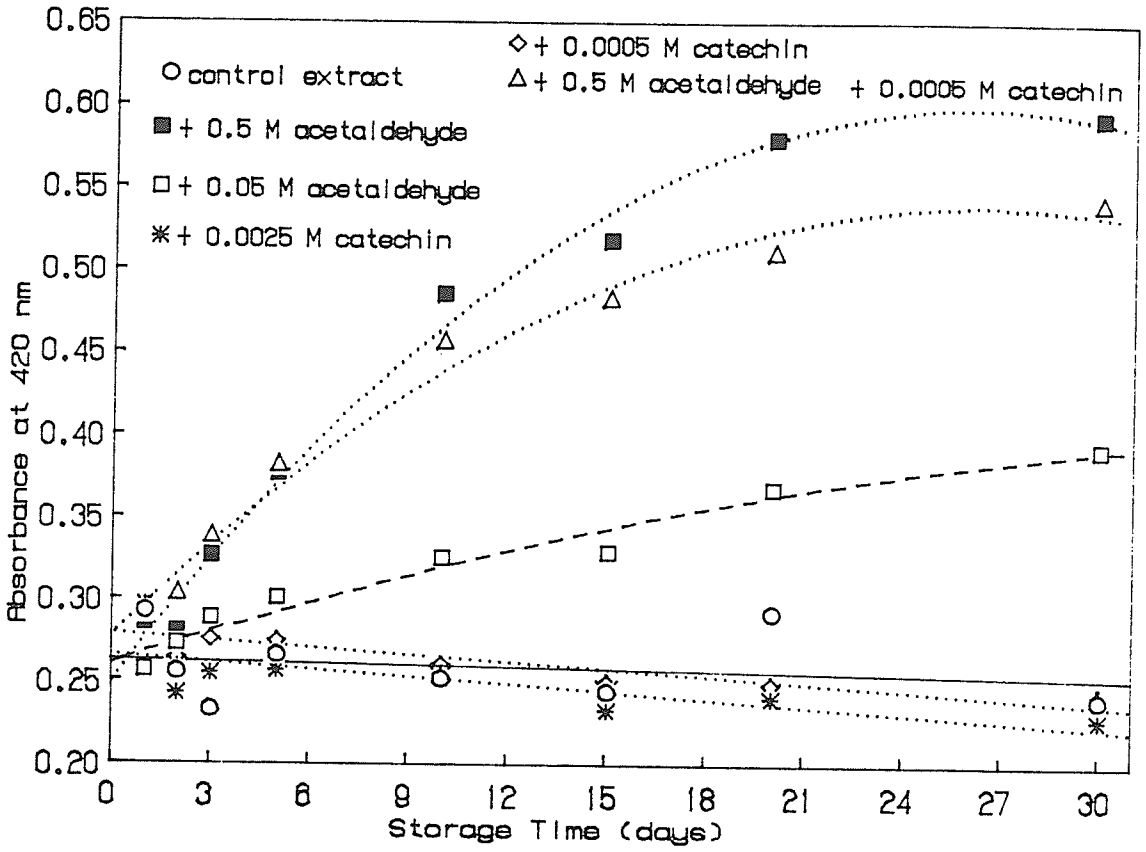

Figure 37. Effect of acetaldehyde and catechin on absorbance at 420 nm of saskatoon berry alcoholic extracts at 23 ± 1°C in the dark. S.E. of any two treatment means at a given time ± 0.02.

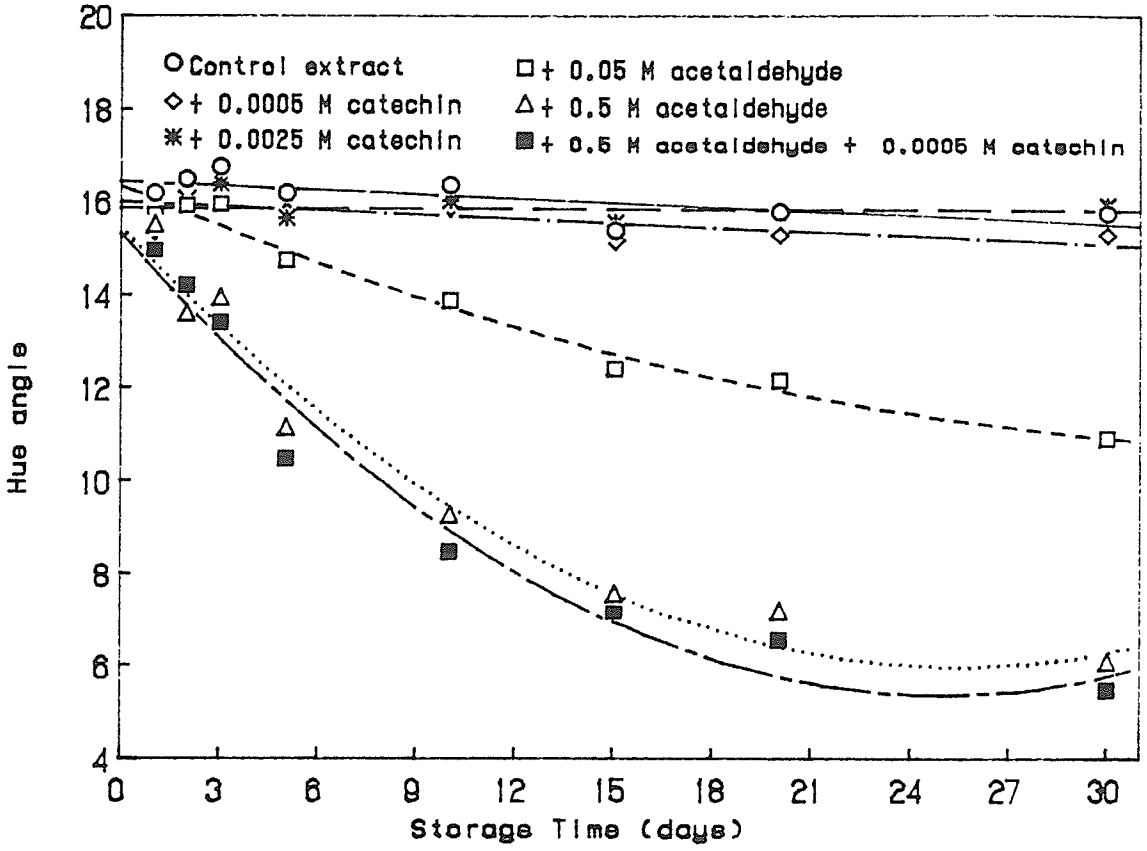

Figure 38. Effect of acetaldehyde and catechin on hue angle of saskatoon berry alcoholic extracts at 23 ± 1°C in the dark. S.E. between any two treatment means at a given time ± 0.8.

acetaldehyde caused the θ to decrease during storage with the magnitude of change increasing with the initial concentration of acetaldehyde in the extract. Also, the θ accurately reflected visual observations on the extracts (Figure 39).

The results indicate that colour intensification through acetaldehyde-induced condensation of anthocyanins and phenolic compounds may occur in saskatoon berry alcoholic systems such as wines and liqueurs. In wine, acetaldehyde is produced from microbial action during fermentation and more slowly from ethanol by coupled oxidation of certain phenolic compounds (Wildenradt and Singleton, 1974; Jones et al., 1986). The level of acetaldehyde found in grape wines has been reported to be approximately 4.1×10^{-3} M (Jones et al., 1986), considerably less than the quantity added to the alcohol extracts studied. However, it is likely that similar reactions would occur, although more slowly. The acetaldehyde-induced colour intensification should impart a purple tint to saskatoon berry wines and liqueurs. Whether the colour of the product remains enhanced or is diminished by precipitation of the complex polymers will depend on the relative concentrations of the anthocyanins, other phenolic constituents and free acetaldehyde in the system.

Figure 39. Saskatoon berry alcoholic extracts after 10 days at 23 ± 1°C in the dark: Pure (control) extract (A); extract + 5 x 10^{-1} M acetaldehyde (B); extract + 2.5 x 10^{-3} M catechin (C); and extract + 5 x 10^{-1} M acetaldehyde + 5 x 10^{-4} M catechin.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The influence of saskatoon berry ripeness on anthocyanin level and other physical and chemical parameters was investigated. In maturing from the red to dark-purple colour stage anthocyanins, phenolic compounds, sugars, sugar-acid ratios and anthocyanin-phenolic compound ratios increase with ripening of the berry, whereas pH and titratable acidity show little change. Since large sugar-acid ratios and high anthocyanin levels are favourable parameters in processed fruit products such as jam, jelly ,juice and wine, saskatoon berries should be harvested after they reach the purple colour stage.

The anthocyanin content of saskatoon berries was shown to be comparable to or higher than that of other commercial fruits such as blueberry and cranberry. Therefore, the use of saskatoon berries as a source of natural colourants could be considered. Anthocyanin levels in saskatoon berries vary with the cultivar and those which contain the highest pigment levels are Honeywood, Northline and Porter.

Favourable parameters reported to increase the stability of anthocyanins, specifically, high levels of phenolic compounds and acidity and low pH and sugar-acid ratios also provide more favourable conditions for their synthesis. The measurement of one or more of these

parameters may be used to predict anthocyanin content in saskatoon berries.

This study has shown that acetaldehyde-induced condensation of anthocyanins and catechin-type phenolic constituents produces new colour intensified compounds. The extent of reaction and thus, colour enhancement may be controlled by adjustment of the acetaldehyde-anthocyanin ratio. The augmentation of anthocyanin colour has implications in the colouring of foods and beverages. If further studies confirm that the colour intensified compounds are stable to conditions of food processing and preparation, they may be feasible replacements for synthetic food dyes.

5.2 RECOMMENDATIONS

This study has determined the relationship of anthocyanins with colour and various chemical parameters of saskatoon berries and has characterized colour intensification of anthocyanin solutions in aqueous and alcohol environments. In order to increase further the potential of saskatoon berries and anthocyanins, it is recommended that future studies include the following:

1) Characterize any differences in types and levels of specific anthocyanins and other phenolic compounds among the saskatoon berry cultivars by determining the HPLC profiles of the pigment crude extracts.

2) Determine the level of acetaldehyde in ripe saskatoon berries as well as processed products from saskatoon berry. The relative contribution of each colour intensification/degradation mechanism can then be estimated.

3) Prepare, isolate and characterize the new, intensely coloured compounds formed in the saskatoon berry extracts which contain added acetaldehyde.

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APPENDICES

Appendix 1. Preparation of Reagents for Measurement of Colour.

Buffers for determination of anthocyanin content

pH 1.0 buffer:
125 mL 0.2 N KCl
+ 385 mL 0.2 N HCl
+ 490 mL distilled water

pH 4.5 buffer:
440 mL 1.0 M sodium acetate
+ 200 mL 1.0 N HCl
+ 360 mL distilled water

The pH of the buffers was adjusted as required to obtain final pH values of 1.0 and 4.5.

Twenty percent potassium metabisulfite solution for determination of polymeric colour

Two g of $K_2S_2O_5$ were dissolved in 10 mL distilled water. This reagent must be prepared daily; otherwise, it develops a yellow colour which will contribute to absorbance.

Reference: Wrolstad, 1976.

Appendix 2. Calculation of Anthocyanin Content in Saskatoon Berries

Steps:

1) T.A. = A x DF

where: T.A. = Total absorbance
A = the absorbance of the diluted sample
DF = dilution factor

2) ΔA = T.A. pH 1.0 - T.A. pH 4.5

where ΔA = change in absorbance between pH 1.0 and pH 4.5

3) $$TAcy = \frac{\Delta A}{\frac{E^{1\%}_{1cm}}{10}}$$

where: TAcy = total anthocyanin content
$E^{1\%}_{1cm}$ = molar extinction coefficient
($E^{1\%}_{1cm}$ cyanidin 3-glucoside =765)

Example:

Five hundred mL of methanol extract were obtained from 100 g dark 1983 Honeywood saskatoon berries. 2.0 mL of the extract was diluted to 100 mL with the respective buffers.

Absorbance values were as follows:

A_{510nm} pH 1.0 = 0.55 A_{510nm} pH 4.5 = 0.07
A_{700nm} pH 1.0 = 0.01 A_{700nm} pH 4.5 = 0.01

T.A. pH 1.0 = (0.55 - 0.01) x 500/2 x 100 = 13500
T.A. pH 4.5 = (0.07 - 0.01) x 500/2 x 100 = 1500

ΔA = 13500 - 1500 = 12000

$$TAcy = \frac{12000}{765/10}$$

= 12000/76.5
= 156.86 mg/100 g berries

Reference: Fuleki and Francis, 1968b.

Appendix 3. Sample Calculation for Total Phenolic Compounds

Steps:

$$T = \frac{\text{mg tannic acid}}{\text{mL extract}} \quad \text{(obtain from standard curve)}$$

$$\% \text{ phenolic compounds} = T \times \frac{1\ g}{1000\ mg} \times \frac{1000\ mL\ extract}{150\ g\ berries} \times 100$$

$$= T \times 0.667$$

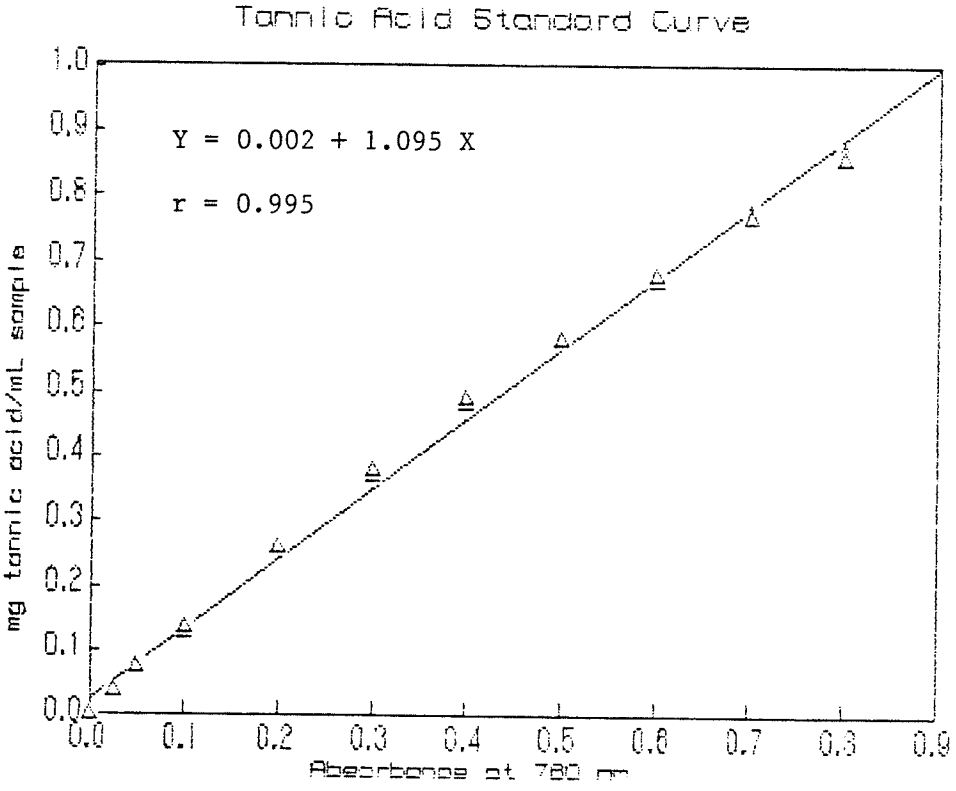
Example:

Observed absorbance = 0.45

Therefore; from standard curve T = 0.391 mg tannic acid/mL extract

$$\% \text{ phenolic compounds} = 0.391 \times 0.667$$
$$= 0.26\%$$

Reference: AOAC, 1980.



Appendix 4. Preparation of 0.2 M sodium acetate - 1 % phosphoric acid buffer.

All reagents were prepared in distilled water. Approximate volumes of the buffer components are shown below. Minor volume adjustment may be necessary to obtain a final pH of 3.5.

Reagent	Volume (mL)
0.2 M sodium acetate	338.9
1 % o-phosphoric acid	661.0
propionic acid	0.1
	1000.0

Appendix 5. Chemical parameters of saskatoon berry cultivars not separated into maturity levels.

Parameter	Cultivar							
	Beaverlodge		Parkhill		Success		Northline	Smoky
	1983	1984	1983	1984	1983	1984	1983	1984
Anthocyanins (mg/100 g)	56.1±1.9[a]	76.8±3.3	86.9±3.9	66.5±5.6	81.2±2.5	63.2±5.0	107.1±2.3	86.2±5.0
Anthocyanin DI	1.01±0.02	0.96±0.01	1.00±0.01	0.97±0.01	0.99±0.00	0.95±0.03	0.99±0.01	0.96±0.01
Total phenolics (% tannic acid)	0.27±0.01	0.27±0.01	0.32±0.03	0.30±0.03	0.29±0.01	0.25±0.01	0.36±0.00	0.32±0.02
Anth/Ph ratio	0.21±0.01	0.28±0.01	0.27±0.03	0.22±0.03	0.28±0.01	0.25±0.03	0.30±0.01	0.27±0.02
pH	4.4±0.0	4.2±0.0	4.4±0.0	4.0±0.0	4.4±0.0	4.1±0.0	3.8±0.0	4.0±0.0
Titratable acidity (% malic)	0.27±0.00	0.34±0.02	0.32±0.02	0.43±0.01	0.27±0.01	0.41±0.01	0.64±0.01	0.41±0.02
Total solids (% wt.)	19.9±0.2	20.8±0.1	19.6±0.5	22.1±0.2	16.5±0.2	21.3±0.1	20.3±0.1	21.3±0.3
Soluble solids (% sucrose)	14.4±0.4	14.4±0.0	12.6±0.4	16.2±0.6	10.4±0.4	17.3±0.7	13.5±0.1	16.6±0.9
SS/Ac	53.5±1.5	42.7±2.0	39.1±2.1	37.7±1.2	37.9±1.5	42.4±1.6	21.0±0.1	40.5±3.8

[a] Standard deviation of the mean of 3 determinations.

Appendix 6. Colour parameters of saskatoon berry cultivars not separated into maturity levels.

Parameter	Cultivar							
	Beaverlodge		Parkhill		Success		Northline	Smoky
	1983	1984	1983	1984	1983	1984	1983	1984
Colour density	4.00±0.40[a]	3.60±0.44	6.58±0.66	7.20±1.39	6.63±0.28	4.27±0.58	7.20±0.29	4.36±0.03
Browning	2.65±0.20	2.27±0.26	3.96±0.62	4.62±0.89	4.05±0.14	2.78±0.33	3.22±0.11	2.65±0.04
Polymeric colour	2.78±0.17	1.80±0.24	4.25±0.94	5.87±1.16	4.29±0.17	2.96±0.34	1.93±0.18	1.96±0.10
% tannin	69.6±2.8	49.9±2.6	64.1±7.5	81.4±0.4	64.9±4.6	69.5±3.8	26.9±2.9	44.9±2.5
Berry L	10.6±0.0	15.8±0.6	13.6±0.2	16.6±1.2	16.6±0.8	17.3±0.7	10.4±0.1	16.7±0.5
Berry a	3.7±0.1	7.6±0.6	7.6±0.5	9.4±0.4	8.3±0.2	9.7±0.8	3.7±0.4	7.5±0.4
Berry b	1.1±0.2	2.1±0.1	2.3±0.1	2.7±0.1	1.9±0.1	2.8±0.2	1.2±0.2	1.8±0.2
Berry hue angle	16.8±1.8	15.7±1.0	16.6±1.0	16.0±0.5	12.9±0.9	16.0±0.1	18.0±1.1	13.4±1.0
Extract[b] L	16.6±0.6	11.8±0.1	11.4±0.1	4.7±0.1	11.0±0.1	11.4±0.1	14.6±0.9	11.8±0.2
Extract a	9.0±0.6	6.6±0.2	4.7±0.0	5.7±0.3	4.6±0.3	5.7±0.1	9.0±1.4	6.4±0.2
Extract b	2.4±0.6	2.2±0.2	1.4±0.1	1.9±0.2	1.4±0.1	1.9±0.1	2.6±0.4	2.2±0.1
Extract hue angle	14.5±2.7	18.5±1.3	17.0±0.6	18.6±2.1	17.4±1.3	18.1±0.8	16.1±1.7	18.7±0.5

[a] Standard deviation of the mean of 3 determinations.
[b] Alcoholic extracts.

Appendix 7. Statistical summary of visible λmax of cyanidin 3-glucoside model systems stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	13.6417	2.18$^{N.S.}$
Treatment	3	294.3438	46.48****
Error 'a'	6	6.3333	
Day	7	22.0589	8.00****
Treatment x Day	21	33.6692	12.21****
Error 'b'	56	2.7574	
Total	95		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Cyanidin 3-glucoside (control)	N.S.	N.S.
Pigment + acetaldehyde	**	N.S.
Pigment + catechin	**	N.S.
Pigment + acetaldehyde + catechin	****	**

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 8. Statistical summary of absorbance at visible λmax of cyanidin 3-glucoside model systems stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	2.5179	384.38****
Treatment	3	0.1114	17.01***
Error 'a'	6	0.0066	
Day	7	0.0010	0.95N.S.
Treatment x Day	21	0.0107	10.51****
Error 'b'	56	0.0010	
Total	95		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Cyanidin 3-glucoside (control)	N.S.	N.S.
Pigment + acetaldehyde	N.S.	N.S.
Pigment + catechin	N.S.	N.S.
Pigment + acetaldehyde + catechin	N.S.	N.S.

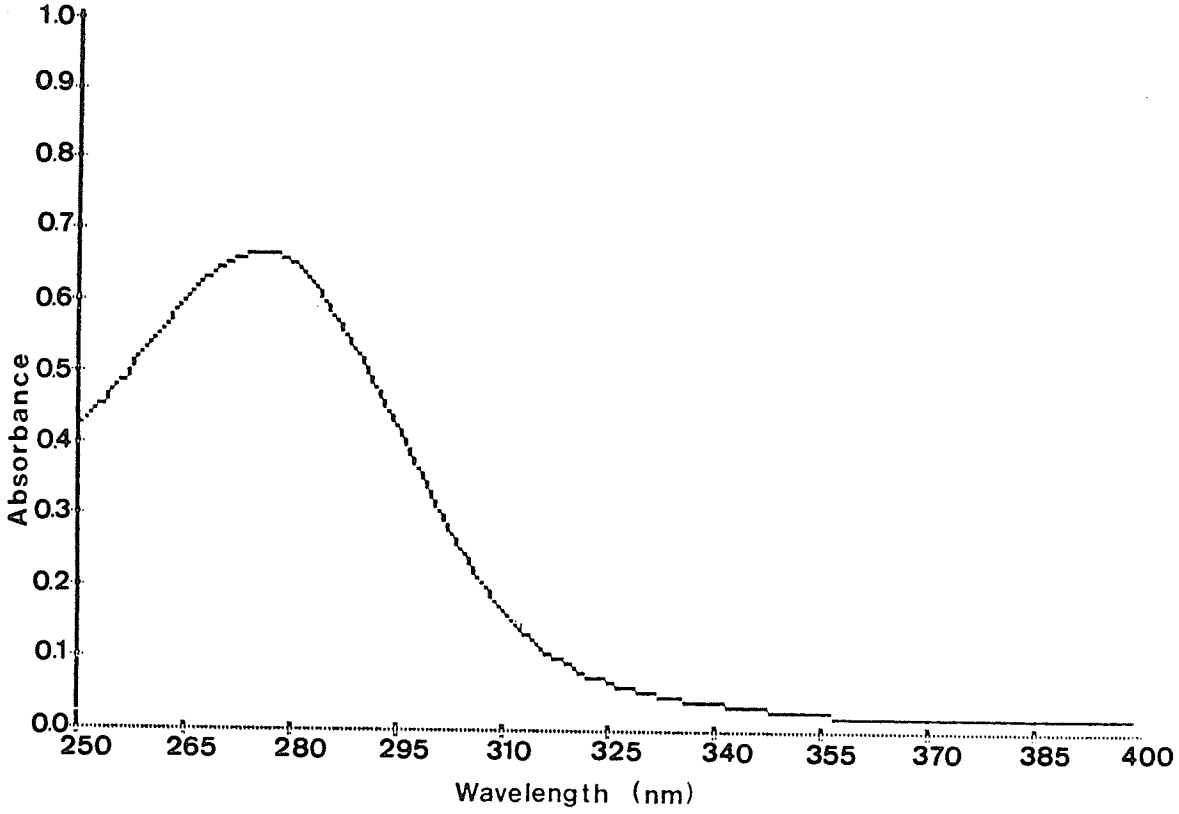
N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 9. Linear and quadratic effects of storage time on new HPLC augmentation peak area for cyanidin 3-glucoside model system containing acetaldehyde and catechin.

Peak Number	Significance	
	Linear effect	Quadratic effect
1	****	*
2	****	***
3	****	*
4	****	N.S.
5	***	N.S.
6	***	**
Total peak area	****	N.S.

N.S. = Not significant; * = $P < 0.10$; ** = $P < 0.05$; *** = $P < 0.01$; **** = $P < 0.001$.

Appendix 10. UV spectrum of acetaldehyde.



Appendix 11. Statistical summary of the effect of acetaldehyde and catechin on absorbance at 420 nm of cyanidin 3-glucoside model systems stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	0.3340	141.91****
Treatment	3	0.0368	15.66***
Error 'a'	6	0.0024	
Day	7	0.0034	5.03****
Treatment x Day	21	0.0056	8.22****
Error 'b'	56	0.0007	
Total	95		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Cyanidin 3-glucoside (control)	N.S.	N.S.
Pigment + acetaldehyde	N.S.	N.S.
Pigment + catechin	N.S.	N.S.
Pigment + acetaldehyde + catechin	***	N.S.

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 12. Statistical summary of the effect of acetaldehyde and catechin on visible λmax of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	29.9915	6.49**
Treatment	5	496.9692	107.57****
Error 'a'	10	4.6199	
Day	7	88.0778	24.82****
Treatment x Day	30	44.1829	12.45****
Error 'b'	74	3.5490	
Total	128		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	***	N.S.
Extract + 1.26 x 10^{-1} M acetaldehyde	***	****
Extract + 1.26 x 10^{-2} M acetaldehyde	****	N.S.
Extract + 6.48 x 10^{-4} M catechin	****	***
Extract + 1.62 x 10^{-4} M catechin	**	*
Extract + 1.26 x 10^{-1} M acetaldehyde + 1.62 x 10^{-4} M catechin	****	****

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 13. Statistical summary of the effect of acetaldehyde and catechin on absorbance at visible λmax of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	0.0812	101.09****
Treatment	5	0.0341	41.97****
Error 'a'	10	0.0008	
Day	7	0.0174	37.57****
Treatment x Day	30	0.0032	6.88****
Error 'b'	74	0.0005	
Total	128		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	****	N.S.
Extract + 1.26 x 10^{-1} M acetaldehyde	N.S.	**
Extract + 1.26 x 10^{-2} M acetaldehyde	N.S.	N.S.
Extract + 6.48 x 10^{-4} M catechin	****	N.S.
Extract + 1.62 x 10^{-4} M catechin	****	N.S.
Extract + 1.26 x 10^{-1} M acetaldehyde + 1.62 x 10^{-4} M catechin	N.S.	**

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 14. Statistical summary of the effect of acetaldehyde and catechin on absorbance at 700 nm of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	0.0024	$1.79^{N.S.}$
Treatment	5	0.0472	35.30^{****}
Error 'a'	10	0.0013	
Day	7	0.0396	31.51^{****}
Treatment x Day	30	0.0107	8.48^{****}
Error 'b'	74	0.0013	
Total	128		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	**	N.S.
Extract + 1.26 x 10^{-1} M acetaldehyde	****	****
Extract + 1.26 x 10^{-2} M acetaldehyde	N.S.	N.S.
Extract + 6.48 x 10^{-4} M catechin	**	N.S.
Extract + 1.62 x 10^{-4} M catechin	N.S.	N.S.
Extract + 1.26 x 10^{-1} M acetaldehyde + 1.62 x 10^{-4} M catechin	****	N.S.

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 15. Statistical summary of the effect of acetaldehyde and catechin on absorbance at 420 nm of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	0.0180	45.00****
Treatment	5	0.0728	182.00****
Error 'a'	10	0.0004	
Day	7	0.0254	127.00****
Treatment x Day	30	0.0049	24.50****
Error 'b'	74	0.0002	
Total	128		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	N.S.	N.S.
Extract + 1.26 x 10^{-1} M acetaldehyde	****	****
Extract + 1.26 x 10^{-2} M acetaldehyde	***	**
Extract + 6.48 x 10^{-4} M catechin	***	N.S.
Extract + 1.62 x 10^{-4} M catechin	N.S.	N.S.
Extract + 1.26 x 10^{-1} M acetaldehyde + 1.62 x 10^{-4} M catechin	****	****

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 16. Statistical summary of the effect of acetaldehyde and catechin on hue angle of saskatoon berry aqueous extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	29.8185	17.79***
Treatment	5	404.0757	241.13****
Error 'a'	10	1.6757	
Day	7	23.0074	48.72****
Treatment x Day	35	25.8369	54.71****
Error 'b'	84	0.4723	
Total	143		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	****	*
Extract + 1.26 x 10^{-1} M acetaldehyde	****	****
Extract + 1.26 x 10^{-2} M acetaldehyde	****	**
Extract + 6.48 x 10^{-4} M catechin	****	N.S.
Extract + 1.62 x 10^{-4} M catechin	****	**
Extract + 1.26 x 10^{-1} M acetaldehyde + 1.62 x 10^{-4} M catechin	****	****

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 17. Statistical summary of the effect of acetaldehyde and catechin on visible λmax of saskatoon berry alcohol extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	0.1460	0.11$^{N.S.}$
Treatment	5	85.3830	64.24****
Error 'a'	10	11.3292	
Day	7	26.1753	16.44****
Treatment x Day	35	9.1290	5.73****
Error 'b'	84	1.5921	
Total	143		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	***	N.S.
Extract + 5 x 10^{-1} M acetaldehyde	****	***
Extract + 5 x 10^{-2} M acetaldehyde	****	N.S.
Extract + 2.5 x 10^{-3} M catechin	N.S.	***
Extract + 5 x 10^{-4} M catechin	N.S.	N.S.
Extract + 5 x 10^{-1} M acetaldehyde + 5 x 10^{-4} M catechin	****	****

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 18. Statistical summary of the effect of acetaldehyde and catechin on absorbance at visible λmax of saskatoon berry alcohol extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	0.1014	12.59^{***}
Treatment	5	0.3580	44.45^{****}
Error 'a'	10	0.0080	
Day	7	0.0132	2.41^{**}
Treatment x Day	35	0.0422	7.72^{****}
Error 'b'	84	0.0055	
Total	143		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	**	N.S.
Extract + 5 x 10^{-1} M acetaldehyde	****	**
Extract + 5 x 10^{-2} M acetaldehyde	N.S.	N.S.
Extract + 2.5 x 10^{-3} M catechin	****	**
Extract + 5 x 10^{-4} M catechin	****	**
Extract + 5 x 10^{-1} M acetaldehyde + 5 x 10^{-4} M catechin	****	***

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 19. The pH of saskatoon berry alcoholic extracts during storage at 23 ± 1°C in the dark.

Day of storage	pH
0	3.50
2	3.50
3	3.55
5	3.65
7	3.75
10	3.80
15	3.85
20	3.90
30	4.00

Appendix 20. Statistical summary of the effect of acetaldehyde and catechin on HPLC peak area of chlorogenic acid of saskatoon berry alcohol extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	2.149×10^{13}	25.43^{***}
Treatment	3	3.957×10^{13}	46.82^{****}
Error 'a'	6	8.452×10^{11}	
Day	7	1.314×10^{12}	8.42^{****}
Treatment x Day	21	2.511×10^{11}	1.61^{*}
Error 'b'	55	1.560×10^{11}	
Total	94		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	N.S.	N.S.
Extract + 5×10^{-1} M acetaldehyde	***	N.S.
Extract + 2.5×10^{-3} M catechin	N.S.	N.S.
Extract + 5×10^{-1} M acetaldehyde + 5×10^{-4} M catechin	***	N.S.

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 21. Statistical summary of the effect of acetaldehyde and catechin on absorbance at 420 nm of saskatoon berry alcohol extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	0.0445	59.77****
Treatment	5	0.1559	209.29****
Error 'a'	10	0.0007	
Day	7	0.0295	39.93****
Treatment x Day	35	0.0119	16.08****
Error 'b'	84	0.0007	
Total	143		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	N.S.	N.S.
Extract + 5 x 10^{-1} M acetaldehyde	****	****
Extract + 5 x 10^{-2} M acetaldehyde	****	N.S.
Extract + 2.5 x 10^{-3} M catechin	**	N.S.
Extract + 5 x 10^{-4} M catechin	*	N.S.
Extract + 5 x 10^{-1} M acetaldehyde + 5 x 10^{-4} M catechin	****	****

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.

Appendix 22. Statistical summary of the effect of acetaldehyde and catechin on hue angle of saskatoon berry alcohol extracts stored at 23 ± 1°C in the dark for 20 days.

a: Analysis of variance

Source	df	mean square	F-value
Replications	2	15.38	5.99**
Treatment	5	180.38	70.24****
Error 'a'	10	2.57	
Day	7	49.14	68.44****
Treatment x Day	35	8.61	12.00****
Error 'b'	84	0.72	
Total	143		

b: Linear and quadratic effects of storage time

Treatment	Significance	
	Linear effect	Quadratic effect
Control extract	N.S.	N.S.
Extract + 5 x 10^{-1} M acetaldehyde	****	***
Extract + 5 x 10^{-2} M acetaldehyde	****	N.S.
Extract + 2.5 x 10^{-3} M catechin	N.S.	N.S.
Extract + 5 x 10^{-4} M catechin	N.S.	N.S.
Extract + 5 x 10^{-1} M acetaldehyde + 5 x 10^{-4} M catechin	****	****

N.S. Not significant; * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$.