AN ABSTRACT OF THE THESIS OF

Luis Enrique Rodriguez-Saona for the degree of Doctor of Philisophy in Food Science and Technology presented June 4, 1998. Title: The Potato: Composition, Non-Enzymatic Browning and Anthocyanins

Abstract approved: _____
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Chipping varieties and model systems were used to determine the role of potato constituents on chip color. Composition was evaluated by HPLC and chip color measured using a ColorQuest colorimeter. Reducing sugar (RS) content did not completely explain color quality when present in low concentrations (<60 mg/100g). Levels of ascorbic acid, glutamine and a chlorogenic acid isomer, along with RS, showed high correlation with color. Sucrose was a poor estimator of chip color.

Model systems used leached potato slices infiltrated with solutions containing sucrose, RS, ascorbic, chlorogenic and amino acids. Linear association of RS with L* and hue angle and quadratic relationship with chroma of chips were found. Ascorbic acid affected chroma and hue at low RS levels while chlorogenic acid was not involved in color development.

Red potatoes (*Solanum tuberosum* and *Solanum stenotomum*) were evaluated as potential source of natural red colorant. Cultivars (33) were screened for anthocyanin content and qualitative composition. Monomeric anthocyanin content, determined by pH differential, ranged between 4 and 40 mg/100g fresh weight (fw) tuber. Varieties 5847-1 and ND04069-4 showed high anthocyanin content (>35 mg/100g). Anthocyanin composition was characterized by HPLC, spectral analysis and Mass Spectroscopy (MS). The major anthocyanin was pelargonidin-3-rutinoside-5-glucoside acylated with *p*-coumaric acid.

The presence of glycoalkaloids (α-solanine and α-chaconine) was detected by MS and quantified by HPLC. Varieties NDO4069-4 and 5847-1 showed glycoalkaloid

levels of 13 and 7 mg/100g fw, respectively. Glycoalkaloids were precipitated from pigment concentrates by alkaline treatment. The best results were obtained at pH 8.0 with 30% monomeric anthocyanin degradation and 90% glycoalkaloid precipitation.

The color and pigment stability of chemically related anthocyanin extracts (red-fleshed potatoes and radishes), the effect of pigment purity, and temperature were evaluated in model juices (pH 3.5). Color (CIELch) and anthocyanin degradation was monitored for 65 wks of storage. All model juices showed color similar to FD&C Red # 40. Excellent stability was obtained with all treatments in refrigeration. Anthocyanin structure and extraction method affected pigment stability. At 25°C, higher stability was obtained on juices colored with chemically purified radish anthocyanins (22 wk half-life) and lowest with potato vegetable juice (10 wk half-life).

The Potato: Composition, Non-Enzymatic Browning and Anthocyanins

by

Luis Enrique Rodriguez-Saona

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed June 4, 1998
Commencement June, 1999

<u>Doctor of Philosophy</u> thesis of <u>Luis Enrique Rodriguez-Saona</u> presented on <u>June 4,</u>
<u>1998</u>

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Luis Enrique Rodriguez-Saona

ACKNOWLEDGEMENTS

CONTRIBUTION OF AUTHORS

Dr. Ronald E. Wrolstad was involved in the design, data analysis and writing of each chapter. Cliff Pereira was involved in the experimental design and data analysis of chapter 4 and the data analysis and interpretation of chapter 6. Experimental design, methodology, and data analysis in chapters 5 and 7 were done with the assistance of M. Mónica Giusti.

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Con mucho amor se lo dedico a

Mónica y Danella

THE POTATO: COMPOSITION, NON-ENZYMATIC BROWNING AND ANTHOCYANINS

CHAPTER 1: INTRODUCTION

Potato is native to the western part of South America (Perú, Bolivia, Ecuador and Chile) and for centuries was the primary food source of the Andean people. Upon introduction of the potato plant into Europe by the Spanish conquerors in the 16th century, it became an important food crop in Europe and from there it spread to all over the world.

Potatoes play an important role in human nutrition around the world because of the significant amounts consumed and its composition, being an excellent source of carbohydrates, essential amino acids, vitamins, and minerals. Potato is ranked as one of the four major food crops in the world, along with rice, wheat and maize. Attributes such as high yield, low cost of production, adaptability to a wide variety of soils and climates, secure production under stress or short crop seasons, wide variety of cultivars, acceptance as daily food, and excellent nutritive value, have made the potato tuber the mainstay in the diet of people in many parts of the world (Leszcynski, 1989).

Since the 1960s, the areas planted with potato and their yield have increased dramatically, especially in developing countries. The major producers of potato are the European countries (Soviet Union, Poland, Netherlands, UK), China and the US. In developing countries, the potato is becoming an increasingly important source of food, rural employment, and income for the growing populations and it is projected that by the year 2000, these countries will produce more than one-third of the world's potato crop. For the period 1991-93, the world potato production was estimated at 275,355,000 MT, with a cultivated area of 18,133 Ha and an estimated yield of 15 MT/Ha.

The United States is among the world's highest producer of potatoes with an annual production of 19,227,000 MT and yields of 36 MT/Ha. Potato is a very versatile commodity, being commercialized as fresh produce as well as in multiple processed products i.e. dehydrated potatoes, starch production, chips, French fries, and novelty red potatoes for salads. The increased demand in processed potato products has been the

driving force for the continued expansion in potato production in the United States (CIP, 1998), with more than half of the annual potato crop being utilized in the processing of French fries and potato chips.

The first part of this thesis research involves the effect of different potato constituents in color development of potato chips. The color and flavor of potato chips and the factors affecting these attributes are of major concern for the potato chip industry. The desirable yellow-brown color and distinctive flavor of potato chips are a consequence of Maillard reactions during frying. Excessive darkening and development of off-flavors produce unacceptable potato products. Color and flavor is determined by the chemical composition of the tubers and its control is difficult because tuber composition will depend on variety, growing conditions, cultural practices, maturity at harvest, post-harvest handling, conditioning techniques and compositional changes during storage, among other factors (Roe and Faulks, 1990,1991; Smith, 1987).

The potato industry relies on reducing sugar levels, often in conjunction with a fry test, as predictive tests of the suitability of the material for processing. Processors and researchers have found that potatoes can develop a fry color different from that expected from its reducing sugar content (Khanbari and Thompson, 1993; Fuller and Hughes, 1984; Habib and Brown, 1956). Amino acids are important substrates in the Maillard reaction but their participation in chip color development has been reported as marginal since their concentration is rarely a limiting factor (Marquez and Añon, 1986). However, the amino acid composition of the potato tubers could play an important role in color development (Hughes and Fuller, 1984), especially due to the accumulation of amino acids such as glutamine, lysine, glycine, arginine with their strong browning potentials (Khanbari and Thompson, 1993). Other potato constituents that might be involved in color development of chips include ascorbic acid reacting with amino acids during frying, phenolic acids (i.e. chlorogenic acid) oxidation which is favored by alkaline pH, and organic acids (Cilliers and Singleton, 1989; Smith, 1987; Wolfrom et al., 1974).

The objectives of this part of the study were to develop and evaluate analytical methods for measuring concentrations of compounds which play an important role in browning of potato chips; to determine the contribution of different potato constituents to color quality; and to determine the individual contribution of ascorbic acid, amino acids (glutamine and asparagine), phenolic acids (chlorogenic acid), sucrose and reducing sugars to the development of potato chip color by carefully controlling their concentrations through the use of model systems.

The second part of this thesis research involved the evaluation of different potato breeding clones as potential colorants. Breeding colored potato cultivars as novelty products have yielded tubers with a wide range of flesh and skin coloration. Investigations on potatoes as source of colorants have been focused on cultivars which are very rich in anthocyanin pigments but purple in color. Cultivars such as Urenika, Congo and Negresse have been studied because of their highly pigmented flesh, being their major anthocyanins malvidin and petunidin derivatives (Lewis, 1996; Harborne, 1960). Since natural red colorants, as alternatives to FD&C Red # 40 or the banned FD&C Red # 2, are of greatest demand by the industry (LaBell, 1990), our research interest was directed towards a red-fleshed potato selection with high pigment content and containing pelargonidin (orange/red hue) as major the aglycon. A limitation to the use of potatoes as a source of colorant is the presence of alkaloids, potent neuro-toxins, that if present in the anthocyanin extracts could result in a potential health hazard.

We characterized and measured the concentration of anthocyanin pigments in red-fleshed potatoes as potential red food colorants. Different cultivars were evaluated in terms of total pigment content, their qualitative anthocyanin patterns and phenolic compositions. Comparisons were also made between potato skin and flesh with respect to anthocyanin pigment and phenolic composition. The color potato anthocyanin extract was compared to FD&C Red # 40 and radish anthocyanin extracts. The identity and content of glycoalkaloids (α-solanine and α-chaconine) in potato varieties and anthocyanin extracts were determined. Alkaline treatments were evaluated for the

precipitation of glycoalkaloids from anthocyanin containing extract. Finally, color and pigment stability of two chemically related anthocyanins (red-fleshed potato and red radish), obtained using 2 extraction methods (chemical purification vs physical juice processing), in a juice model system over 65 wks of storage at 25°C and 2°C were evaluated.

REFERENCES

Cilliers, J.J.L. Singleton, V.L. 1989. Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. J. Agric. Food Chem. 37:890-896.

CIP. 1998. Potato production. In *Post-harvest and marketing*.
http://www.cipotato.org/ph&mkt

Fuller, T.J. and Hughes, J.C. 1984. Factors influrncing the relationships between reducing sugars and fry colour of potato tubers of cv. Record. J. Food Technol. 19:455-467.

Giusti, M.M. and Wrolstad, R.E. 1996b. Radish anthocyanin extract as a natural red colorant for maraschino cherries. J. Food Sci. 61:688-694.

Habib, A.T. and Brown, H.D. 1956. Factors influencing the color of potato chips. Food Tech. 10(7):332-336.

Harborne, J.B. 1960. Plant polyphenols: 1. anthocyanin production in the cultivated potato. Biochem. J. 74: 262-269.

Hughes, J.C. and Fuller, T.J. 1984. The effect of nitrogen during growth on sugar content and fry colour of cv. Record during extended storage at 10°C. Abstracts of conference papers of the 9th Triennial Conference of the European Association of Potato Research.

Khanbari, O.S. and Thompson, A.K. 1993. Effects of amino acids and glucose on the fry colour of potato crisps. Potato Research 36:359-364.

LaBell, F. 1990. Technology diversifies use of natural colors. New cabbage coloring extends stability. Food Processing. 51(4):69-70.

Leszczynski, W. 1989a. Introduction. In *Potato Science and Technology*. Chp. 2. G. Lisinska and W. Leszczynski Eds. Elsevier Applied Science. London. Great Britain.

Lewis, C.E. 1996. Biochemistry and regulation of anthocyanin synthesis in potato and other tuber-bearing *Solanum* species. Ph. D. Thesis. Department of Plant and Plant Microbial Science, University of Canterbury, Christchurch, New Zealand.

Marquez, G. and Añon, M.C. 1986. Influence of reducing sugars and amino acids in the color development of fried potatoes. J. Food Sci. 51:157-160.

Roe, M.A. and Faulks, R.M. 1991. Color development in a model system during frying: Role of individual amino acids and sugars. J. Food Sci. 56:1711-1713.

Smith, O. 1987. Potato chips. In *Potato processing*. 4th edition. W.F. Talburt and O. Smith Eds. The AVI Publishing Co., Inc. Wetport, CT.

Wolfrom, M.L.; Kashimura, N. and Horton, D. 1974. Factors affecting the Maillard browning reaction between sugars and amino acids. Studies on the non-enzymic browning of dehydrated orange juice. J. Agric. Food Chem. 22:796-800.

CHAPTER 2. PART I
COMPOSITIONAL FACTORS THAT INFLUENCE THE NON-ENZYMIC BROWNING OF POTATO CHIPS AND PRODUCTION OF AN ANTHOCYANIN-CONTAINING RED COLORANT

THE CULTIVATED POTATO SPECIES

Potato, as the genus *Solanum* includes over 2000 species distributed throughout the world. Only eight species (Fig. 2.1) are used in cultivation while all others are wild species. Wild potatoes are widely distributed in tropical and subtropical regions, and have a wide range of adaptation to altitude (ranging from sea level to over 4000 m), temperature regimes, day lengths, water supply, and ecological diversity. Wild potatoes considerably augment the gene pool available to the potato breeder and provide important traits like resistance to late blight (*S. demissum*), viruses (*S. acaule* and *S. stoloniferum*), cyst nematodes (*S. vernei, S. kurtzianum* and *S. multidissectum*), and frost (*S. acaule* and *S. megistacrolobum*), however, they often contain unacceptable high levels of alkaloids (Hawkes, 1990; Burton, 1989).

Potato tubers and roots have formed part of the diet of the Andean people for several thousands of years. Ancient food remains from high Chilca canyon (Lima, Perú) was dated to about 7000 BC (Hawkes, 1990). The area of origin and diversity of the cultivated potato lies in the central Andes of Perd and Bolivia, and it is assumed that *S. stenotomum* is the most primitive cultivated specie (Hawkes, 1990).

The most important event in cultivated potato evolution was the hybridization between *S. stenotomum* and *S. sparsipilum* (wild potato) to form *S. tuberosum* which is the most productive and widespread of the cultivated potato species.

Most of the cultivated potatoes originated over centuries of hybridization in the South American highlands giving rise to the present mixture of cultivated species. According to Burton (1990) the cultivation of different potato species depended on the locality.
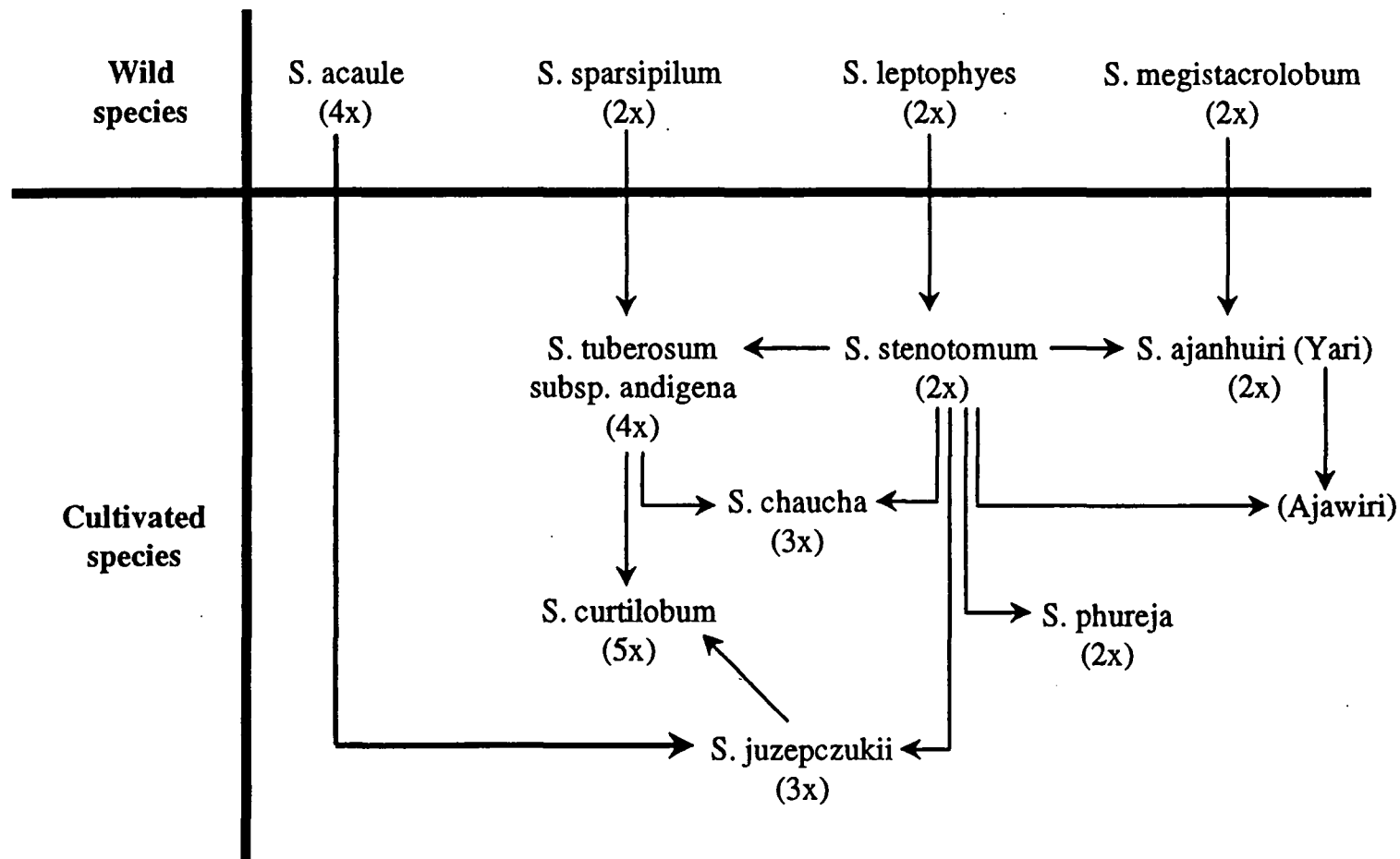
Figure 2.1: Evolutionary relationship of cultivated potatoes and their ploidy levels (n=12)

The cultivation of *S. ajanhuiri*, *S. juzepczukii* and *S. curtilobum* was favored in places with frost and hail while in more favorable localities the production of *S. tuberosum* subsp. *andigena* was chosen for yield and eating quality.

The propagation of potatoes can be either generative from seeds (True Potato Seed, TPS) or vegetative from tubers (Lesczczynski, 1989a). Uniformity of the clones is very difficult to obtain by TPS because the species themselves are highly variable due to their complex ancestry and heterozygous nature; while the characteristics of any one variety are retained by using vegetative reproduction from tubers, unless mutation occurred (Burton, 1990; Jackson, 1987). Vegetative propagation to the present day is the normal method practiced by the Andean cultivators, but natural reproduction by true seed must have been frequent in the crop, resulting in much variability. The Andean farmer did not segregate cultivars since growing a mixture, with a range of resistances, will ensure at least some crop in case of a natural catastrophe may occur.

Potato Cytology

Potato species show polyploid series with a base chromosome number of 12 (Smith, 1927). Despite the presence of polyploids in the tuberous *Solanum* species the majority (about 75%) are diploid, though quite a large number of tetraploids and some hexaploids also occur (Hawkes, 1990). Triploids are normally derived from spontaneous crosses between diploids and tetraploids species while pentaploids are either the result of crosses between tetraploids and hexaploid species or diploids with hexaploids.

In general, diploids, tetraploids and hexaploids are sexually fertile and the odd-number polyploids (triploids and pentaploids) are sterile and are maintained entirely by vegetative propagation (Hawkes, 1990).

Cultivated potato species occur in a range of chromosome numbers from 24 (diploid), 36 (triploid), 48 (tetraploid) and 60 (pentaploid). The diploid specie *S. stenotomum*, the first cultivated specie, gave rise to the diploids *S. phureja* and *S. gonocalyx* by possible mutation and selection; the diploid *S. ajanhuiri* is a natural hybrid

between *S. stenotomum* and the wild diploid *S. megistacrolobum*; the triploid *S. juzepczukii* is a natural hybrid between *S. stenotomum* and the wild tetraploid *S. acaule*; the tetraploid *S. tuberosum* is an amphiploid hybrid between *S. stenotomum* and the wild diploid *S. sparsipilum*; the triplod *S. chaucha* is a natural hybrid between *S. stenotomum* and the wild diploid *S. tuberosum*; and the pentaploid *S. curtilobum* is a hybrid between *S. juzepczukii* and *S. tuberosum* (Burton, 1990).

Spread of the Potato

Potatoes are the mainstay in the diet of people in many parts of the world because of their high yield, low cost of production, adaptability to a wide variety of soils and climates, secure production under stress or short crop seasons, wide variety of cultivars, acceptance as daily food, and excellent nutritive value (Niederhauser, 1993; Talburt, 1987).

For centuries potatoes have served as the primary food source of the Indians inhabiting the Andean areas of what is now Perú, Chile, Bolivia and Ecuador. Potatoes were dehydrated by allowing them to freeze at night and to dry in the sun (freeze dried potatoes), making a product called *chuño*, that provided sustenance during droughts or product shortages. After the Spanish explorers arrived to the New World, the potato was quite widely distributed throughout South and Central America, Mexico and the southern part of United States (Brown, 1993; Talburt, 1987).

The potato was introduced into Europe by the Spanish in the 16th century. As a strange plant, it was subjected to nearly 100 years of botanical curiosity and slow acceptance as a food crop (Niederhauser, 1993).

Based on early herbarium specimens, the potatoes originally introduced to Europe resembles *S. tuberosum* subsp. *andigena* brought from the northern highlands of South America and that climatic and daylength conditions and selection of seedlings would have led in the course of time to form *S. tuberosum* subsp. *tuberosum*, which is distributed world-wide (Burton, 1990; Hawkes, 1990).

The spread of potatoes in Europe was due to the interplay of several factors,

being hunger during war the most important. Potato yielded better than cereals, grew on marginal soils, and its subterranean location prevented pilferage and destruction by armies. Potato become the "bread of the poor" of thousands of people suffering from starvation (Brown, 1993; Leszczynski, 1989b). The 18th century was the century of acceptance of the potato as a foodstuff throughout Europe, while the 19th century was the century of its ascendancy (Burton, 1989). During 1845 and 1846 the potato crop failed in Europe because of the attack by the fungus *Phytophthora infestans* (blight or late blight) and led to hunger, several deaths and a vast emigration. The potato crop emerged in Europe after 1859 thanks to breeding and selection of new potato varieties resistant to diseases (Burton, 1989; Leszczynski, 1989b). From Europe, the potato spread all over the world primarily through the colonial powers. Thus, in remarkably a brief period of 3 centuries, the potato attained its current role as one of the four major food crops in the world, along with rice, wheat and maize (Niederhauser, 1993).

NON-ENZYMATIC BROWNING REACTIONS IN FOOD AND DEVELOPMENT OF COLORED PRODUCTS

The development of color, flavor and aroma during storage and heat processing of foods is due to products of the Maillard reaction that results from carbonyl-amino reactions. The Maillard reaction, also known as non-enzymatic browning, involve a complex series of chemical reactions that lead to the formation of many heterocyclic compounds with distinctive flavor impact, and to the production of colored polymeric pigments, known as melanoidins (Fig. 2.2). Depending on the food product, the significance of Maillard reaction products (MRP) varied from unpleasant and deleterious to its contribution as desirable attributes of certain foods such as chocolate, coffee, roast beef, bread and fried potatoes.

The rate, type and properties of the products generated by Maillard reaction will depend on the nature and concentration of the reactants, temperature, reaction time, water activity and pH. An enormous diversity of products are possible because of the large number of interactions, reactivity of products and possible reaction routes.

Reducing sugars and α-amino acids

↓

N-glucosylamines or N-fructosylamines

Furfural
(from pentoses)

↓

Amadori and Heyn's intermediates — Dehydration

Hydroxymethyl-5-furfural
(from hexoses)

↓

Reductones and dehydro-reductones —

| NH₃ | H₂S | | | amino acids | Strecker degradation |

Retro-aldolization

↓

3-furanones
4-pyranones
Pyrroles
Thiophenes

Hydroxyacetone
Dihydroxyacetone
Hydroxyacetyl
Cyclotene

Glyoxal
Pyruvaldehyde
Glycoaldehyse
Glyceraldehyde

aldehydes + a-aminoketones
(+ methional from methionine)
(+ H₂S and NH₃ from cysteine)

Acetoin

Heterocyclization

↓

Pyridines   Thiazoles
Pyrazines   Pyrroles
Oxazoles   Imidazoles

Figure 2.2: Formation of Maillard compounds in Foods

Besides their significance in the development of color and flavor attributes in foods, MRP are responsible for reduction of nutritional value from loss of essential amino acids, decreased protein digestibility (development of cross-links), chelation of trace metals, and inhibition of digestive enzymes. Mutagenic and carcinogenic effects have been reported due to the formation of heterocyclic amines (HCAs).

MRPs have also been involved in the formation of beneficial compounds such as antimutagenic melanoidin products that can interact with certain mutagens (Yen and Hsieh, 1994; Namiki, 1988) or scavenge active oxygen species (Hayase, 1996; Aeschbacher, 1990). Also melanoidin products act as antioxidants displaying strong metal chelating abilities, reducing activity properties (Hayase, 1996; Namiki, 1988); and some compounds show antimicrobial effects (Lingnert, 1990).

Non-enzymatic browning may also result from caramelization, oxidative degradation of ascorbic acid and further reaction of the carbonyl compounds, or non-enzymatic oxidation of phenolic compounds to form quinones (Cilliers and Singleton, 1989; Dao and Friedman, 1992; Sapers, 1993).

The present review will attempt to describe the reaction pathways and factors involved in Maillard reaction with especial attention to the role of potato constituents.

The Maillard Reaction Pathways

In 1912, while attempting to determine the biological synthesis of proteins, Louis-Camile Maillard observed the formation of brown pigments and development of odors following the heating of a concentrated solution of D-glucose and amino acids (Eskin, 1990; Danehy, 1986). This reaction was subsequently referred as the Maillard reaction and covers all those reactions involving compounds with amino groups (amino acids, proteins, amines) and carbonyl groups (reducing sugars, ascorbic acid, aldehydes, ketones, products of lipid oxidation). Maillard reaction products have been established as causative factors for flavor, color and nutritional changes in foods (Rizzi, 1994; Eskin, 1990).

Hodge (1953) integrated the available information and proposed a general browning reaction mechanism, which is still considered the most appropriate description of the Maillard reaction. Hayashi and Namiki (1986) have proposed an alternative pathway with the production of C2 and C3 sugar fragments under alkaline conditions.

The sugar-amine browning reaction was classified into three phases by Hodge (1953), involving seven different types of reactions.

I. Initial phase: colorless products with no absorption in near-ultraviolet spectrum.

    A. Sugar-amine condensation

    B. Amadori rearrangement

II. Intermediate phase: colorless or yellow products with a strong absorption in near-ultraviolet spectrum.

    C. Sugar dehydration

    D. Sugar fragmentation

    E. Strecker degradation

III. Final phase: production of melanoidins that are highly colored polymeric compounds.

    F. Aldol condensation

    G. Aldehyde-amine polymerization

Maillard reactions have continued to be a significant focus of attention in the chemistry of foods and biological systems. A number of subsequent contributions have made possible the better understanding of the early stage of Maillard reaction, both with regard to the reactions occurring and to their products.

Initial Phase of Maillard Reaction in Foods

The first step in the reaction consists of the condensation between the carbonyl group of the sugar and the amino functional group. This mechanism involves opening of the ring form of the sugar, addition of the amine to the carbonyl group and subsequent elimination of a molecule of water to form the N-substituted glycosylamine (Fig. 2.3).

Top row:

HC=O
HC-OH + RNH₂ ⇌ HO~C~N-R (H) ⇌ HC=N-R ⇌ HC-N-R (H) ⇌ HC-N-R (H H)
HO-CH   HC-OH   HC-OH   HC-OH   HC=O
                      HO-CH   H₂O   HO-CH   HO-CH   HO-CH

Aldose   [Schiff base]   1-amino-1-deoxy-2-ketose
                                                                   Amadori compound

Bottom row:

H₂C-OH
C=O + RNH₂ ⇌ H₂C-OH, HO~C~N-R (H) ⇌ H₂C-OH, C=N-R ⇌ H₂C-OH, C-N-R (H) ⇌ HC=O, HC-N-R (H)
HO-CH   HO-CH   HO-CH   HO-CH   HO-CH
                          H₂O

                                                [Schiff base]   2-amino-2-deoxyaldose
                                                                 Heyn's compound
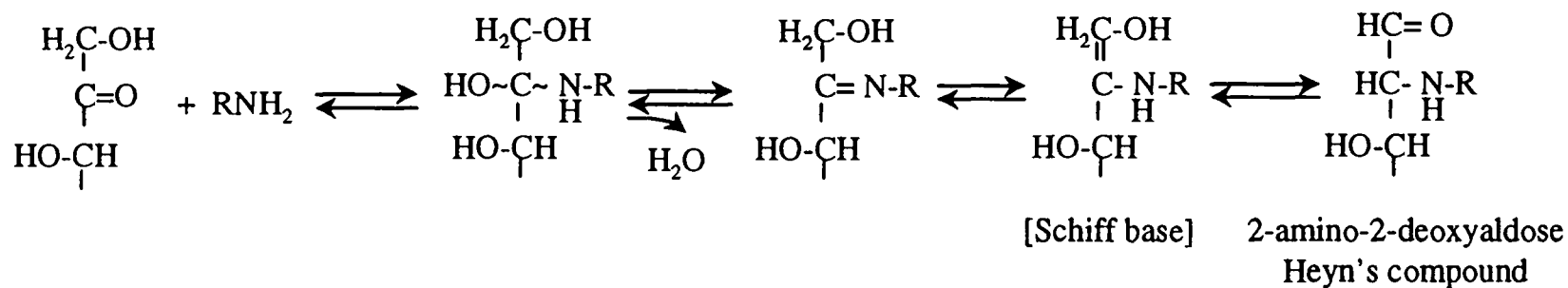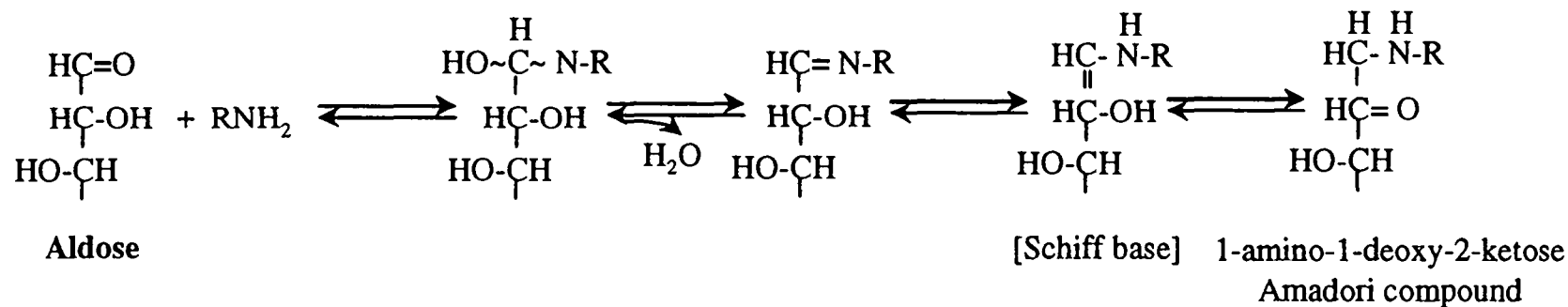
Figure 2.3: Formation of Amadori and Heyn's compounds during the initial phase of Maillard reaction

Amino acids in the presence of reducing sugars can act as nucleophiles or as acid/base catalysts for enolization reactions. As acids or bases, they can catalyze the transformation of ketoses and aldoses into α-dicarbonyl compounds without the formation of aminoketoses (Yaylayan and Huyghues-Despointes, 1994). As nucleophiles, amino acids can initiate the formation of Amadori rearrangement products that in turn decompose into α-dicarbonyl intermediates. Both catalytic and nucleophillic pathways produce common intermediates. The significance of amines in the Maillard reaction lies in their ability to catalyze the sugar rearrangements under pH conditions normally found in foods and living organisms (Ledl and Schleicher, 1990). Sugars require extreme pH conditions (pH> 8; pH< 3) or temperatures (>130°C) to produce a-dicarbonyl reactive intermediates, the significance of amines in the Maillard reaction lies in their ability to catalyze the sugar rearrangements under conditions normally found in foods and living organisms (Ledl and Schleicher, 1990).

During Maillard reaction, the condensation reaction is initiated by an attack of a nucleophillic amino nitrogen, with an unshared electron pair, on the carbonyl carbon. The rate of condensation reaches a maximum at a weakly acidic pH. Protonation of the carbonyl group enhances its reactivity to the nucleophillic reagent, while protonation of the nitrogen of the amino group inhibits the attack on the carbonyl carbon (Namiki, 1988). The reaction is facilitated when the pH of the medium is above the isoelectric point of the amino group (Eskin, 1990).

Elimination of water gives a Schiff base that cyclizes to give the corresponding N-substituted glycosylamine (Whitfield, 1992). Glycosylamines of amino acids or aliphatic amines are usually only detectable in very small amounts in sugar-amine reaction mixtures since they quickly undergo further reactions and rearrange via aminoenol into the aminoketose (Amadori compounds) and aminoaldoses (Heyns products) (Ledl and Schleicher, 1990; Ledl, 1990). Relatively stable glycosylamines are obtained from aromatic and heterocyclic amines. The reactions leading up to the formation of Amadori and Heyns products are reversible (Eskin, 1990).
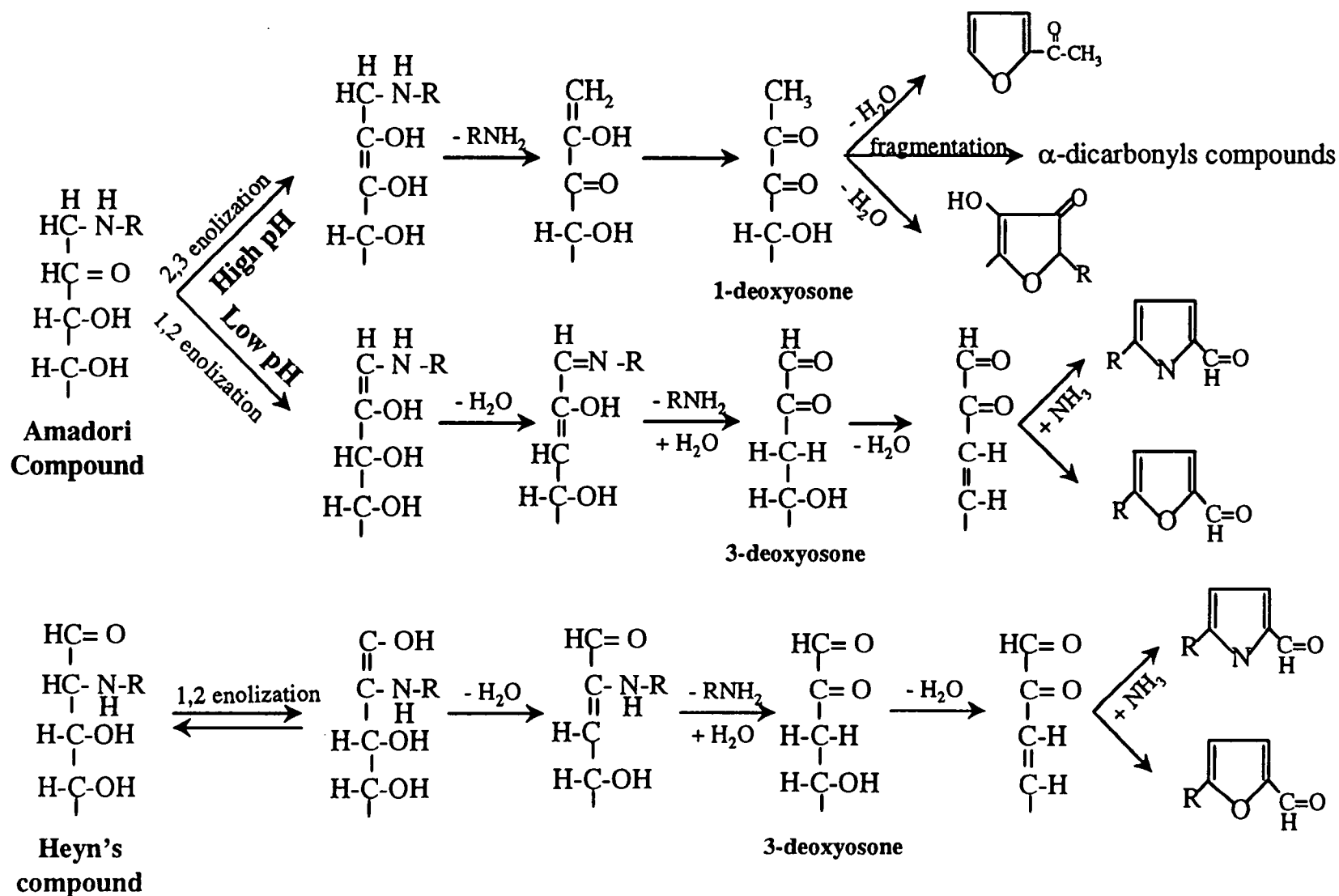
Figure 2.4: Decomposition of Amadori and Heyn's Products in the Maillard Reaction

Intermediate Phase of Browning

Amadori and Heyns intermediates do not contribute to flavor or color, however, they are important precursors. Amadori and Heyns products decompose in a similar way (Fig. 2.4); they are thermally unstable and undergo dehydration and deamination to give reductones, furfural, and fission products (Mottram, 1994; Ledl and Schleicher, 1990).

The breakdown of Amadori and Heyns compounds is strongly affected by pH. A low pH (pH < 5) tends to favor 1,2-enolization, and the loss of the amino compound from the 1,2- eneaminol gives a 3-deoxyosone; elimination of water and cyclization yields furfural from a pentose and 5-hydroxymethylfurfural and 5-methylfurfural from a hexose. At higher pH (pH 5 to 7) a 2,3-enolization is favored and elimination of the amine gives a 1-deoxyosone. Further dehydration and intramolecular cyclizations can lead to 5-methyl-4-hydroxy-3(2H)-furanone from pentoses or the 2,5-dimethyl homologue from hexoses. Fragmentation of the carbohydrate chains of the deoxyosone intermediates can lead to a variety of α-dicarbonyls compounds (Mottram, 1994; Eskin, 1990).

In addition to enolization, dehydration and fragmentation reactions, Amadori rearrangement products themselves can act as nucleophiles and react with a second sugar molecule to form diglycated Amadori products (Yaylayan and Huyghues-Despointes, 1994).

Alternative pathway for the browning reaction

Hayashi and Namiki proposed a new pathway for browning that involves the sugar fragmentation of the Schiff base during the initial stages of Maillard reaction under neutral or alkaline conditions to give very reactive enaminol compounds responsible for the formation of colored melanoidins pigments (Namiki and Hayashi, 1975, 1981, 1983; Hayashi and Namiki, 1980, 1981; 1986).

From ESR analysis, the presence of a free radical product, N,N'-disubstituted pyrazine cation derivative, was suggested during early stages of the sugar-amino

browning reaction. However, the presence of such pyrazinium derivatives has rarely been demonstrated probably because of their high instability (Namiki, 1988; Namiki and Hayashi, 1983; Hayashi et al., 1977; Hayashi and Namiki, 1981). Upon oxidation of the pyridinium radical, glyoxal or the corresponding nitrogenous analogs are produced and immediately transformed into colored compounds.

Namiki and Hayashi (1983) proposed a pathway for browning reaction involving the formation of two-carbon enaminol compound by sugar fragmentation and subsequent dimerization (Fig. 2.5) without the formation of Amadori compounds.

Degradation of 3-deoxyosones and 1-deoxyosones

The deoxyosones are the precursors of several heterocyclic and carbocyclic compounds, many of which are considered to be major sources of aroma volatiles. A complete review of the different degradation products of deoxyosones are presented by Ledl (1990), Ledl and Schleicher (1990), Feather (1994), Ledl et al. (1986).

The best known products derived from 3-deoxyosones (Fig. 5) are furan derivatives such as furfural and 5-hydroxymethyl-2-furfural (5-HMF). 3-deoxyosones can lead to the formation of furanones which have extremely low aroma thresholds and exhibit characteristic sweet, caramel aroma notes (Rizzi, 1994), or the lactone under reaction conditions at pH 4-7 (Ledl, 1990). A pyranone structure can be formed when the hydroxy group of the carbon 5 adds to the aldehyde function of the 3-doxyosone and water is eliminated. A condensation product of pyranone and 5-HMF leads to the formation of a yellow colored compound (Ledl, 1990).

In the presence of amines, the formation of 5-HMF can be largely suppressed in favor of nitrogen containing products, pyrrolaldehydes (Fig. 2.4) and pyridiniumbetaines. The formation of pyrroles is particularly important because of the reactivity of the hydroxymethyl carbon toward nucleophillic substitution reactions that may lead to protein cross-linking (Yaylayan and Huyghues-Despointes, 1994).

Ledl and Schleicher (1990) reported the formation of pyrrolaldehydes and pyridiniumbetaines by reaction of maltose and lactose with primary amino acids.
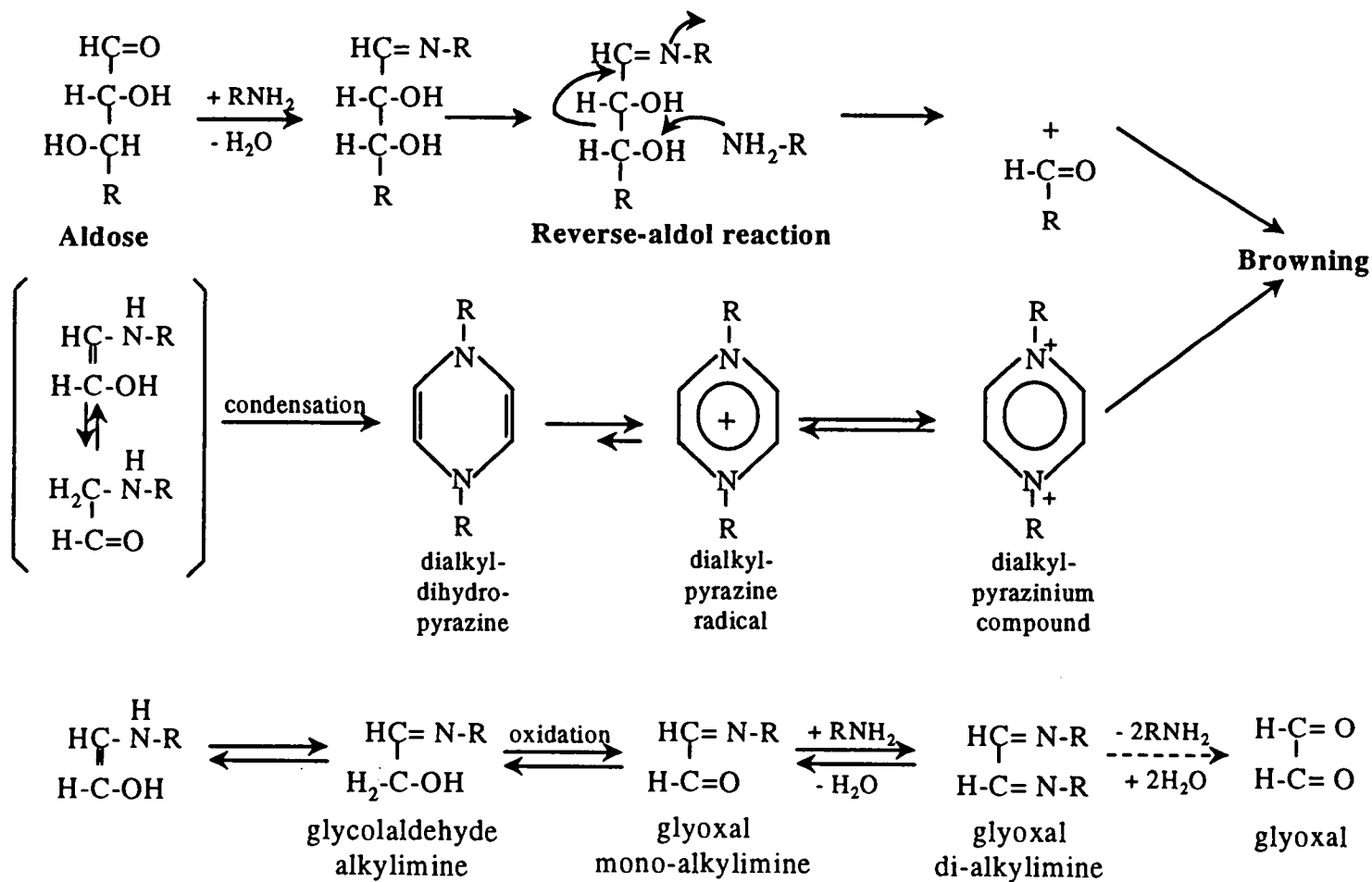
Figure 2.5: Proposed Pathway for Browning Reaction involving C2 sugar fragmentation and free radicals

The browning reaction involved loss of the oxygen atom attached to C4, regardless of whether the galactosyl or glucosyl residue is attached.

Other nitrogen containing heterocyclic compounds include maltoxazine from the reaction of 3-deoxyosone and a secondary amine (proline); and pyrazines and imidazoles from Amadori compounds and 3-deoxyosone when ammonia is heated with sugars (Ledl and Schleicher, 1990; Ledl, 1990).1-Deoxyosones are important precursors of a number of food flavors and aromas. Cyclization between the hydroxy group in position 5 and the C2 keto function leads to the formation of furanones, important aroma compounds in the Maillard reaction (Ledl, 1990). Furanones play an important role in the browning of pentoses (Ledl and Schleicher, 1990); 6-deoxyhexoses and pentoses produce the hydroxyfuranone known as furaneol, which is an important aroma compound not only in Maillard systems but also in fruits (Hodge et al. 1963).

Pyranones, only possible with hexoses, are formed from 1-deoxyosones by enolization and loss of hydroxy group from C2. This pyranone (Fig. 2.6) has been detected in many heated and stored foods (Ledl, 1990) and has been proposed as indicator substance of Maillard reaction, since hexoses occur in virtually all foods (Ledl and Schleicher, 1990). Primary amines are easily incorporated into the ring structure leading to pyrrolinones that exhibit yellow and blue fluorescent properties (Ledl, 1990).

Ledl and Schleicher (1990) reported differences in the reactions of 1-deoxyosones from monosaccharides and disaccharides (Fig. 2.6). A γ-pyranone is a characteristic intermediate of disaccharide degradation, and have been found in heated milk (R = α-gal) and steamed ginseng root (R= α-glu). Maltol and isomaltol are main degradation products of maltose and lactose, respectively, and are major contributors to pastry flavor (Feather, 1994; Ledl and Schleicher (1990).

Other products formed from degradation of 1-deoxyosones include pyridones, with metal binding capabilities, and furan derivatives among others (Ledl and Schleicher, 1990).

The property of the pyrrole (from 3-deoxyosones) and pyrrolidones (from 1-deoxyosones) to dimerize or substitute the hydroxy group with other nucleophiles finds
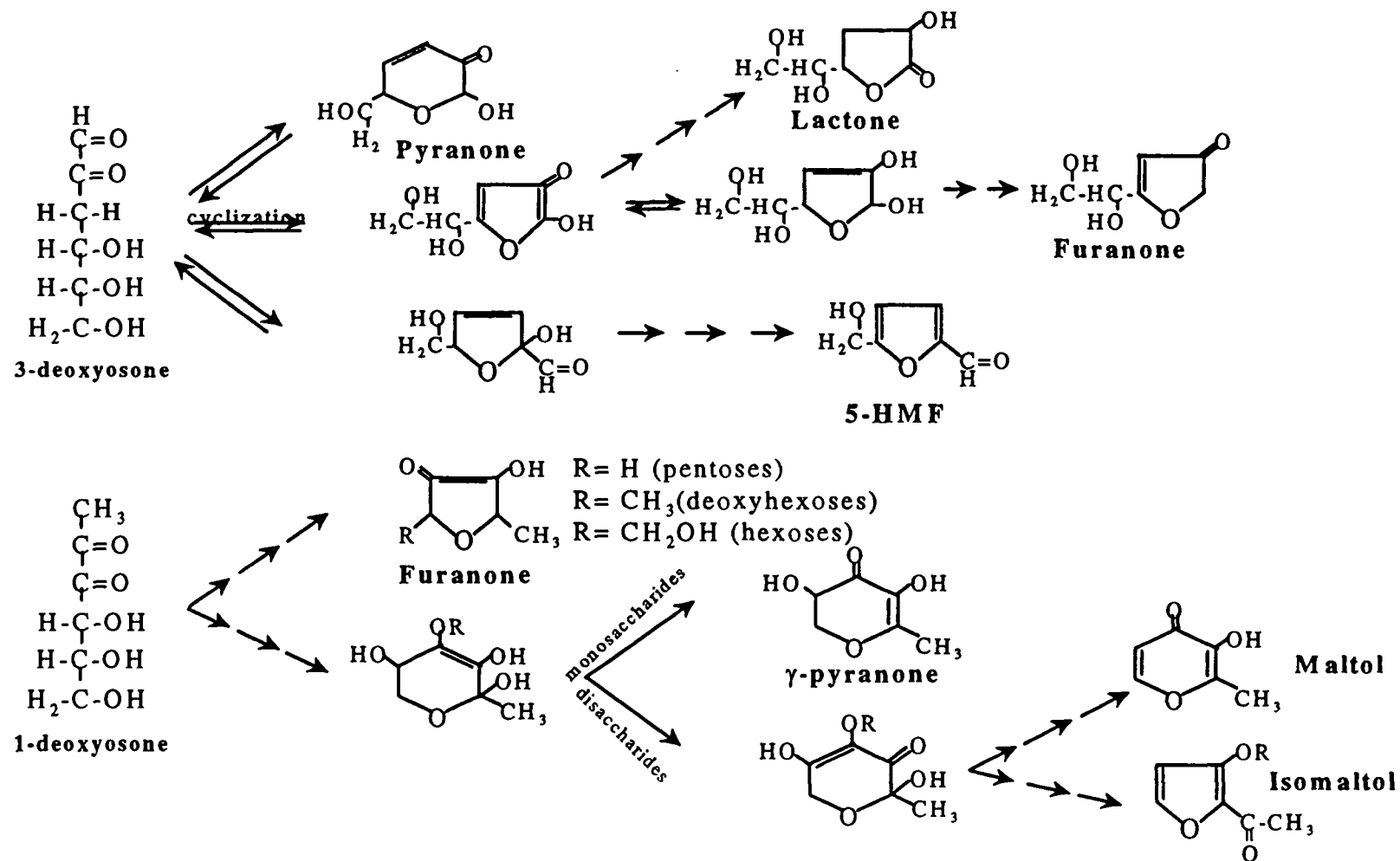
Figure 2.6: Degradation Pathways for Deoxyosones and Formation of Flavor Compounds

increasing interest because of their potential for crosslinking proteins, changing the protein function in the organism (Ledl, 1990).

Sugar fragmentation

Sugar fragmentation products play a considerable role in aroma formation. Sugars and many of their derivatives can undergo retro-aldol cleavage, in some cases followed by oxidation and dehydration to produce very reactive compounds that can undergo further condensation reactions (Ledl and Schleicher, 1990).

Three carbon (methylglyoxal , glyceraldehyde, glycolaldehyde, etc. or their imine derivatives) and other low molecular sugar fragmentary products are produced through scission of the C-C bonds after enolization of the Amadori product (Hayashi and Namiki, 1986; Hayase and Kato, 1986).

The reaction pathways for the formation of browning products is greatly influenced by pH (Fig. 2.7). Sugar fragmentation is negligible at acidic pH, observable at neutral pH and increased greatly at alkaline pH. Although the yields of C2 and C3 products are very low as compared to those of Amadori products even under alkaline conditions, their browning abilities are estimated to be several hundred times higher than Amadori products. Thus, the increase in the formation rates of fragmentation products might contribute significantly to browning (Hayashi and Namiki, 1986).

Strecker degradation

The Strecker degradation is one of the most important reactions leading to final aroma compounds and generation of reducing compounds essential for formation of colored products in heated foods. The reaction involves initial Schiff base formation of an a-dicarbonyls compound with an amino acid, rearrangement, decarboxylation and hydrolysis to produce an α-amino carbonyl compound and aldehydes (Fig. 2.8). The significance of the Strecker degradation lies in the fact that amino acids furnish ammonia or aminoenols that participate in the formation of important heterocyclic compounds,

sugar + amino acid, amine or protein

Glycosylamino compounds

C-2 compounds

Amadori products

C-3 compounds

C-2 imine

Osones

Free radicals

Furfural

Browning

Melanoidins

Browning

| 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | pH |
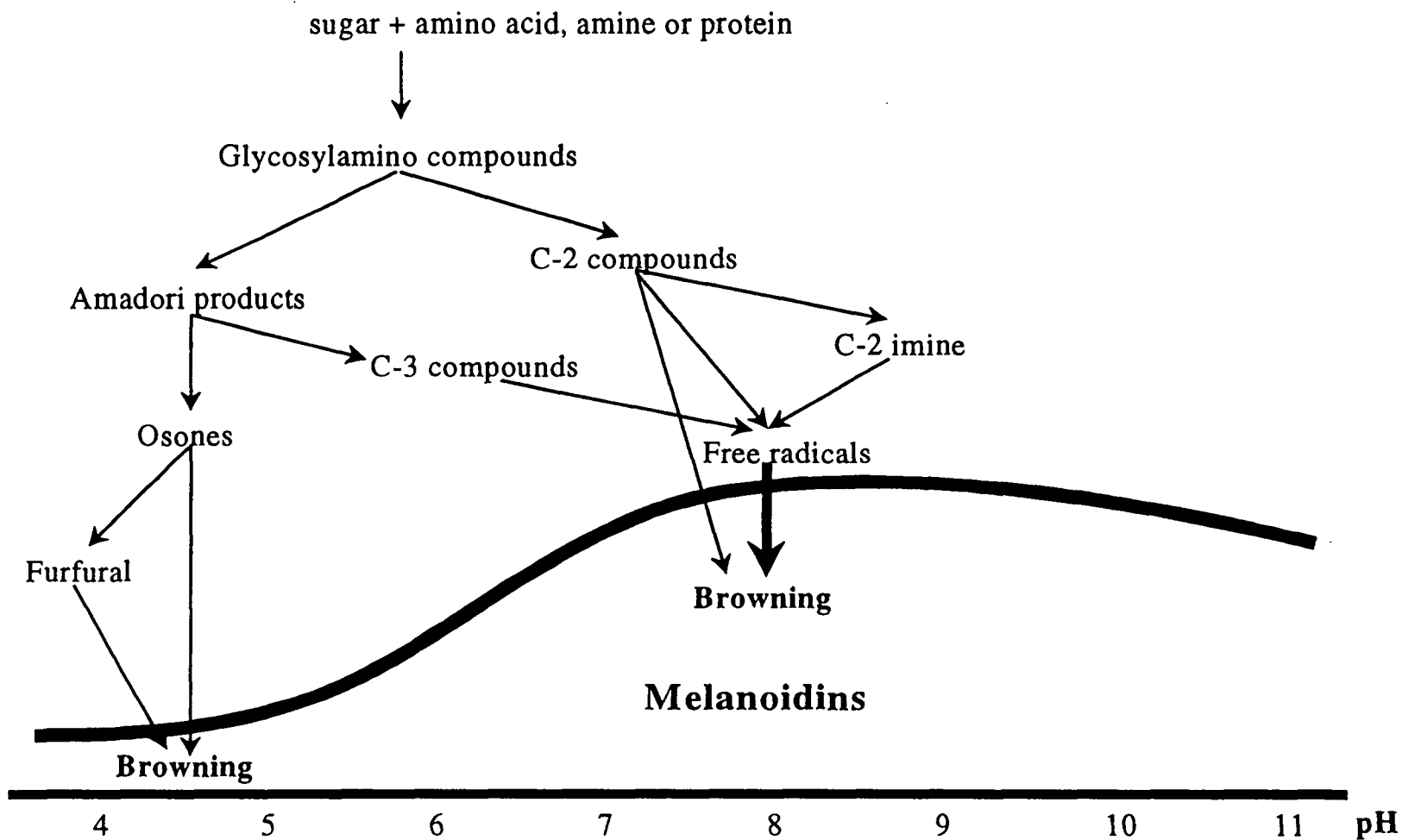
Figure 2.7 Effect of pH on Melanoidin Formation
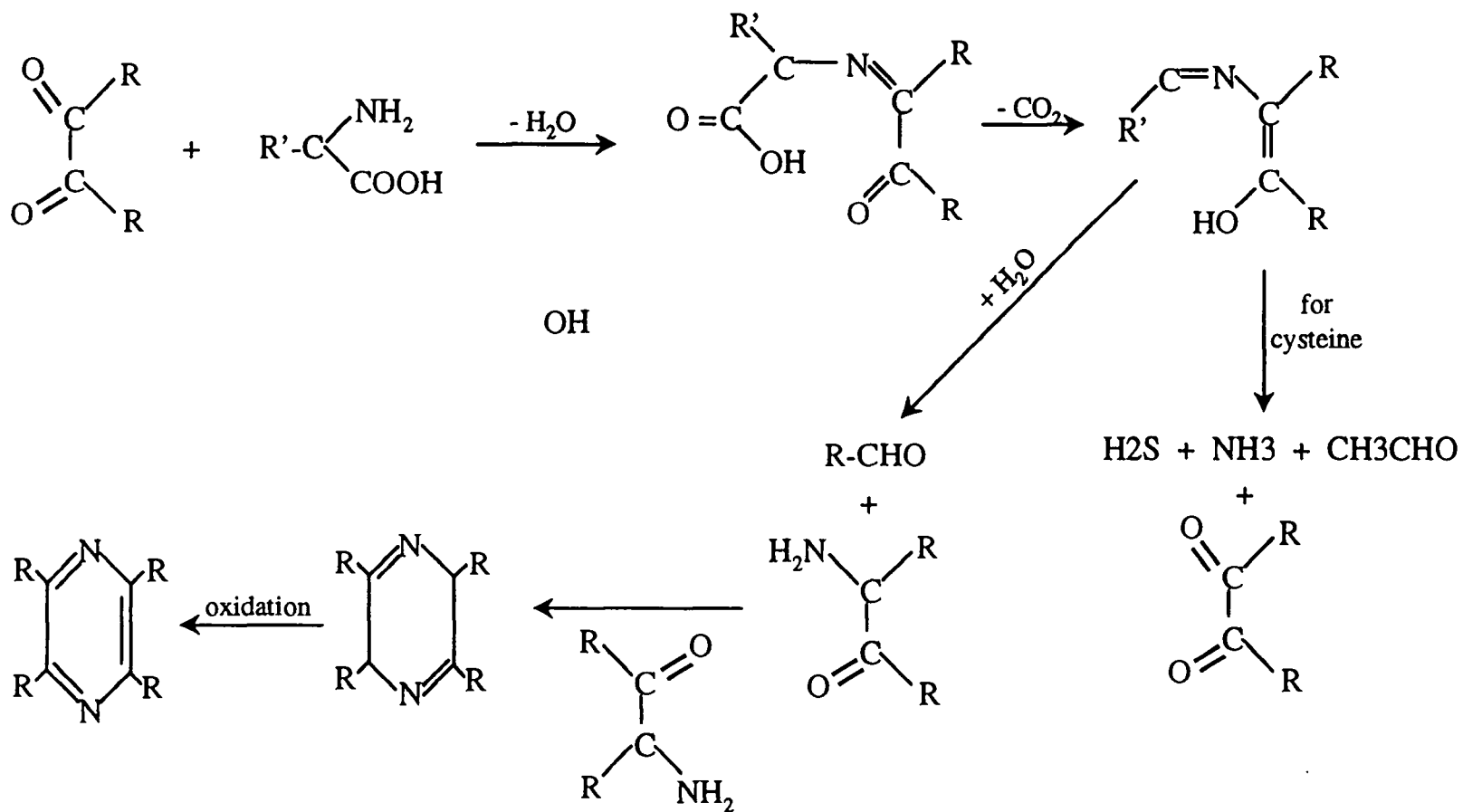
Figure 2.8: Strecker Degradation of α-aminoacids and Formation of Alkylpyrazines

pyrazines, pyrrolines, oxazoles, oxazolines and thiazoles derivatives responsible for the flavor of heated foods. Furthermore, many Strecker aldehydes are responsible for the flavor of roasted foods such as methional for potato products and isovaldehyde for cocoa (Ho, 1996; Eskin, 1990; Ledl and Schleicher, 1990).

Strecker aldehydes can undergo aldol condensation with other reductones and browning products leading to the formation of brown pigments (Hodge, 1953).

Significance of intermediate Maillard reaction products in foods

The Maillard reaction contributes to formation of color, aroma and flavor of several foods. Color formation can lead to desirable attributes or lowering of the quality of foods.

Deoxyosones are important intermediates in the formation of brown substances. 3-Deoxyosones have potential for condensation with a wide range of carbonyl compounds resulting in a multitude of browning products, such as the yellow compounds formed by condensation of HMF with a pyranone (Ledl and Schleicher, 1990). 1-Deoxyosones also causes colored compounds, such as yellow compounds formed by the interaction of Amadori products with carbonyl compounds or reaction of hydroxyfuranone with furfural and pyrrolaldehyde, respectively (Ledl and Schleicher, 1990).

Cleavage products, such as dihydroxyacetone and methylglyoxal, are found in the structure of colored compounds. Maillard products with a carbonyl function next to an enol group often give colored compounds with metal ions. Aldehydes and aminoenols formed by Strecker degradation also produce colored condensation products (Ledl and Schleicher, 1990).

Maillard reaction products are responsible for the bitter taste in overheated products, especially from the reaction of imino acid (proline) and sugars. Maillard reaction generates many compounds of low molecular weight which are very important in flavor and off-flavor production of many stored or heated foods.

Nursten (1980) classified the aroma compounds into 3 groups depending on the origin of the volatile compounds derived from the Maillard reaction in foods.

1. Simple sugar dehydration/fragmentation products: Furans, pyrones, cyclopentenes, carbonyl compounds, and acids.

2. Simple amino acid degradation products: Aldehydes, sulfur compounds (H_2S, methanethiol), and nitrogen compounds (ammonia, amines).

3. Volatiles produced by further interactions: Pyrroles, pyridines, pyrazines, imidazoles, oxazoles, compounds from aldol condensations, thiazoles, thiophenes, di- and trithiolanes, di- and trithianes and furanthioles.

Several reviews have dealt with the significance of aroma flavors generated by Maillard reactions, with a detailed information on formation pathways, interactions with other products and sensory properties (Ho, 1996; Mottram, 1994; Whitfield, 1992; Eskin, 1990; Baltes, 1990; Reneccius, 1990; Vernin and Parkanyi, 1982).

Final phase: Formation of high molecular weight colored melanoidin compounds

Very little is still known about the structure of high molecular weight melanoidin substances, mainly due to their inhomogeneous nature (Ledl and Schleicher, 1990; Rizzi, 1994). During the final stage of Maillard reaction, colored intermediates and other precursor products (such as enaminol products, sugar fragmentation products and unsaturated carbonyl products) proceed to condense and polymerize to form brown polymers (Namiki, 1988). As mentioned before, the formation of melanoidins is affected by the pH of the system. Melanoidins have been reported to be formed via pyrazinium radicals under neutral to alkaline pH, via osazones, furfurals and six-carbon heterocyclic compounds at neutral to acidic pH conditions (Hayase, 1996).

Elucidation of the chemical structure of melanoidin pigments has been attempted using ^{13}C and ^{15}N-Cross-Polarized-Magnetic angle spinning (CP- MAS) NMR. Benzing-Purdie and Ratcliffe (1986) suggested the presence of secondary amides, pyrroles and indole-like nitrogens in melanoidins formed on a Xyl-Gly system, while they discarded

the presence of pyridine and pyrazine-type nitrogens in melanoidins. Hayase et al. (1986) grouped the CP-MAS NMR signals into six major regions:

I. Saturated carbons joined to carbon or nitrogen atoms or methyl carbons (10-50 ppm),

II. Saturated atoms bound with oxygen or nitrogen (60-75 ppm),

III. Unsaturated carbons (105-115 ppm),

IV. Aromatic carbons (139-140 ppm),

V. Amide or carboxyl groups (170-180 ppm),

VI. Carbonyl carbons (190-205 ppm).

Ozone treatment suggests that saturated and aliphatic carbons (regions I, II and V) form the backbone of melanoidins and that C=C and C=N bonds (regions III and IV) are responsible for the structure of the chromophore (Hayase et al. 1986; Kim et al. 1985). Kato and Tsuchida (1981) proposed that the chemical structure of melanoidins has a repeating unit involving conjugated carbon double bonds and tertiary nitrogen. Also, the contents of heterocyclic compounds in melanoidins are thought to be less abundant (Hayase et al. 1996). The monomers involved in the condensation of melanoidins are still unknown and further work is required.

Properties of Melanoidin Compounds

The complexity in chemical composition of foods renders study of many chemical and biological properties of browning reactions extremely difficult. Since large amounts of browning compounds are consumed as dietary components, the effect of MRPs formed in heated sugar-amino acid models has been investigated.

The antioxidative activity observed in Maillard reaction mixtures is assumed to be present mainly in melanoidins due to the presence of amino reductone structures (Namiki, 1988). Reductones are commonly considered to be effective antioxidants due to their reducing activity and metal chelating activity (Namiki, 1988). Melanoidin pigments have a strong scavenging activity against active oxygen species such as hydrogen peroxides, hydroxyl radicals and superoxides (Hayase et al., 1990), and metal

chelating ability through the binding of Fe, Cu and other heavy metal ions involved in lipid peroxidation (Namiki, 1988; Johnson et al., 1983).

The presence of an amino compound is required to induce antioxidative activity since neither sugar nor their pyrolytic products alone show activity (Namiki, 1988). Low molecular weight sugars and some dicarbonyl compounds are very reactive in producing Maillard browning and inducing potent antioxidant activity. Furthermore, Lingnert and Eriksson (1980) reported that the antioxidative activity is enhanced in MRPs obtained from basic amino acids (arginine, histidine and lysine).

The antimutagenicity of Maillard reaction products has been reported in several studies with different model systems using sugars and amino acids (Chan et al., 1982, Yen et al., 1992, Yen and Tsai, 1993). Results from epidemiological, animal, and short term assay studies suggest that naturally occurring antioxidants (tocopherols, phenolic compounds, ascorbic acid) can inhibit the carcinogenesis and mutagenesis (Powrie et al., 1986) because of their ability to scavenge free radicals or induce detoxifying enzymes (Hochstein and Atallah, 1988). The antimutagenic activity of Maillard reaction products seems to be due to the specific reductone structure of melanoidins and their ability to scavenge active oxygen species and chelate metals (Hayase, 1996; Yen and Tsai, 1993). Yen and Hsieh (1994) reported that a possible mechanism for the antimutagenic effects of xylose-lysine MRPs towards 2-amino-3-methylimidazol[4,5-f]quinoline (IQ) might be their interaction with proximate metabolites of IQ to form inactive adducts.

The formation of heterocyclic amines during Maillard reactions has motivated concern due to their potential mutagenic, nutritional and toxicological impact in foods (O'Brien and Morrissey, 1989; Eksari et al., 1989; Quattrucci, 1988; Jagerstad et al., 1986; and Aaeschbacher, 1986). Several studies have indicated the *in vitro* mutagenicity of some MRPs such as 1,2 dicarbonyl compounds (Bjeldanes and Chew, 1979), dihydroxyacetone, glyceraldehyde, glyoxal, glyoxilic acid (Yamaguchi and Nakata, 1983), pyrazine and its derivatives, furan and furan derivatives, thiazoles, imidazoles and pyrazines (Wong and Shibamoto, 1996; Stich et al., 1981) and caramelization products (Stich et al.,1981).

Factors involved in the formation of mutagenic heterocyclic amines include cooking method, temperature, time and reactants (creatinine, amino acid and sugar content). During high temperature cooking processes (e.g. frying, broiling and grilling), mutagenic pyrolysates from amino acids are formed (Wong and Shibamoto, 1996). In cooked muscle foods (meat and fish), heat treatment causes cyclization of creatine to creatinine to form the imidazole moiety that can participate in the formation of carcinogenic and mutagenic heterocyclic aromatic amines (Wong and Shibamoto, 1996). Weisburger (1994) stated that Maillard reactions with foods containing creatinine lead to the production of powerful mutagens and genotoxic carcinogens associated with a risk of important types of cancer including the breast, colon and pancreas.

Melanoidins have shown desmutagenicity against heterocyclic amines as well as other mutagenic aromatic and heterocyclic compounds such as aflatoxin B_1, benzo[a]pyrene, 2-aminofluorene, 4-aminobiphenyl and 2-aminonaphtalene (Lee et al., 1994; Kim et al., 1986). The proposed desmutagenic activity of melanoidins is by reacting with the NHOH group of the amines or by scavenging the active oxygen species (Hayase, 1996).

Factors influencing the rate and extent of Maillard reaction

The rate of Maillard reaction is dependent on temperature, extent of the reaction, pH, water activity, metals and nature of the reactants. It is very difficult to isolate the effect of only one factor influencing the Maillard reaction since the different variables are always interrelated, resulting in a very complex reaction system which make results from different model studies complicated and sometimes conflicting (Lingnert, 1990).

Effect of temperature

Mixtures of sugars and amino acids stored at refrigerated temperatures can show signs of Maillard browning and the reaction increases markedly with temperature (Baxter, 1995; Mottram, 1994). The highest generation of brown color and flavor is

generally achieved at the elevated temperatures associated with cooking (Mottram, 1994). The temperature and extent of reaction strongly will influence the sensory properties of foods; very different flavors can be obtained from the same reaction system simply by changing the temperature or time of heating (Reineccius, 1990).

Maillard browning involves numerous chemical reactions with the formation of a wide variety of products. Each of these reactions has its own activation energy which can range from 10-160 KJ/mol, depending on the system and type of reaction measured (Reineccius, 1990; Lingnert, 1990). The activation energy for the Maillard reaction is highly dependent on water activity, pH and the participating reactants (Lingnert, 1990).

Effect of pH

Maillard reaction rates and product formation is strongly influenced by pH. The rate of browning is favored under alkaline conditions, exhibiting a maximum rate at about pH 10 (Eskin, 1990; O'Brien and Morrissey, 1989). As mentioned before, pH will affect the degradation of Amadori compounds through different enolization pathways and sugar fragmentation. Furthermore, under neutral or alkaline conditions, an alternate pathway involving the fragmentation of the Schiff base occurs (Hayashi and Namiki, 1986).

Effect of water activity and metals

Water in food systems has an important influence in Maillard reaction by controlling the liquid phase viscosity which will affect the dissolution, concentration or dilution of reactants (O'Brien and Morrissey, 1989). The browning reaction rate and generation of volatile flavors will depend on the water activity (a_w) of the system and it has been generally assumed that the a_w optimum for the reaction ranges between 0.65-0.75 (Mottram, 1994; Reineccius, 1990). The activation energy for the browning reaction is usually increased at low a_w.

The presence of metals affects the Maillard reaction. Copper and iron salts catalyze the browning reaction while tin and manganese appear to inhibit it (Kato et al., 1981; Bohart and Carson, 1955).

Nature of the reactants

The effect of the system composition in Maillard reaction is going to be dealt in more detail. The essential ingredients in Maillard reaction are the reducing moiety and free amino groups. In foods, the reducing agents involved are reducing sugars (ribose, xylose, glucose, fructose, lactose, maltose), ascorbic acid, aldehydes and ketones, orthophenols and sucrose when reacted under acidic conditions. The reacting amines can comprise free amino acids, secondary amines, N-terminal amine group on a protein, and the ε-amino group on lysine in a protein (Labuza, 1994).

Reducing sugars

The initial rate of the reaction is dependent on the rate at which the sugar ring opens to the oxo or reducible form, which is the only reactive specie capable of reacting with amino compounds (Yaylayan and Huyghues-Despointes, 1994; Eskin, 1990). In considering the reactivity of sugars in the Maillard reaction two factors are important: the amount of acyclic form and the rate at which the open-chain form interconverts with cyclic forms (mutarotation rate). Although sugars having higher rates of mutarotation do not necessarily have higher contents of acyclic forms, such higher rates of mutarotation might expose the amino acid to higher concentrations of open-chain forms per unit of time and hence speed up the rate of reaction (Yaylayan and Forage, 1992).

In general, pentoses yield stronger color intensities than hexoses, which in turn are more reactive than reducing disaccharides (Lingnert, 1990; Spark, 1969). Among hexoses, their browning reactivity decreased in the order of D-galactose > D-mannose > D-glucose (Spark, 1969). Although mannose and glucose add to amino acids to give the same Amadori product, solutions of mannose/amino acid brown faster than those of

glucose/amino acid. The greater browning potential of mannose could be attributed to its higher percentage of the acyclic form and its higher rate of mutarotation. It has also been proposed that the Amadori product formed can react further in the presence of the more reactive sugar (mannose) to form diglycated Amadori products, which seems to provide a more efficient pathway for browning. Diglycated products are very reactive and decompose readily to produce the brown color (Yaylayan and Forage, 1992).

Aldoses appear to be more reactive than ketoses as a consequence of the more sterically hindered carbonyl group of ketoses (O'Brien and Morrissey, 1989). Baxter (1995) reported that glucose showed a higher browning reactivity than fructose in the presence of a solution containing several amino acids at pH 7.5 and stored at 50°C. Fructose has been reported to brown at a faster rate than glucose during the initial stages of browning but was subsequently taken over by glucose (Wolfrom et al., 1974; Shallenberger and Birch, 1975; Bobbio et al., 1973), presumably due the greater polymerization of glucose-derived melanoidins (Reyes et al., 1982). Also, faster rates of Maillard cross-linking of proteins by fructose than by glucose have been reported in vitro (Dills, 1993). The increased rates of fructose in Maillard reactions might be due to its presence in greater extent in the open chain form compared to glucose an other aldoses (Dills, 1993).

As mentioned before, disaccharides participate in the Maillard reaction (Ledl and Schleicher, 1990) and produce specific browning products. In general, disaccharides (maltose and lactose) show similar browning reactivities and brown at a slower rate than monosaccharides (Baxter, 1995; Rao and Rao, 1972). However, Ashoor and Zent (1984) reported that α-lactose reacts at a faster rate than D-ribose, D-glucose and D-fructose. Sucrose, a non-reducing sugar, does not participate in the browning reaction unless the conditions favor its hydrolysis.

The concentration of the reactive open chain form of sugars is usually very low. At room temperature the formation of Amadori rearrangement products proceeds very slow because for most monosaccharides the acyclic form represents less than 1% of the total sugar in solution. However, the percent open chain form and the rate of

mutarotation increases with temperature and pH, and if the rate of mutarotation is faster than the rate of the reaction, the amount of open chain forms can be replenished (Yaylayan and Huyghues-Despointes, 1994).

Amino compounds

Amino groups act as nucleophiles during the Maillard reaction, accelerating the breakdown of sugar to produce brown color. The nature of the amino compound affects the rate of Maillard browning, and varies among different studies depending on the conditions (c.a. pH, temperature, reaction time) and specific reactants used in the experiment. Labuza (1990) emphasized the importance of multiple measurements during the browning reaction in order to obtain valid kinetic parameters to avoid error due to the recycling of the amino acid. Since each step in the overall reaction has a different activation energy, which may be different for each amino acid and sugar used, using only one condition to generalize about browning or amino acid reactivity is an error (Labuza, 1990).

Lysine has been proposed as the most reactive amino acid due to the presence of the ε-amino group (O'Brien and Morrisey, 1989), however, tryptophan has been reported to be over 30 times more reactive (Fry and Stegink, 1982). In general, basic and hydroxy amino acids react strongly with reducing compounds to yield dark products, whereas the acidic nonpolar amino acids react to a lesser extent (Vernin and Parkanyi, 1982). Amino acids with high browning reactivities include lysine, glycine, tryptophan and tyrosine, average browning reactivities include leucine, serine, alanine, phenylalanine, proline, while the low browning amino acids include aspartic acid, glutamic acid and cysteine (Baltes, 1990; Ashoor and Zent, 1984).

Some contradictory results have been reported for the basic amino acids histidine and arginine. Arginine and histidine have been shown to brown at similar rates as glycine, tryptophan and lysine in several model systems (Baxter, 1995; Baltes, 1990; Wolfrom et al., 1974). However, Ashoor and Zent (1984) classified these basic amino

acids as low browning intensity compounds after heating solutions for 10 min @ 121°C in the presence of reducing sugars.

The browning conditions used in the model system plays an important role in the rate of the reaction. Einarsson (1987) reported a pH dependency in the browning reaction involving an arginine/glucose system, in which the initial rate of color formation was faster at pH 11 but the final color intensity was highest at pH 3.

Interestingly, aspartic acid, glutamic acid, and cysteine have been reported to reduce sugar-amine browning (Pham and Cheftel, 1990; Nafisi and Markakis, 1983).

The concentration of lysine in the protein has been closely related to its propensity to brown (O'Brien and Morrisey, 1989); this higher reactivity of lysine in a protein has been attributed to the availability of the ε-amino group for reaction (Labuza, 1994). Protein-bound tryptophan reacts with reducing sugars only to a limited extent and is hardly damaged as compared to lysine (Ledl and Schleicher, 1990).

Amino acids also play a significant role in the development of aroma compounds, mainly through Strecker degradation reactions. Strecker products include the formation of aldehydes and α-aminoketones which are important in the formation of heterocyclic compounds. The aminoketones are intermediates in the formation of several classes of heterocyclic compounds such as pyrazines, pyridines, pyrroles, oxazoles, thiazoles and imidazoles (Mottram, 1994; Rizzi, 1994). Strecker aldehydes contribute to the desired aroma characteristics of heated foods but also can be responsible for off-flavor development (Baltes, 1990; Eichner and Wolf, 1983). Sulfur containing compounds, formed by degradation of cysteine, cystine and methionine, are considered to be the most important contributors to roast aromas because of their low threshold values (Baltes, 1990). Reaction of dicarbonyls with amino acids or ammonia can yield furan-like volatiles such as pyrroles, pyrrolines and pyrrolidines (Mottram, 1994).

L-ascorbic acid

L-ascorbic acid (AA), also known as vitamin C, is widely used in food processing because of its nutritional value and antioxidant activity. However, AA

degrades during food processing or heating, leading to the formation of dark polymeric products or melanoidins. The degradation of AA has been associated with browning and loss of quality in fruit juices and concentrates, such as lemon, grapefruit and orange (Handwerk and Coleman, 1988; Eskin, 1990).

Degradation of AA is mainly oxidative, but anaerobic decomposition also occurs (Namiki, 1988). Non-oxidative decomposition of AA under strong acidic conditions yield mainly furfural. It is assumed that the carbonyl 3-deoxypentosulose is the intermediate product of the non-oxidative reaction (Namiki, 1988). The most important factors in the oxidative degradation of AA are pH and the presence of metal ions (Cu^{2+}, Fe^{3+}). Decomposition is rapid in alkaline medium while is much slower at pH less than 7 in the absence of metal catalysts. The proposed oxidation pathway is summarized in Fig. 2.9.

The oxidation involves the oxidation of AA to give L-dehydroascorbic acid (DHA) which produces 2,3 diketo-L-gulonic acid (DKG) by ring opening. DKG is highly unstable and further breaks down into a wide variety of compounds, including DKG d-lactone which is considered an important browning intermediate (Namiki, 1988; Ohtsuka et al., 1986). Ascorbic acid has been shown to be involved in protein cross-linking under aerobic conditions, however, protein dimerization occurs at the same extent under anaerobic conditions in the presence of DHA (Larish et al., 1996).

Reaction of DHA with primary aliphatic amines under oxidative and non-oxidative conditions yielded the following aminoreductones as important degradation products: 2-deoxy-2-(propylamino) ascorbic acid, 3-deoxy-3-(alkylamino) ascorbic acid, and mono- and di-amines of oxalic acid. These degradation products were detected at a wide range of reaction temperature (40-100°C), being produced faster at higher temperatures. The authors concluded that these types of products may be responsible for protein glycation that occurs during food processing or under physiological conditions in the presence of AA (Larisch et al. 1996).
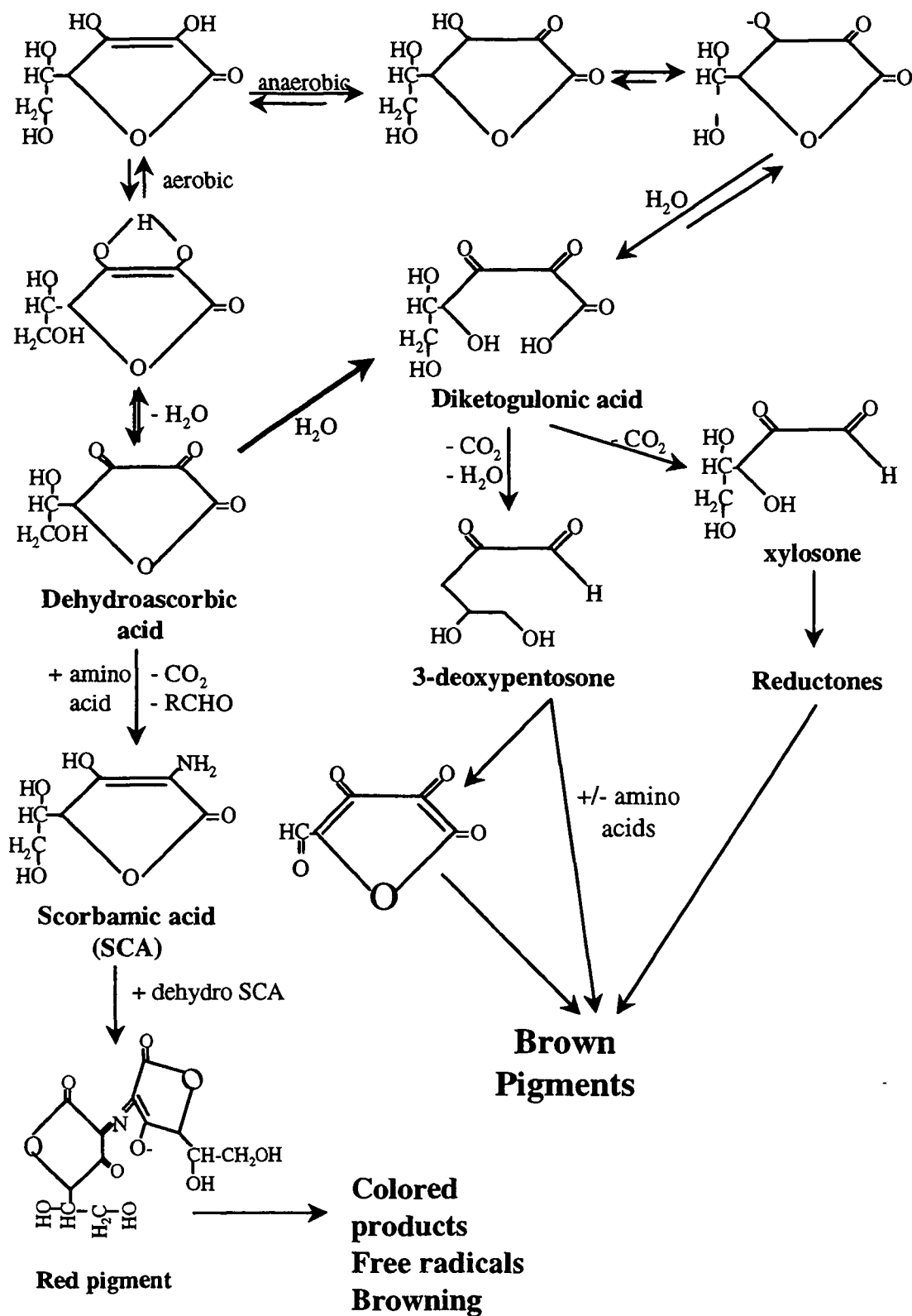
Figure 2.9: Non-enzymatic degradative pathways of ascorbic acid

The formation of red pigments in stored dried fruits and vegetables has been attributed to the reaction of AA and α-amino acids (Kurata et al., 1973; Nakabayashi and Shibata, 1967; Koppanyi et al., 1945). Kurata et al. (1973) reported that the formation mechanism of the red coloration was by an amino-carbonyl reaction of DHA with a-amino acids involving Strecker degradation. The authors attributed the generation of red color to the formation of 2,2'-nitrilo di-2-(2)-deoxy-L-ascorbic acid (NDA). Hayashi et al. (1983) proposed a complete scheme for the formation of the colored products, free radicals and browning in the reaction of DHA-amino acid system which involves complicated redox reactions. The reaction of DHA and amino acids also produces yellow (Hayashi et al., 1983) and purple (Hayashi et al., 1984) pigments as well as antioxidative products (Namiki, 1988).The formation of the red pigment has been achieved with almost all α-amino acids except L-proline (Kurata et al., 1973) and proteins (Hayashi et al., 1985).

Phenolic compounds

Enzymatic oxidation of phenolic compounds has been extensively reviewed because is an important cause for quality and nutritional loss in damaged fruits and vegetables. This reaction is catalyzed by polyphenol oxidases and results in the oxidation of polyphenolic compounds to orthoquinones which rapidly polymerize to form brown polymeric pigments (melanoidins) or can participate in polymerization reactions with protein functional groups to form cross-linked polymers (Friedman, 1997; Sapers, 1993; Richardson and Hyslop, 1985; Hurrel and Finot , 1984; Joslyn and Ponting, 1951).

In processed foods where the enzyme has been inactivated, the development of undesirable brown compounds during processing or storage can still take place (Cilliers and Singleton, 1991). This reaction is known as non-enzymatic autoxidative phenolic browning and occurs in the presence of oxygen and alkaline conditions or metal ions (Cilliers and Singleton, 1989; Pandell, 1983; Hurrel et al., 1982). Food undergoing processing at a high pH will be susceptible to rapid phenolic oxidation. Non-enzymatic autoxiodation of polyphenols produce *o*-semiquinone radicals or *o*-quinone molecules

that react further with amino acids and proteins or polymerize due to its strong electrophillic nature (Cilliers and Singleton, 1989; Kalyanaraman et al., 1987).

Cilliers and Singleton (1991; 1990 and 1989) have investigated the autoxidation of a caffeic acid model system. The rate of the reaction is very dependent on pH and occurs extremely rapid at pH 8.0 or higher. The fact that the reaction showed relatively constant energies of activation (c.a. 50 KJ/mol) and a high pH dependency suggested the involvement of phenolate anions as intermediates. The oxidation products have been characterized as dimers and trimers of caffeic acid in which enough conjugation is present to produce browning. After phenolate ion is formed, the reaction with oxygen produces the semiquinones that can undergo further coupling reactions to form different structural isomers, known as caffeicins. High color production can be obtained at intermediate pH (pH 6-7) due to the formation of more conjugated products at a slower reaction rates. Caffeic acid derivatives can become autoxidized under storage conditions even at low pH's and temperatures (Cilliers and Singleton, 1989).

Other compounds involved in Maillard reaction

Degradation products of lipid oxidation and organic acids can also participate in the browning reaction. Lipid derived products such as aldehydes, ketones and alcohols can interact with Maillard intermediates to produce specific heterocyclic compounds with alkyl substituents. Interactions between lipid degradation products and Maillard intermediates produce volatiles that affect the balance of aroma compounds produced during cooking (Mottram, 1994). Some important volatiles formed include pentyl- and hexyl-substituted pyrazines and long-chain sulfur containing heterocyclic compounds such as alkylthiozoles and alkylthiophenes (Ho, 1996).

Oragnic acids acting as buffering compounds can enhance the color formation by preventing the pH drop that results from removing the basic amino group from the amine during carbonyl-amine condensation (Handwerk and Coleman, 1988). According to Spark (1969), buffering a sugar and amino acid system will accelerate the formation

of ketosyl- and aldosyl-amines. Also, it has been reported that compounds with active methylene groups are effective promoters of Amadori and Heyns rearragements (Hodge and Rist, 1953). Citrate ions, phosphates, citric acid and malonic acid are among the acids reported to accelerate browning reactions (Bhalerao, 1993; Wolfrom et al., 1974; Spark, 1969). The ability of citric acid to enhance color formation has been proposed to be its buffering capacity across a broad range of pH, catalytic activity to form breakdown products from sugars, or interaction with specific Maillard intermediates in the formation of brown pigment (Bhalerao, 1993; Handwerk and Coleman, 1988).

FACTORS AFFECTING COLOR DEVELOPMENT IN POTATO CHIPS

Maillard reactants in potato tubers

The levels of sugars, in particular reducing sugars, and amino acids in potato tubers have an important effect on color development of fried potatoes. The formation of brown colored products and bitter compounds upon frying, due to Maillard reactions, are critical for the potato processing industry because it severely decreases the quality of the end products, e.g. potato chips and French fries (Adam et al., 1991; Roe and Faults, 1991; Smith, 1987; Schallenberger et al., 1959).

<u>Sugars</u>

The sugar content in potatoes is highly variable and may range from only trace amounts to up to 10% of the dry weight of the tuber depending on factors such as cultivars, temperature, pre-condition and handling of the tubers (Talburt et al., 1987; Sieczka and Maatta, 1986). The sugars consist mainly of sucrose, glucose and fructose with trace amounts of ketoheptose, melibiose, melezitose and raffinose. The presence of rhamnose and galactose have been inferred from their occurrence in structures such as alkaloids and anthocyanins (Burton, 1989).

Potatoes obtain their carbon for biosynthesis and energy as sucrose, delivered via the translocation stream from the foliage (ap Rees and Morrell, 1990; Burton, 1989). On arrival, sucrose is partitioned between starch, structural polysaccharide, or stored as sucrose or hexose, which enters the respiratory pathways to provide ATP, reducing power, and carbon skeletons for synthesis of amino acids, organic acids and lipids (ap Rees and Morrell, 1990). The fate of sucrose will be determined by the relative activities of proteins in the cytosol such as alkaline invertase, sucrose synthase and tonoplast proteins responsible for transport of sucrose from cytosol to vacuole. In the vacuole, sucrose is stored unless it is hydrolyzed by vacuolar acid invertase to give hexoses that return to the cytosol for subsequent metabolism (ap Rees and Morrell, 1990).

The levels of free sugars in potato tubers depends on the interaction of several pathways of carbohydrate metabolism (Fig. 2.10) including starch synthesis, glycolysis, mitochondrial respiration and gluconeogenesis (Sowokinos, 1994). The larger proportion of sucrose is incorporated into starch by a series of enzymatic reactions (Fig. 2.11). In the mature tuber, the greater part (c.a. 98%) of the carbohydrate is in the insoluble form of starch (Burton, 1989).

The sugar content in developing tubers varies widely within individual tubers, between tubers of the same plant, and between cultivars (Table 2.1). Other factors such as the growth conditions, growing season, cultural practices and storage conditions will influence sugar accumulation (ap Rees and Morrell, 1990; Burton, 1989).

These factors will affect the supply of sucrose, the rate and pathway of sucrose breakdown, relative rates of phosphorylation of glucose and fructose, and the activity of vacuolar acid invertase.

The suitability of a cultivar for processing is dependent on its quality at harvest and its response to storage conditions (Burton and Wilson, 1978). During storage, sugars accumulate due to sprouting, aging of the tubers (senescent sweetening), and low temperature sweetening (van Es and Hartmans, 1981).

Cold-induced sweetening is a major problem for the potato industry since it results in the accumulation of reducing sugars rendering tubers unsuitable for
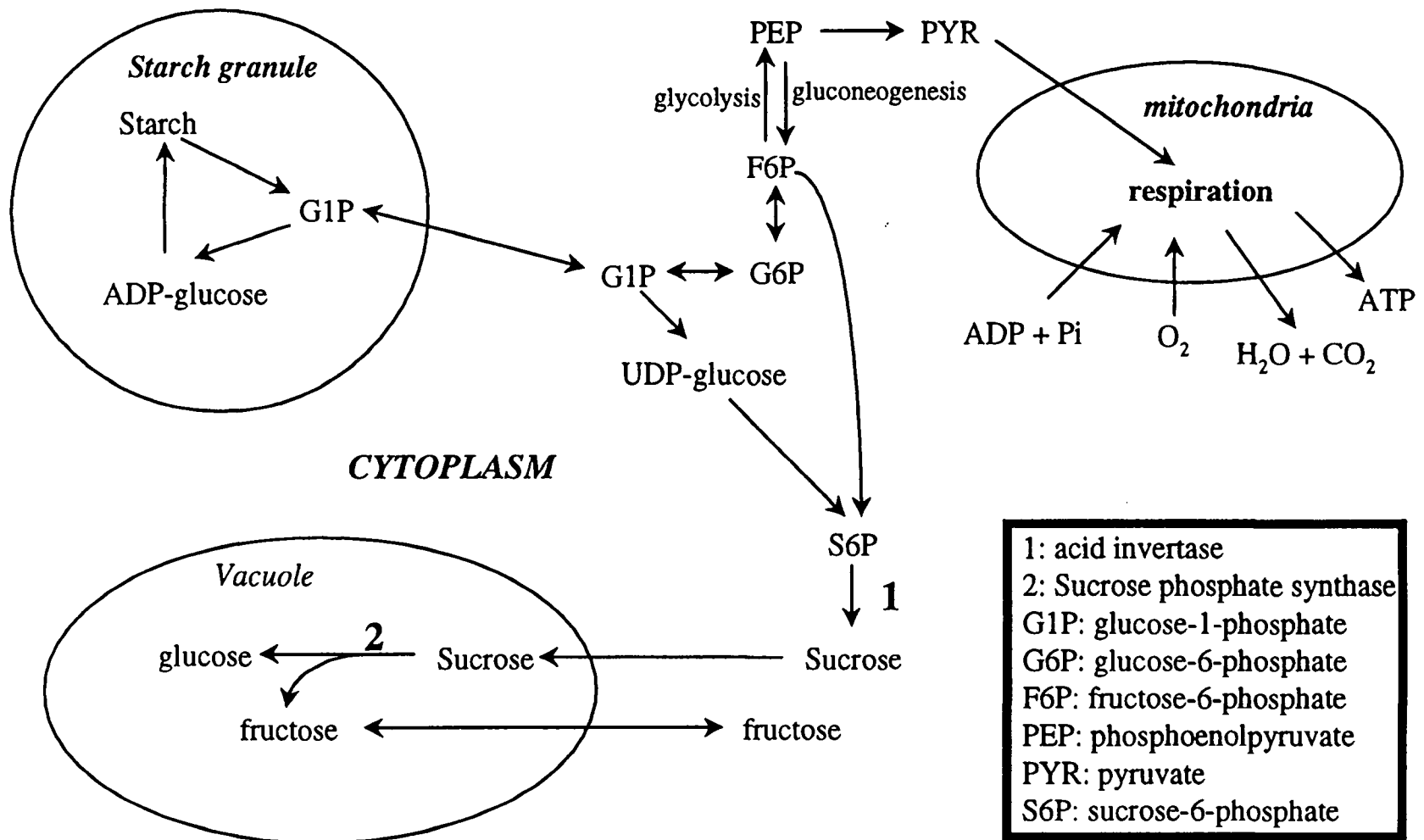
Figure 2.10: Scheme for Carbon Movement in Potato Tubers

Legend box:

1: acid invertase
2: Sucrose phosphate synthase
G1P: glucose-1-phosphate
G6P: glucose-6-phosphate
F6P: fructose-6-phosphate
PEP: phosphoenolpyruvate
PYR: pyruvate
S6P: sucrose-6-phosphate

Sucrose $\xrightarrow[\text{+ UDP}]{\text{Sucrose synthase}}$ UDP-glucose + fructose $\xrightarrow[\text{+ PPi}]{\text{Pyrophosphorilase}}$ Glucose phosphate +UTP

Pyrophosphorilase | + ATP

Starch synthase
Starch ◄―― ◄―― ◄―― ADP-glucose + Phosphate

Figure 2.11: Synthesis of starch in the potato tuber

processing. The use of low temperature storage (< 5°C) offers several benefits such as control of sprout growth, maintenance of high humidity atmosphere, reduction of senescent sweetening and minimization of storage diseases and pests. However, the associated accumulation of sugars in most cultivars overwhelms the benefits (Duplessis et al., 1996).

Mature tubers, stored at low temperatures, accumulate sugars through the degradation of starch. The initial degradation of starch during cold-induced sugar accumulation is catalyzed by phosphorylases that releases hexose phosphates (Claassen et al., 1993). Amylases facilitate starch degradation (Davies and Viola, 1992; Cottrell et al., 1993). Hexose phosphates are converted to sucrose by sucrose phosphate synthase (SPS). The onset of sucrose accumulation is triggered by regulation of SPS during cold storage (Hill et al., 1996). The concentration of hexose phosphates and UDP-glucose in the cytosol for SPS activity and the cold-induced inhibition of some glycolytic enzymes (phosphofructokinase and pyrophosphate-phosphotransferase) that divert hexose phosphates towards sucrose synthesis are among other factors that influence accumulation of sugars in tubers (Hill et al., 1996; Trevanion and Kruger, 1991; ap Rees et al., 1988).

Sucrose is hydrolyzed to glucose and fructose by an acid invertase resulting in accumulation of reducing sugars from the inactivation of an invertase inhibitor during

cold storage (Isla et al., 1991; Isherwood, 1976; Pressey, 1969). Invertase activity is modulated by its products and is involved in the regulation of the ratio of hexose to sucrose (Duplessis et al., 1996).

Table 2.1: Sugar content and chip color (Agtron value) of chipping potato varieties at different harvest dates and growing season

Variety	Harvest period (days)	1988 Season			1989 Season		
		Sucrose	Glucose	Agtron	Sucrose	Glucose	Agtron
		(mg/100g fw)		value	(mg/100g fw)		value
Atlantic	98	0.26	0.11	71	0.59	0.10	61
	138	0.59	0.08	71	0.38	0.05	70
Eramosa	98	0.17	0.32	51	0.49	1.15	33
	138	1.03	0.34	41	0.19	1.21	35
Kanona	98	0.80	0.15	70	0.76	0.03	66
	138	0.58	0.10	69	0.50	0.03	69
Norchip	98	0.19	0.11	79	0.58	**0.04**	**58**
	138	0.58	0.10	70	0.49	0.05	61
Onaway	98	0.45	**0.18**	**49**	0.66	1.28	30
	138	0.95	0.53	38	0.19	1.34	27
Saginaw-Gold	98	0.28	0.14	70	1.04	**0.12**	**71**
	138	0.38	**0.17**	**80**	0.36	0.06	73
Spartan Pearl	98	0.08	0.16	76	0.68	0.09	.60
	138	0.22	0.10	60	0.38	0.05	63
Snowden	98	0.76	0.12	72	0.80	0.05	64
	138	0.58	**0.08**	**78**	0.45	**0.03**	**76**

An alternate pathway involving cyanide resistant respiration that leads to decreased ATP levels and increased in sucrose concentrations has been suggested (Duplessis et al., 1996).

Senescent sweetening is an irreversible process that results in increased sugar content during prolonged storage of tubers. Cold-induced sweetening is a reversible process to a large extent by raising the temperature to 10-20°C due to re-formation of starch from sugars, however, this conditioning process may alter significantly the structure of the starch granule and the sugar conversion to starch is not complete (Jadhav et al., 1991; Burton, 1989).

The rate at which reducing sugars disappear during conditioning depends on variety and the length and temperature of previous storage. Herrman et al. (1996) reported that potatoes receiving a cold-storage treatment, involving a combination of temperature regimes that starts at 10°C (1 mo), gradually decreases the temperature to 4°C, followed by 6 mo at 4°C, brought back gradually to 10°C and stored for 3 mo at 10°C, contained less sugar concentration and produced lighter chips than tubers stored at 10°C for 12 mo.

Amino acids and amides

Potato tubers are an excellent source of dietary nitrogen and essential amino acids. Potatoes supply a high-quality crude protein, representing c.a. 10% of the tuber dry weight which is similar to the protein content of most cereals (McKay et al., 1987).

Only 50% of the total nitrogen in potatoes is derived from proteins; the remaining nitrogen consists of free amino acids (15%), amides associated with asparagine and glutamine (23%), and glycoalkaloids and secondary metabolites (12%).

Transport of nitrogenous substances from plant foliage to the tuber is mainly in the forms of amides, amino acids and ureides, particularly in the form of glutamine and asparagine (Burton, 1989). In the potato tuber, glutamine and asparagine readily change into glutamic and aspartic acid, respectively, and upon binding of excess toxic ammonia they are transformed back to the amide form (Leszczynski, 1989a).

Potatoes provide an excellent source of lysine, but are deficient in sulfur-containing amino acids (methionine and cystine) which limits their nutritive value (Friedman, 1996). Asparagine and glutamine represent the major constituents of soluble non-protein nitrogen. The main free amino acids present in the potato tubers are aspartic acid, glutamic acid, valine, and 4-aminobutyric acid.

Free amino acids are present in potato tubers in different amounts depending on variety and environmental factors (Leszczynski, 1989a; Davies, 1977). These factors result in high variability and wide range of amino acid content in potato tubers (Table 2.2).

Nitrogen fertilization is the major source of variation in the levels of free amino acids in the tuber (Burton, 1989). Different amino acids respond differently to fertilization. The levels of glutamine, asparagine, arginine, alanine and proline are increased upon application of fertilizer, while tyrosine and phenylalanine give practically no response (Eppendorfer and Billie, 1996; Hoff et al., 1971).

The effect of phosphorus- and potassium-deficient conditions varied among the different free amino acids (Eppendorfer and Billie, 1996; Mulder and Bakema, 1956). Location, growing season, the health of the plant, weather conditions, and soil conditions are other factors that affect the pool of soluble non-protein nitrogen (Burton, 1989).

Talley and Porter (1970) reported that storage time had a relatively minor effect on the amino acid composition of the tuber. However, amino acid content varied inversely with the specific gravity of the tuber.

Ascorbic acid

Vitamin C occurs as L(+) ascorbic acid (L-AA) and dehydroascorbic acid (DHAA), the oxidation product of L-AA, which possesses 80-100% of the vitamin activity of L-AA (Cooke, 1974). Ascorbic acid is of importance for the food industry because of its nutritional value but also because its inhibitory effect of enzymatic browning and antioxidant ability. Potatoes are an important source of vitamin C in the

diet because of its high rate of consumption and contribute between 20-25% of the total dietary vitamin C requirements (Wills, 1987).

Table 2.2: Free amino acid content in potato tubers and chip color produced in model systems

Amino acid	Average content (mg/ 100g dry matter)		Hue angle *	Grayness **
	Talley et al., 1970	Davies, 1977	(degrees)	
Aspartic acid	226 (113 - 588)	184 (32 - 376)	60.6	
Asparagine	1530 (552 - 2647)	1487 (371 - 3490)	62.7	144.5
Threonine	60 (24 - 180)	48 (14 - 143)		
Serine	65 (29 - 154)	60 (15 - 128)		
Glutamine	959 (117 - 2045)	1820 (220 - 9122)	58.4	134.5
Glutamic acid	284 (110 - 462)	294 (225-371)	64.7	
Proline	93 (9 - 1049)	88 (0 - 484)		144.7
Glycine	18 (8 - 37)	12 (1 - 35)	55.1	
Alanine	58 (12 - 122)	32 (6 - 118)		
Valine	212 (95 - 376)	133 (15 - 370)		143.2
Methionine	88 (1 - 140)	55 (9 - 108)		148.1
Isoleucine	96 (38 - 186)	58 (14 - 165)		
Leucine	50 (13 - 217)	33 (8 - 1331)		
Tyrosine	121 (36 - 313)	95 (17 - 316)		145.0
Phenylalanine	108 (43 - 220)	77 (0 - 204)	60.3	
Tryptophan	? 17 (3 - 46)	66 (7 - 174)		
Lysine	110 (41 - 221)	65 (9 - 319)	50.5	
Histidine	60 (23 - 107)	117 (17 - 328)		143.1
Arginine	259 (122 - 536)	251 (60 - 736)	59.5	140.6
4-aminobutyric acid	220 (86 - 352)	156 (15 - 448)	52.3	

Ascorbic acid concentration in mature potato tubers after harvest of about 20 mg/100g fw is typical. Factors such as yield, cultivar, harvest, storage conditions and length of storage will affect the ascorbic acid content resulting in levels between 8-30 mg/100g fw (Smith, 1968). During the first 11 wks following planting, the ascorbic acid content increases with the growth and development of the potato tuber but tends to decrease thereafter with increasing maturity (Mondy and Munshi, 1993). Cultural practices and growing conditions can have a strong effect on the concentration of L-AA in potatoes possibly by affecting tuber maturity. Zhang et al. (1997) reported that irrigation tends to reduce L-AA levels.

Vitamin C is very labile and the level of fresh potato tends to fall dramatically during storage (Zhang et al., 1997; Augustin et al., 1978). The concentration of L-AA decreases on storage, showing a rapid decline during the first months of storage and then continues to diminish at a lower rate (Mondy and Munshi, 1993; Linnemann et al., 1985). Degradation of L-AA during storage depends mostly on pH and temperature, also is affected by the availability of oxygen, metal ions and the presence of enzymes (Kinkal and Giray, 1987). Linnemann et al. (1985) reported a decrease of L-AA at low storage temperature (7°C), however, an increase in the L-AA content in tubers stored at 16°C and 28°C was noted and attributed to processes involving sprouting and increased respiration.

Thermal processes such as blanching, steaming, boiling, and frying will decrease the vitamin C content of potato products, with losses in the order of 30% (Wills and Silalahi, 1990; Artz et al., 1983). The losses of vitamin C during processing are aggravated by subsequent storage of the final product (Burton, 1989).

Phenolic acids

Polyphenolic compounds are secondary metabolites that appear to be involved in the defense of plants against invading pathogens (bacteria, fungi and viruses) through their oxidation products (Friedman, 1997). When the potato tissues are damaged, polyphenols are enzymatically oxidized to quinones which then polymerize to dark

pigments. The polymerized phenolic compounds help to seal the injured plant surface and begin the healing process (Friedman, 1997). The rate of pigment formation seems to impart resistance. Friedman (1997) published an excellent review on the chemistry, biochemistry and dietary role of polyphenols in potato.

Potato tubers contain free and conjugated phenolic acids which are distributed mostly in the outer part of the tubers, cortex and skin tissues (Malmberg and Theander, 1984; Reeve et al., 1969). The amount of phenolic acids in potato tubers is usually small but they tend to accumulate in high levels in wounded tissues and sites of infection (Ramamurthy et al., 1992; Malmberg and Theander, 1984).

Chlorogenic acid (5-O-caffeoylquinic acid) an its isomers, 3- and 4-O-caffeoylquinic acid, represent the major phenolic acid and constitutes up to 90% of the total phenolic content of potato tubers (Friedman, 1997; Ramamurthy et al., 1992; Dao and Friedman, 1992). Chlorogenic acid content in fresh potatoes have been reported to range from 4.5 to 19 mg/100 g fw (Ramamurthy et al., 1992; Dao and Friedman, 1992). Other phenolic compounds reported in potato tubers are caffeic acid, *p*-coumaric acid, ferulic acid and protocatechuic acid (Ramamurthy et al., 1992; Malmberg and Theander, 1984). The phenolic acid composition of potato peels is more complex with the presence of additional phenolic compounds such as *p*-hydroxybenzoic acid, vanillic acid, syringic acid, gallic acid and phenolic aldehydes (Onyeneho and Hettiarachchy, 1993; Malmberg and Theander, 1984).

Accumulation of phenolic acids is induced as a response to infection, bruising, stress conditions, and during wound healing. Other factors affecting the accumulation of polyphenolic compounds involve storage of potato tubers, especially at low temperatures where lower PPO activity is observed (Friedman, 1997; Mondy and Gosselin, 1989) and light exposure (Griffiths et al., 1995; Dao and Friedman, 1994). Conflicting results have been reported on the effect of g-radiation on accumulation of phenolic compounds. Irradiation effectively prevents sprouting and protects potatoes during storage against damage by fungi and other phytopathogens (Swallow, 1989). Irradiation results in the reduction of both total phenolics and chlorogenic acid contents

due to impaired phenolic biosynthesis (Penharkar and Nair, 1995; Penharkar and Nair, 1987). However, Ramamurthy et al. (1992) reported that irradiation, even though produced tubers with significantly lower levels of chlorogenic acid and its isomers as compared to control potatoes, increased the phenolic acid content during wound healing storage (25°C). Mondy and Gosselin, 1989 showed that irradiated tubers increased their phenolic content during storage and produced higher discoloration, especially when stored at 5°C as compared to 20°C. Also, the storage period from harvest to irradiation affects the potato discoloration, being suggested that tubers should be stored at ambient temperature for 1 mo before irradiation to minimize browning (Ogawa and Uritani, 1979).

Maillard reaction and color formation in potato chips

The effect of different potato constituents on color development of potato chips has been studied using correlation parameters between the tuber composition and color (Roe et al., 1990; Marquez and Añon, 1986; Mazza, 1983; Habib and Brown, 1956) , paper disks (Roe and Faults, 1991; Smith, 1987; Leszkowiat et al., 1990; Shallenberger et al., 1959) or leached potato slices (Khanbari and Thompson, 1993) saturated with controlled levels of reactants.

The desirable yellow-brown color and distinctive flavor of potato chips are consequence of Maillard reactions. However, excessive browning during frying produces undesirable color and bitter taste, which is unacceptable (Roe et al., 1990). Color development of potato chips during frying results from the participation of amino acids and reducing sugars. The extent of browning has been mainly attributed to the levels of reducing sugars in the tubers, since the amount of amino nitrogen is rarely limiting (Marquez and Añon, 1986).

Mazza (1983) showed that the development of dark color in fried chips is closely correlated with the reducing sugar content. Glucose and fructose react similarly with amino acids and produced comparable color during frying in model systems (Roe and Faults, 1991). However, a closer association between chip color and glucose levels as

compared to total reducing sugars has been reported for potato tubers (Pritchard and Adam, 1994). This result could be attributed to the often higher concentration of glucose than fructose in stored potato tubers (Davies and Viola, 1994). The reported glucose:fructose ratios range from 1:1 to as high 10:1 depending on variety, sucrose concentration at harvest, partial or complete starch breakdown by amylases, starch synthesizing potential and fructokinase activity (Davies and Viola, 1994; Pritchard and Adam, 1994; Renz et al., 1993). Sowokinos and Preston (1988) suggested maximum tolerable levels (SAFE values) of sucrose and glucose of 1.0 and 0.035 mg/100g fw, respectively.

The potato industry uses reducing sugar levels, often in conjunction with a fry test, as a predictive test of the suitability of the material for processing (Fuller and Hughes, 1984). However, processors have found that potatoes (Table 2.2) can develop a fry color different from that expected from its reducing sugar content (Khanbari and Thompson, 1993). Although the level of reducing sugars may explain the most of the variation in color, the presence of other tuber constituents and pH may influence color formation (Fuller and Hughes, 1984).

Potatoes grown under different levels of nitrogen can have different fry colors for the same amount of sugar (Hughes and Fuller, 1984). Increasing nitrogen fertilization has been shown to reduce the amount of reducing sugars in potatoes and increase the free amino acid content, although not all amino acids are affected to the same extent (Hughes and Fuller, 1984; Hoff et al., 1971). Roe et al. (1990) showed that decreasing the level of fertilization leads to a decrease in free amino acid content but to an increase in reducing sugars which results in increased color development.

The free amino acid composition of the potato tuber must be considered in addition to the reducing sugar content when evaluating any variety for chipping (Habib and Brown, 1956). Lysine, γ-aminobutyric acid, glycine, and glutamine show strong browning potential, the effect of arginine depends on the reducing sugar concentration, while aspartic acid, glutamic acid, asparagine, methionine and tyrosine produce light brown color (Khanbari and Thompson, 1993; Roe and Faults, 1991). Glutamine has an

important role in dark color development at low reducing sugar concentrations while asparagine decreases the gray color intensity (Khanbari and Thompson, 1993).

The role of sucrose in the browning reaction is only marginal. Sucrose enters the Maillard reaction through thermal hydrolysis to yield glucose and fructose, however, the reactions do not go to completion (Leszkowiat et al., 1990; Shallenberger et al., 1959). The hydrolysis reaction is influenced by pH and temperature conditions, being favored by acidic pH (< 4.0)(Roe and Faults, 1991; Leszkowiat et al., 1990). Since the average pH for the potato sap varies between pH 5.5 and 6.5 (Burton, 1966), a slight hydrolysis of sucrose might occur and participate ion the browning reaction (Roe and Faults, 1991). In potato tubers, sucrose levels do not correlate well with chip color (Marquez and Añon, 1986; Sinha et al., 1992). Mazza (1983) found significant correlations between chip color and sucrose levels, however, high variability in the correlation coefficients were reported for cultivars and growing season.

Ascorbic acid can undergo non-enzymatic browning via its oxidative degradation and further reaction with carbonyl compounds or amino groups to yield brown pigments (Sapers, 1993). In model systems, reaction of ascorbic acid and amino acids develops dark coloration during frying (Smith, 1987). However, it has been suggested that ascorbic acid is present in the potato tubers in low concentration to cause unacceptably dark color (Smith, 1987). Mazza (1983) reported low or non-significant correlation between chip color and ascorbic acid.

Reducing sugars and free amino acid content of potato tubers are the major components responsible for color development in fried potatoes. Sucrose and ascorbic acid can participate in the browning reaction under frying conditions while there is lack of information about the contribution of polyphenolic compounds to potato chip color. A better understanding of the role of different potato tuber constituents along with reducing sugars in the non-enzymatic browning reaction would allow the color optimization of potato chips.

REFERENCES

Adam, S.S.; Dhumal, S.S. and Jambhale, N.D. 1991. Structure, nutritional composition, and quality. In *Potato: processing, production, and products*. D.K. Salunkhe, S.S. Adam and S.J. Jadhav Eds. CRC Press. USA.

Aeschbacher, H.U. 1986. Genetic toxicology of browning and caramelization products. In *Genetic toxicology of the diet* - proceedings of a satallite symposium of the 4th international conference of environmental mutagens. Ib Knudsen Ed. Copenhagen, Denmark.

Aeschbacher, H.U. 1990. Anticarcinogenic effect of browning reaction products. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Ap Rees, T. and Morrell, S. 1990. Carbohydrate metabolism in developing potatoes. Am. Potato J. 67:835-847.

Artz, W.E.; Pettibone, C.A.; Augustin, J. and Swanson, B.G. 1983. Vitamin C retention of potato fries blanched in water. J. Food Sci. 48:272-273.

Ashoor, S.H. and Zent, J.B. 1984. Maillard browning of common amino acids and sugars. J. Food Sci. 49:1206-1207.

Augustin, J.; Johnson, S.R.; Teitzel, C.; Toma, R.B.; Shaw, R.L.; True, R.H.; Hogan, J.M. and Deutsch, R.M. 1978. Vitamin composition of freshly harvested and stored potatoes. J. Food Sci. 40:1295-1299.

Baltes, W. 1990. Roest aroma formation - The role of amino acids during the Maillard reaction. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Baxter, J. 1995. Free amino acid stability in reducing sugar systems. J. Food Sci. 60:405-408.

Benzig-Purdie, L.M. and Ratcliff, C.I. 1986. A study of the Maillard reaction by ^{13}C and ^{15}N CP-MAS NMR: Influence of time, temperature, and reactants on major products. Dev. Food Sci. 13:193-205.

Bhalerao, S.D. 1993. A factorial approach to identify reactive constituents and their interactions in multicomponent highly acidic model systems simulating lime juice concentrate (32/B). J. Sci. Food Agric. 63(1):91-94.

Bjeldanes, L.F. and Chew, H. 1979. Mutagenicity of 1,2-dicarbonyl compounds: maltol, kojic acid, diacetyl and related substances. Mutat. Res. 67, 367-371.

Bobbio, P.A.; Bobbio, F.O. and Trevisan, L.M.V. 1973. Estudos sobre a reacao de Maillard. I. Efeitos da temperatura e do pH. An. Acad. Brasil. Cienc. 45:419.

Bohart, G.S. and Carson, J.F. 1955. Effect of trace metals, oxygen and light on the glucose-glycine browning reaction. Nature 175:470.

Brown, C.R. 1993. Origin and history of the potato. Am. Potato J. 70:363.

Burton, W.G. 1966. *The Potato*. 2nd edition. Veenman, Wageningen.

Burton, W.G. 1989. *The Potato*. 3rd Edition. Longman Scientific & Technical. England

Burton, W.G. Wilson, A.R. 1978. The sugar content and sprout growth of tubers of potato cultivar Record, grown in different localities, when stored at 10, 2 and 20°C. Potato Res. 21:145-161.

Chan, R.I. Stich, H.F. Rosin, M.P. and Powrie, W.D. 1982. Antimutagenic activity of browning reaction products. Can. lett. 15: 27-33.

Cilliers, J.J.L. Singleton, V.L. 1989. Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. J. Agric. Food Chem. 37:890-896.

Cilliers, J.J.L. Singleton, V.L. 1990. Nonenzymic autoxidative reactions of caffeic acid in wine. Am. J. Enol. Vitic. 41:84-86.

Cilliers, J.J.L. Singleton, V.L.1991.Characterization of the products of nonenzymic autoxidative phenolic reactions in a caffeic acid model system. J. Agric Food Chem. 39: 98-1303.

Claassen, P.A.M.; Budde, M.A.W. and Van Calker, M.H. 1993. Increase in phosphorylase activity during cold-induced sugar accumulation in potato tubers. Potato Res. 36:205-217.

Cooke, J.R. 1974. The chemical estimation of vitamin C. In *Vitamin C*. G.G. Birch and K.J. Parker Eds. Applied Science Publishers, Ltd. London.

Cottrell, J.E.; Duffus, C.M.; Paterson, L.; Mackay, G.R.; Allison, M.J. and Bain, H. 1993. The effect of storage temperature on reducing sugar accumulation and the

activities of three amylolytic enzymes in tubers of the cultivated potato, *Solanum tuberosum* L. Potato Res. 36:107-117.

Danehy, J.P. 1986. Maillard reactions: nonenzymatic browning in food systems with special reference to the development of flavor. Adv. Food Res. 30:77-138.

Dao, L. and Friedman, M. 1992. Chlorogenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry. J. Agric. Food Chem. 40:2152-2156.

Dao, L. and Friedman, M. 1994. Chlorophyll, chlorogenic acid, glycoalkaloid and protease inhibitor content of fresh and green potatoes. J. Agric. Food Chem. 42:633-639.

Davies, A.M.C. 1977. The free amino acids of tubers of potato varieties grown in England and Ireland. Potato Res. 20:9-21.

Davies, H.V. and Viola, R. 1994. Control of sugar balance in potato tubers. In *The molecular and cellular biology of potato*. 2nd Edition. W.R. Belknap, M.E. Vayda and W.D. Park Eds: CAB International, Wallingford, UK

Dillls, W.L. 1993. Protein fructosylation: fructose and the Maillard reaction. Am. J. Clin. Nutr. 779S-787S.

Duplessis, P.M.; Marangoni, A.G. and Yada, R.Y. 1996. A mechanism for low temperature induced sugar accumulation in stored potato tubers: the potential role of the alternative pathway and invertase. Am. Potato J. 73:483-494.

Eichner, K. Wolf, W. 1983. Maillard reaction products as indicator compounds for optimizing drying and storage conditions Carrots, varieties. ACS Symposium series - American Chemical Society. 215:317-333.

Einarsson, H. 1987. Lebensm. Wiss. Und Technol. 20:51-55.

Eksari, I. Bonestroo, M.H. Jongen, W.M.F. and Pilnik, W. 1989. Mutagenicity and possible occurrence of flavonol aglycones in heated orange juices. Food Chem. 31(4), 289-294.

Eppendorfer, W.H. and Billie, S.W. 1996. Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower and potatoes as influenced by nitrogen fertilization and phosphorous and potassium deficiency. J. Sci. Food Agric. 71:449-458.

Es, A. van. Hartmans, K.J. 1981. Sugars and starch during tuberization, storage and sprouting. In *Storage of potatoes: post-harvest behaviour, store design, storage practice, handling*. Wageningen, Centre for Agricultural Publ. and Documentation.

Eskin, N.M., 1990. Biochemistry of food processing: browning reactions in foods. In *Biochemistry of Foods*. Second Edition. Academic Press Inc. USA.

Feather, M.S. 1994. Dicarbonyl sugar derivatives and their role in the Maillard reaction. In *ACS Symposium series: Thermally generated flavors - Maillard, microwave and extrusion processes*. T.H. Parliament, M.J. Morello and R.J. McGorrin Eds. American Chemical Society. Washington DC, USA.

Friedman, M. 1996. Nutritional value of proteins from different food sources. J. Agric. Food Chem. 44:6-29.

Friedman, M. 1997. Chemistry, biochemistry and dietary role of potato polyphenols. J. Agric. Food Chem. 45:1523-1540.

Fry, L.K. and Stegink, L.D. 1982. Formation of Maillard reaction products in parenteral alimentation solutions. J. Nutrition 112:1631-1637.

Fuller, T.J. and Hughes, J.C. 1984. Factors influencing the relationships between reducing sugars and fry colour of potato tubers of cv. Record. J. Food Technol. 19:455-467.

Griffiths, D.W.; Bain, H. and Dale, M.F.B. 1995. Photo-induced changes in the total chlorogenic acid content of potato (Solanum tuberosum) tubers. J. Sci. Food Agric. 68:105-110.

Habib, A.T. and Brown, H.D. 1956. Factors influencing the color of potato chips. Food Tech. 10(7):332-336.

Handwerk, R.L. and Coleman, R.L. 1988. Approaches to the citrus browning problem. J. Agric Food Chem. 36 (1):231-236.

Hayase, F. 1996. Scavenging of active oxygen by melanoidins. In *The Maillard Reaction - Consequences for the chemical and life sciences*. Chp. 5. R. Ikan Ed. John Wiley & Sons. West Sussex. Great Britain.

Hayase, F. And Kato, H. 1986. Low molecular Maillard reaction products and their formation mechanisms. In *Amino-carbonyl reactions in food and biological systems*. Proceedings of the 3rd international symposium on the Maillard reaction. M. Fujimaki, M. Namiki, and H. Kato Eds. Shizuoka. Japan.

Hayase, F.; Kim, S.B. and Kato, H. 1986. Analyses of the chemical structures of melanoidins by ^{13}C NMR, ^{13}C and ^{15}N CP-MAS spectrometry. Agric. Biol. Chem. 50:1951-1957.

Hayase, F.; Hirashima, S.; Okamoto, G. and Kato, H. 1990. Scavenging of active oxygens by melanoidin. In *The Maillard reaction in food processing, human nutrition and physiology.* P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Hayashi, T. and Namiki, M. 1980. Formation of two carbon sugar fragment at an early stage of the browning reaction with amine. Agric. Biol. Chem. 44:2575-2580.

Hayashi, T. and Namiki, M. 1981. On the mechanism of free radical formation during browning reaction of sugar with amino compounds. Agric. Biol. Chem. 45:933-939.

Hayashi, T. and Namiki, M. 1986. Role of sugar fragmentation in the Maillard reaction. In *Amino-carbonyl reactions in food and biological systems.* Proceedings of the 3rd international symposium on the Maillard reaction. M. Fujimaki, M. Namiki, and H. Kato Eds. Shizuoka. Japan.

Hayashi, T.; Ohta, Y.; Namiki, M. 1977. Electron spin resonance spectral study on the structure of novel free radical products formed by the reactions of sugars with amino acids. J. Agric. Food Chem. 25:1282-1287.

Hayashi, T.; Namiki, M. and Tsuji, K. 1983. Formation mechanism of the free radical product and its precursor by the reaction of dehydro-L-ascorbic acid with amino acid. Agric. Biol. Chem. 47:1955-1960.

Hayashi, T.; Hoshi, Y. and Namiki, M. 1984. Purple pigment produced by the reaction of dehydro-L-ascorbic acid with phenylalanine. Agric. Biol. Chem. 48:2377-2378.

Hayashi, T.; Mase, S. and Namiki, M. 1985. Formation of the N,N=-dialkylpyrazine cation radical from glyoxal dialkylimine produced on reaction of a sugar with an amine or amino acid. Agric. Biol. Chem. 49:3131-3137.

Hawkes, J.G. 1990. The Potato - Evolution, Biodiversity and Genetic Resources. Belhaven Press. London, Great Britain.

Herrman, T.J.; Love, S.L.; Shafii, B. and Dwelle, R.B. 1996. Chipping performance of three processing potato cultivars during long-term storage at two temperature regimes. Am. Potato J. 73:411-425.

Hill, L.M.; Reimholz, Rroder, R.; Nielsen, T.H. and Stitt, M. 1996. The onset of sucrose accumulation in cold stored potato tubers is caused by an increase rate of sucrose synthesis and coincides with low levels of hexose-phosphates, an activation of sucrose phosphate synthase and the appearance of an new form of amylase. Plant Cell Env. 19:1223-1237.

Ho, C. 1996. Thermal generation of Maillard aromas. In *The Maillard Reaction - Consequences for the chemical and life sciences*. Chp. 5. R. Ikan Ed. John Wiley & Sons. West Sussex. Great Britain.

Hodge, J.E. 1953. Dehydrated foods: chemistry of browning reactions in model systemsric. Agric. Food Chem. 1:929-943.

Hodge, J.E. and Rist, C.E. 1953. The Amadori rearrangement under new conditions and its significance for non-enzymatic browning reactions. J. Am. Chem. Soc. 75:316-322.

Hodge, J.E.; Fisher, B.E. and Nelson, E.C. 1963. Dicarbonyls, reductones and heterocyclics produced by the reactions of reducing sugars with secondary amine salts. Proc. Am. Soc. Brew. Chem. 84-92.

Hoff, J.E.; Jones, C.M.; Wicox, C.E. and Castro, M.D. 1971. The effect of nitrogen fertilization on the composition of the free amino acid pool of potato tubers. Am. Potato J. 48:390-394.

Hughes, J.C. and Fuller, T.J. 1984. The effect of nitrogen during growth on sugar content and fry colour of cv. Record during extended storage at 10/C. Abstracts of conference papers of the 9th Triennial Conference of the European Association of Potato Research.

Hurrel, R.F. and Finot, P.A. 1984. Nutritional consequences of the reactions between proteins and oxidized polyphenols. In Nutritional and toxicological aspects of food safety. M. Friedman Ed. Plenum, NY.

Hurrel, R.F.; Finot, P.A.; and Cuq, J.L. 1982. Protein-polyphenol reactions. 1. Nutritional and metabolic consequences of the reaction between oxidized caffeic acid and the lysine residues of casein. Br. J. Nutr. 47:191-211.

Isherwood, F A. 1976. Mechanism of starch-sugar interconversion in solanum tuberosum [potatoes]. Phytochem. 15:33-41.

Isla, M.I. Vattuone, M.A. Sampietro, A.R. 1991. Proteinaceous inhibitor from Solanum tuberosum invertase. Phytochem. 30:739-743.

Jackson, M.T. 1987. Breeding strategies for True Potato Seed. In *The production of new potato varieties*. G.J. Jellis and D.E. Richardson Eds. Cambridge University Press. Great Britain.

Jadhav, S.J.; Mazza, G. and Desai, U.T. 1991. Post-harvest handling and storage. In *Potato: production, processing, and products*. D. K. Salunkhe, S. S Adam, and S. J. Jadhav Eds. CRC Press. Boca Raton, FL.

Jagerstad, M. Grivas, S. Olsson, K. Reutersward, A.L. Negishi, C. and Sato, S. 1986. Formation of food mutagens via Maillard reactions. In *Genetic toxicology of the diet* - proceedings of a satallite symposium of the 4th international conference of environmental mutagens. Ib Knudsen Ed. Copenhagen, Denmark.

Johnson, P.E.; Lykken, G.; Mahalko, J.; Milne, D.; Inman, L.; Garcia, W.J. and Inglett, G.E. 1983. The effect of browned and unbrowned corn products on absorption of zinc, iron, and copper in humans. ACS Symp. Series 215:3349-3360.

Kalyanaraman, B., Premovic, P.I. and Sealy, R.C. 1987. Semiquinone anion radicals from addition of amino acids, peptides, and proteins to quinone derived from oxidation of catechols and catecholamines. J. Biol. Chem. 262:11080-11087.

Kato, H. and Tsuchida, H. 1981. Estimation of melanoidin structure by pyrolysis and oxidation. Prog. Food Nutr. Sci. 5:147-156.

Kato, K.; Watanabe, K. and Sato, Y. 1981. Effect of metals on the Maillard reaction of ovalbumin. J. Agric. Food Chem. 29:540.

Khanbari, O.S. and Thompson, A.K. 1993. Effects of amino acids and glucose on the fry colour of potato crisps. Potato Research 36:359-364.

Kim, S.B.; Hayase, F. and Kato, H. 1985. Decolorization and degradation products of melanoidins on ozonolysis. Agic. Biol. Chem. 49:785-792.

Kim, S.B.; Hayase, F. and Kato, H. 1986. Desmutagenic effects of melanoidins against amino acid and protein pyrolysates. Dev. Food Sci. 13:383-392.

Kincal, N.S. and Giray, C. Kinetics of ascorbic acid degradation in potato blanching. Intl. J. Food Sci. Tech. 22:249-254.

Koppanyi, T.; Vivino, A.E. and Veitch, Jr., F.P. 1945. A reaction of ascorbic acid with amino acids. Science. 101:541-542.

Kurata, T.; Fujimaki, M. and Sakurai, 1973. Red pigment produced by the oxidation of dehydro-L-ascorbic acid with amino acid. Agric. Biol. Chem. 37:1471-1477.

Labuza, T.P. 1994. Interpreting the complexity of the kinetics of the Maillard reaction. In *Maillard reactions in chemistry, food and health*. The proceedings of the 5th international symposium on the Maillard reaction. T.P. Labuza, G.A. Reineccius, Monnier, V., O'Brien, J. and Baynes, J. Eds. The Royal Society of Chemistry. Cambridge, Great Britain.

Larisch, B. Pischetsrieder, M. and Severin, T. 1996. Reactions of dehydroascorbic acid with primary aliphatic amines including N alpha-acetyllysine. J. Agric Food Chem. 44:1630-1634.

Ledl, F. 1990. Chemical pathway of the Maillard reaction. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Ledl, F. and Schleicher, E. 1990. New aspects of the Maillard reaction in foods and in the human body. Angewandte Chemie 29(6):565-706.

Ledl, F.; Fritsch, G.; Hiebl, J.; Pachmayr, O. And Severin, T. 1986. Degradation of Maillard products. In *Amino-carbonyl reactions in food and biological systems*. Proceedings of the 3rd international symposium on the Maillard reaction. M. Fujimaki, M. Namiki, and H. Kato, Eds. Shizuoka. Japan.

Lee, I.E.; Chuyen, N.V.; Hayase, F. and Kato, H. 1994. Desmutagenicity of melanoidins against various kinds of mutagens and activated mutagens. Biosci. Biotechnol. Biochem. 58:18-23.

Leszczynski, W. 1989a. Potato tubers as a raw material for processing and nutrition. In *Potato Science and Technology*. Chp. 2. G. Lisinska and W. Leszczynski Eds. Elsevier Applied Science. London. Great Britain.

Leszczynski, W. 1989b. Introduction. In *Potato Science and Technology*. Chp. 1. G. Lisinska and W. Leszczynski Eds. Elsevier Applied Science. London. Great Britain.

Leszkowiat, M.J.; Baricello, V.; Yada, R.Y.; Coffin, R.H.; Lougheed, E.C. and Stanley, D.W. 1990. Contribution of sucrose to nonenzymatic browning in potato chips. J. Food Sci. 55:281-282.

Lingnert, H. 1990. Development of the Maillard reaction during food processing. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Lingnert, H. and Eriksson, C.E. 1980. Antioxidative Maillard reaction products. I. Products from sugars and free amino acids. J. Food Process. Preserv. 4:161-172.

Linnemann, A.R.; van Es, A. and Hartmans, K.J. 1985. Changes in content of L-ascorbic acid, glucose, fructose, sucrose and total glycoalkaloids in potatoes (cv. Bintje) stored at 7, 16 and 28°C. Potato Research 28:271-278.

Malmberg, A. and Theander, O. 1984. Free and conjugated phenolic acids and aldehydes in potato tubers. Swedish J. Agric. Res. 14:119-125.

Marquez, G. and Añon, M.C. 1986. Influence of reducing sugars and amino acids in the color development of fried potatoes. J. Food Sci. 51:157-160.

Mazza, G. 1983. Correlations between quality parameters of potatoes during growth and long-term storage. Amer. Potato J. 60:145-159.

Mondy, N.I. and Gosselin, B. 1989. Effect of irradiation on discoloration, phenols and lipids of potatoes. J. Food Sci. 54:982-984.

Mondy, N.I. and Munshi, C.B. 1993. Effect of maturity and storage on ascorbic acid and tyrosine concentrations and enzymatic discoloration of potatoes. J. Agric. Food Chem. 41:849-852.

Mottram, D.S. 1994. Flavor compounds formed during the Maillard reaction. In *ACS Symposium series: Thermally generated flavors - Maillard, microwave and extrusion processes*. T.H. Parliament, M.J. Morello and R.J. McGorrin Eds. American Chemical Society. Washington DC, USA.

Mulder, E.G. and Bakena, K. 1956. Effect of nitrogen, phosphorus and magnesium nutrition of potato plants on the content of free amino acids and on the amino acid composition of the protein of the tubers. Plant Soil 7:135-166.

McCay, C.M.; McCay, J.B. and Smith, O. 1987. The nutritive value of potatoes.In *Potato processing*. Fourth edition. W.F. Talburt and O. Smith Eds. The AVI Publishing Co., Inc. Westport, CT.

Nafisi, K. and Markakis, P. 1983. Inhibition of sugar-amine browning by aspartic and glutamic acids. J. Agric. Food Chem. 31:1115-1117.

Nakabayashi, T. and Shibata, A. 1967. Formation of red color by dehydrated food in the presence of ascorbic acid. Nippon Shokuhin Gakkaishi. 14:11-14.

Namiki, M. and Hayashi, T. 1975. Development of novel free radicals during the amino-carbonyl reaction of sugars with amino acids. J. Agric. Food Chem. 23:497-491.

Namiki, M. and Hayashi, T. 1981. Formation of novel free radical products in an early stage of Maillard reaction. Prog. Food Nutr. Sci. 5: 81-91.

Namiki, M. and Hayashi, T. 1983. A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. ACS symposium series 215:21-46.

Namiki, M. 1988. Chemistry of Maillard reactions: Recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. Adv. Food Res. 32:115-184.

Niederhauser, J.S. 1993. International cooperation and the role of the potato in feeding the world. Am. Potato J. 70:385

O'Brien, J. and Morrissey, P.A. 1989. Nutritional and toxicological aspects of the Maillard browning reaction in foods. Crit. Rev. Food Sci. Nutr. 28:211-248.

Ogawa, M. and Uritiani, L. 1979. Tissue browning of potato tubers induced by gamma irradiation. Agric. Biol. Chem. 34:870-877.

Ohtsuka, M.; Kurata, T. and Arakawa, N. 1986. Isolation and characterization of a degradation product derived from 2,3-diketo-L-gulonic acid. Dev. Food Sci. 13:77-84.

Onyeneho, S.N. and Hettiarachchy, N.S. 1993. Antioxidant activity, fatty acids and phenolic acids compositions of potato peels. J. Sci. Food Agric. 62:345-350.

Pandell, A.J. 1983. Mechanism of Fe(III)-catalyzed reaction for pyrocatechase. J. Org. Chem. 48:3908-3912.

Pendharkar, M.B. and Nair, P.M. 1987. Alteration of phenylpropanoid metabolism in gamma irradiated potatoes. Potato Res. 30:589-601.

Pendharkar, M.B. and Nair, P.M. 1995. A comparative study of phenylpropanoid metabolism in gamma irradiated and unirradiated potato tubers. Potato Res. 38:187-198.

Pham, C.B. Cheftel, J.C. 1990. Influence of salts, amino acids and urea on the non-enzymatic browning of the protein-sugar system. Food Chem. 37:251-260.

Pressey, R. 1969. Role of invertase in the accumulation of sugars in coldstored potatoes. Am. Potato J. 46:291-297.

Pritchard, M.K. and Adam, L.R. 1994. Relationships between fry color and sugar concentration in stored Russet Burbank and Shepody potatoes. Amer. Potato J. 71:59-68.

Quattrucci, E. 1988. Heat treatments and nutritional significance of Maillard reaction products. In *Nutritional and toxicological aspects of food processing* - proceedings of an international symposium. Rome, Italy.

Ramamurthy, M.S.; Maiti, B.; Thomas, P. and Nair, P.M. 1992. High-Performance liquid chromatography determination of phenolic acids in potato tubers (*Solanum tuberosum*) during wound healing. J. Agric. Food Chem. 40:569-572.

Rao, N.M. and Rao, M.N. 1972. Effect of non-enzymatic browning on the nutritive value of casein-sugar system. J. Food Sci. Technol. 9:66.

Reeve, R.M.; Hautala, E. and Weaver, M.L. 1969. Anatomy and compositional variation within potatoes. Phenolics, enzymes and other minor components. Am. Potato J. 46:374-386.

Reineccius, G.A. 1990. The influence of Maillard reactions on the sensory properties of foods. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Renz, A.; Merlo, L. and Stitt, M. 1993. Partial purification from potato tubers of three fructokinases and three hexokinases which show differing organ and development specificity. Planta 190:156-165.

Reyes, F.R.; Poocharden, B. and Wrolstad, R.E. 1982. Maillard browning reaction of sugar-glycine model systems: changes in sugar concentration, color and appearance. J. Food Sci. 47:1376-1377.

Richardson, T. and Hyslop, D.B. 1985. "Enzymes" from Food Chemistry. O. R. Fennema Ed. Marcel Dekker, Inc. New York.

Rizzi, G.P. 1994. The Maillard reaction in foods. In *Maillard reactions in chemistry, food and health*. The proceedings of the 5th international symposium on the Maillard reaction. T.P. Labuza, G.A. Reineccius, Monnier, V., O'Brien, J. And Baynes, J. Eds. The Royal Society of Chemistry. Cambridge, Great Britain.

Roe, M.A.; Faults, R.M. and Belsten, J.L. 1990. Role of reducing sugars and amino acids in fry colour of chips from potatoes grown under different nitrogen regimes. J. Sci Food Agric. 52:207-214.

Roe, M.A. and Faults, R.M. 1991. Color development in a model system during frying: Role of individual amino acids and sugars. J. Food Sci. 56:1711-1713.

Sapers, G.M. 1993. Browning of foods: Control by sulfites, antioxidants and other means. Food Technol. 47(10): 75-84.

Shallenberger, R.S., Smith, O. and Treadway, R.H. 1959. Role of the sugars in the browning reaction in potato chips. J. Agric. Food Chem. 7:274-277.

Shallenberger, R.S. and Birch, G.G. 1975. Sugar Chemistry. AVI Publishing Co. Inc., Westport, CT.

Sieczka, J.B. and Maatta, C. 1986. The effects of handling on chip color and sugar content of potato tubers. Am. Potato J. 63:363-372.

Sinha, N.K.; Cash, J.N. and Chase, R.W. 1992. Differences in sugars, chip color, specific gravity and yield of selected potato cultivars grown in Michigan. Am. Potato J. 69:385-389.

Smith, H.B. 1927. Chromosome counts in the varieties of *Solanum tuberosum* and allied wild species. Genetics 12: 84-92.

Smith, O. 1987. Potato chips. In *Potato processing*. 4th edition. W.F. Talburt and O. Smith Eds. The AVI Publishing Co., Inc. Westport, CT.

Sowokinos, J.R. and Preston, D.A. 1988. Maintenance of potato processing quality by chemical maturity monitoring (CMM). Station Bulletin 586. Minnesota Agricultural Experiment Station. University of Minnesota.

Sowokinos, J.R. 1994. Post-harvest regulation of sucrose accumulation in transgenic potatoes: role and properties of potato tuber UDP-glucose pyrophosphorylase. In The molecular and cellular biology of the potato. 2nd Edition. W.R. Belknap, M.E. Vayda and W.D. Park Eds. CAB international. Wallingford, UK

Spark, A.A. 1969. Role of amino acids in non-enzymic browning. J. Sci. Food Agric. 20:308.

Stich, H.F. Rosin, M.P. Wu, C.H. and Powrie, W.D. 1981. Clastogenicity of furans found in food. Cancer letters. 113: 89-95.

Talburt, W.F. 1987. History of potato processing. In *Potato Processing*. 4th Edition. W.F. Talburt and O. Smith Eds.The AVI Publishing Co., Inc. Westport, CT.

Talburt, W.F.; Schwimmer, S. and Burr, H.K. 1987. Structure and chemical composition of the potato tuber. In *Potato processing*. 4th Edition. W.F. Talburt and O. Smith Eds.The AVI Publishing Co., Inc. Westport, CT.

Talley, E.A. and Porter, W.L. 1970. Chemical composition of potatoes. Relationship of the free amino acid concentrations to specific gravity and storage time. Am. Potato J. 47:214-224.

Trevanion, S.J. and Kruger, N.J. 1991. Effect of temperature on the kinetic properties of pyrophosphate: fructose 6-phosphate phosphotransferase from potato tuber. J. Plant Physiol. 137:753-759.

Vernin, G. and Parkanyi, C. 1982. Mechanism of formation of heterocyclic compounds in Maillard and pyrolysis reactions. In *Chemistry of heterocyclic compounds in flavours and aromas*. G. Vernin Ed. Ellis Horwood Limited. Chichester, England.

Weisburger, J.H. 1994. Specific Maillard reactions yield powerful mutagens and carcinogens. In *Maillard reactions in chemistry, food and health*. The proceedings of the 5th international symposium on the Maillard reaction. T.P. Labuza, G.A. Reineccius, Monnier, V., O'Brien, J. and Baynes, J. Eds. The Royal Society of Chemistry. Cambridge, Great Britain.

Whitfield, F.B. 1992. Volatiles from interactions of Maillard reactions and lipids. Crit. Rev. Food Sci. Nutr. 31(1/2):1-58.

Wills, R.B.H. 1987. Composition of Australian fresh fruit and vegetables. Food Tech. Aust. 39:523-526.

Wills, R.B.H. and Silalahi, J. 1990. Effect of storage temperature on loss of vitamin C in potato crisps. J. Sci. Food Agric. 53:275-278.

Wolfrom, M.L.; Kashimura, N. and Horton, D. 1974. Factors affecting the Maillard browning reaction between sugars and amino acids. Studies on the nonenzymic browning of dehydrated orange juice. J. Agric. Food Chem. 22:796-800.

Wong, J.W. and Shibamoto, T. 1996. Genotoxicity of Maillard reaction products. In *The Maillard Reaction - Consequences for the chemical and life sciences*. Chp. 7. R. Ikan Ed. John Wiley & Sons. West Sussex. Great Britain.

Yamaguchi, T. and Nakagawa, K. 1983. Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivatives. Agr. Biol. Chem. 47: 2461-2465.

Yaylayan, V.A. and Forage, N.G. 1992. A kinetic model for the reaction of tryptophan with glucose and mannose - the role of diglycation in the Maillard reaction. Food Chem. 44:201-208.

Yaylayan, V.A. and Hughes-Despointes, A. 1994. Chemistry of Amadori rearrangements products: analysis, synthesis, kinetics, reactions and spectroscopic properties. Crit. Rev. Food Sci. Nutr. 34: 321-369.

Yen, G.C. Tsai, L.C. and Lii, G.C. 1992. Antimutagenic effect of Maillard browning products obtained from amino acids and sugars. Food Chem. Toxicol. 30, 127-132.

Yen G.C. and Tsai, L.C. 1993. Antimutagenicity of a partially fractionated Maillard reaction product. Food Chem. 47: 11-15.

Yen, G.C. and Hsieh, P.P. 1994. Mechanism of antimutagenic effect of Maillard reaction products prepared from xylose and lysine. In *Maillard reactions in chemistry, food and health*. The proceedings of the 5th international symposium on the Maillard reaction. T.P. Labuza, G.A. Reineccius, Monnier, V., O'Brien, J. And Baynes, J. Eds. The Royal Society of Chemistry. Cambridge, Great Britain.

Zhang, L.; Porter, G.A. and Bushway, R.J. 1997. Ascorbic acid and glycoalkaloid content of Atlantic and Superior potato tubers as affected by supplemental irrigation and soil amendments. Am. Potato J. 74:285-304.

CHAPTER 2. PART II
POTENTIAL USE OF ANTHOCYANINS AS NATURAL COLORANTS

COLOR ADDITIVES

Color additives are defined as any dye, pigment or substance capable of imparting color when added or applied to a food, drug or cosmetic (21 CFR Chp 1, Sec. 70.3). Color is a vital constituent of foods because is one of the first characteristics perceived by the senses and is used by consumers for the rapid identification and ultimate acceptance of foods (Noonan, 1972).

Color additives have long been used as a means of enhancing the aesthetic value of foods, beverages and cosmetics. Unfortunately, color additives were also used to adulterate foods and other products (Hallagan, 1991).

The discovery of synthetic dyes, derived from coal-tar, by Sir William Henry Perkins in 1856 prompted the search for other dyes since synthetic dyes were superior to many natural extracts in tinctorial value, stability and uniformity (Newsome, 1986). By 1900, nearly 700 colors had been synthesized and a major industry developed in the field of coal-tar dyes (Rumore et al., 1992). However, there was not a careful selection of food dyes and the public had minimal protection from harmful color additives (Rumore et al, 1992). Toxic mineral salts which contained lead, copper or arsenic were commonly added to foods as coloring compounds, thus representing a significant health risk (Hallagan, 1991).

The first comprehensive legislation to regulate synthetic dyes intended for foods in the U.S. was the Federal Pure Food and Drug Act of 1906. The law permitted seven dyes for use in foods and established voluntary certification (Rumore et al., 1992; Noonan, 1972). The purposes of color regulations were to keep poisons out of foods and to prevent cheating the consumers by deceptive use of color (Damon and Janssen, 1973). The Food, Drug and Cosmetic Act of 1938 superseded the Act of 1906 and broadened the scope of certified colors, creating three categories: FD&C Colors

(acceptable for foods, drugs and cosmetics), D&C Colors (for use in drugs and cosmetics only) and External D&C Colors (for external use only). The Act required that a color be harmless at any level, certification was mandatory and was placed under the jurisdiction of the Food and Drug Administration (FDA). Products that contained unsafe color additives were deemed as adulterated and prohibited from interstate commerce (Rumore et al., 1992; Noonan, 1972).

The Color Additive Amendments of 1960 set up uniform rules for all permitted colors, provided for the listing of color additives which must be certified and color additives exempt from certification, defined the term "natural" color, and included the Delaney clause. The FDA had the authority to classify the color additives as certified and uncertified, determine whether a color additive may be listed for use in foods, drugs and cosmetics, and set tolerances and conditions on their use (Noonan, 1972). The amendment placed the color additives already in use on a provisional list and permitted their continued use pending the completion of scientific investigations needed to determine their suitability for use and petition their permanent listing. No color could be placed in the market until scientific testing had proved that it would not be harmful in the uses and quantities proposed, that it accomplished the intended effect, and that its use would not result in deception of consumers (Rumore et al., 1992; Damon and Janssen, 1973). All colors for use in foods, drugs and cosmetics were required to undergo pre-marketing safety clearance. The burden of proving safety was shifted from government to industry. Because of the great expense involved in pharmacological and chemical experiments necessary for permanent listing, only colors of economical importance for the industry undergo pertinent testing while many of the previously certifiable colors were eliminated by default (Rumore et al., 1992). From 1960 to 1976 the FDA either has approved or terminated the provisional listing of about 75 color additives (Rumore et al., 1992; Noonan, 1972).

The law requires FDA to hold all color additives (certified and exempt from certification) to the same safety standard, although in practice FDA has required much more stringent safety evaluations of certified color additives. Currently approved

certified color additives are among the most thoroughly studied food ingredients (Hallagan, 1991).

Certified Colorants

Certified colors are compounds of known structure that are produced by chemical synthesis and that conform to high purity specifications established by the FDA (Meggos, 1994). A representative sample of each batch must be submitted to the FDA and tested for compliance because of the complex chemical reactions that take place during synthesis. The specifications, uses and restrictions are described in Title 21, Parts 74 and 82 of the *Code of Federal Regulations*.

The color additive certification process assures the safety, quality, consistency and strength of the color additive prior to its use in foods (FDA, 1993).

Certifiable color additives are used widely because their coloring ability is more intense than those derived from natural products. In addition, certifiable color additives are more stable, provide better color uniformity and blend together easily to provide a wide range of hues and generally do not impart undesirable flavors to foods. Regulations known as Good Manufacturing Practices limit the amount of color added to foods.

There are seven approved certified color additives (Table 2.3) that may be added to foods which represent the primary source of commercial colorants (Hallagan, 1991; Newsome, 1986). Certifiable color additives are available for use in food as either FD&C dyes and FD&C lakes.

FD&C dyes

FD&C dyes are water soluble compounds and insoluble in nearly all organic solvents (Noonan, 1972). They are represented by four chemical classes (azo, trimethyl-methane, xanthene and indigoid dyes). Their water solubility is conferred by one or more sulfonic acid groups which also reduces their oil solubility. Unsulfonated dyes would be oil soluble but lack of sulfonation seems to go along with toxicity (Noonan, 1972).

Table 2.3: Regulatory status of the FD&C color additives in the United States

Color Additive	Hue	Dye	Lake	Food Uses
FD&C Red # 40	Orange-red	Permanently listed for all uses at GMP 21 CFR 74.340, 74.1340 and 74.2340	Permanently listed for all uses at GMP 21 CFR 74.340, 74.1340, 74.2340	Gelatins, puddings, dairy products, confections, beverages, condiments
FD&C Blue # 1	Bright blue	Permanently listed for all uses at GMP 21 CFR 74.101, 74.1101 and 74.2101	Provisionally listed for all uses at GMP 21 CFR 82.101	Beverages, condiments confections, icings, syrups dairy products powders
FD&C Blue # 2	Royal Blue	Permanently listed for all uses at GMP 21 CFR 74.102, 74.1102	Provisionally listed for all uses at GMP 21 CFR 82.102	Baked goods, cereals, snack foods, ice-cream, confections, cherries
FD&C Green # 3	Sea Green	Permanently listed for all uses at GMP 21 CFR 74.203, 74.1203, and 74.2203	Provisionally listed for all uses at GMP 21 CFR 82.203	Beverages, puddings, ice-cream, sherbet, cherries, confections, baked goods, dairy products
FD&C Yellow # 5	Lemon Yellow	Permanently listed for all uses at GMP 21 CFR 74.705, 74.1705, and 74.2705	Provisionally listed for all uses at GMP 21 CFR 82.705,	Custards, beverages, ice-cream, confections, preserves, cereals
FD&C Yellow # 6	Orange	Permanently listed for all uses at GMP 21 CFR 74.706, 74.1706, and 74.2706	Provisionally listed for all uses at GMP 21 CFR 82.706,	Cereals, baked goods, snack foods, ice-cream, beverages, dessert powders
FD&C Red # 3	Cherry Red	Permanently listed for all foods and ingested drugs at GMP 21 CFR 74.303, 74.1303	Delisted for all uses Fed. Reg. 55:3516 21 CFR 81.10(u)	Cherries in fruit cocktail and canned fruits for salads, confections, baked goods, dairy products, snack foods

Under anhydrous conditions, the dyes can be dissolved in glycerine and propylene glycol. Good coloring technique recommends that the dyes be solubilized before addition to the colored product. However, it can be added as a dry powder to the batch and its solubility will depend upon the added moisture and heat to dissolve the color during processing (Noonan, 1972).

Dyes are available as powders, granular, plating colors, blends, liquids and pastes. The best form for any specific use will be dictated by the nature of the product to be colored, the process conditions and volume of color used (Noonan, 1972). Good manufacturing practices suggests levels lower than 300 ppm (Newsome, 1986).

Certified food colorants are stable to most processes used by the food industry. However, instability has been shown in combination with reducing agents and retorted protein materials. Color fading occurs in the presence of metals (zinc, tin, aluminum and copper) and ascorbic acid, while azo dyes are capable to cause can corrosion at levels of 50-150 ppm (Noonan, 1972). The pH value must be considered when choosing a colorant since it can result in shifts in shade, variations in shelf life, changes in solubility and loss of tinctorial strength. For example, FD&C Red # 3 precipitates from acid solutions whereas FD&C Green # 3 turns blue under alkaline conditions (Marmion, 1991).

FD&C lakes

FD&C lakes are water insoluble pigments made by chemically adsorbing the dyes onto an insoluble substrate, alumina hydrate or aluminum hydroxide (Meggos, 1994). Lakes impart color by dispersing them in the medium being colored (Marmion, 1991). Important commercial applications include oil-based products and products which do not contain sufficient moisture for dye solubilization (Newsome, 1986).

The shade, dispersibility and tinctorial strength of lakes are highly dependent on the conditions used in their manufacture (pH, temperature, agitation) as well as their physical properties, including their particle size and crystal structure (Marmion, 1991; Dziezak, 1987). Color uniformity requires careful process control and differences in

shade can exist among manufactures (Noonan, 1972).

Properties of lakes that enhance their usefulness include their opacity, ability to be incorporated into products in dry state, relative insolubility and superior stability to heat and light; however, they are generally more expensive than dyes (Meggos, 1994; Marmion, 1991). The tinctorial strength improves with smaller particle size and increased number of particles because of the greater surface area which permits more light to be reflected, leading to enhanced color efficiency (Dziezak, 1987). Lakes are stable in the pH ranges of 3.5 to 9.5 but outside this range the substratum breaks down, releasing the dye. Lakes do not have a specified minimum dye content but typical lakes contain 10-40% pure dye and 15-25% moisture (Marmion, 1991). The use of aluminum hydroxide as substratum results in lakes being insoluble to nearly all solvents (Noonan, 1972).

Safety of FD&C Color Additives

The safety of color additives has been of concern to consumers and has in some instances led the FDA to ban several colors. In 1976, amaranth (FD&C Red # 2), FD&C Red # 4 and carbon black were delisted because of unresolved safety issues (Rumore et al., 1992). Several other provisional colors have been revoked for safety and other reasons (Table 2.4).

The most controversial and publicized regulatory decision concerned the ban of FD&C Red # 2 based on results from questionable experiments that alleged that the color was carcinogenic and embryotoxic. The FDA terminated the provisional listing of FD&C Red # 2 based on insufficient scientific evidence demonstrating that the color is safe. However, Canada, Japan and countries from the European Economic Community allow its use in foods (Newsome, 1986).

The seven approved FD&C colors have undergone a complete battery of toxicity and carcinogenicity tests in a variety of species including studies on lifetime carcinogenesis, reproduction and teratogenecity, metabolism, and others (Hallagan, 1991).

Table 2.4: Examples of banned provisional color additives

Color	Reason
Violet 6B (FD&C Violet # 1)	Safety cannot be shown
Guinea Green (D&C Green # 1)	Inadequate analytical methods
Graphite	Contamination with polynuclear aromatic hydrocarbons
Naphtol Yellow S (D&C Yellow # 1)	Contamination with 4-aminobiphenyl
Amaranth (FD&C Red # 2)	Cancer in rodents
Ponceau SX (FD&C Red # 4)	Safety cannot be shown
Canary Yellow (D&C Yellow # 10)	Contamination with β-naphtylamine
Carmoisin (D&C Red # 19)	Contamination with β-naphtylamine
Geranine 2G (D&C Red # 11)	Contamination with β-naphtylamine
Carbon Black	Safety cannot be shown
D&C Green # 6	No petition to list filed
D&C Orange # 10 and 11	No petition to list filed
D&C Red # 36	Cancer in rodents

Nevertheless, some adverse reactions have been reported for some colorants. FD&C Red # 3 was found to cause benign thyroid tumors in laboratory rats after consuming large amounts (4% or 2464 mg/kg/day) of the dye over their lifetime. No adverse effects were observed at lower dose levels. Studies in humans have failed to identify adverse effects following ingestion of the dye (Newsome, 1986). In 1990, FDA discontinued the provisional listing of all lake forms of FD&C Red # 3 and its dye form used in external drugs and cosmetics.

The FDA stated that any human risk was extremely small and the decision to ban certain uses of FD&C Red # 3 was based less on safety concerns than the legal mandate of the Delaney Clause. FD&C Red # 3 remains permanently listed for use in food and ingested drugs, although FDA has announced its intent to propose rescinding those listings (FDA, 1993; Rumore et al., 1992).

FD&C Yellow # 5 (tartrazine) is responsible for a variety of allergic reactions in few sensitive people (Newsome, 1986). Symptoms include itching, hives, tissue swelling, asthma and rhinitis and has been observed in asthmatic, allergic and aspirin-intolerant individuals rather than the general public (Newsome, 1986). The FDA has approved the use of FD&C Yellow # 5 in foods since it is safe for consumption at the current levels and applications. All products containing this color are required to list it on the ingredient label to allow the small portion of people who may be sensitive to the color to avoid it (FDA, 1993; Newsome, 1986).

Colorants Exempt from Certification

Uncertified color additives (Table 2.5) include colors obtained from natural sources (vegetables, animals or minerals) and *nature identical* compounds which are synthetic counterparts of natural derivatives (Dziezak, 1987).

Uncertified colorants are commonly referred as "natural" colorants, however, the FDA has rejected the description of "natural color" on label declarations and advocated the use of terms such as "artificially colored", "artificial color added" or similar statements, since the color has been added to the food product for a specific purpose. The use of the term "artificial" can be avoided, provided that the declaration identifies the color by its common name or by function (Spears, 1988; Dziezak, 1987).

Although "exempt" colorants are not required to be certified, they are subject to surveillance by FDA to ensure they meet current government specifications (21 CFR Part 73) and are used in accordance with all the provisions of its regulation (Marmion, 1991). These include category of use, specified uses and restrictions, identity, levels of usage, specifications, and labeling requirements (Lipman, 1996).

Table 2.5: Certification-exempt color additives approved for foods

Color Additive	CFR #	EEC #	Level	Restrictions
Annato Extract	73.30	E160b	GMP	From *Bixa orellana* L.; specified extractants
Dehydrated beets	73.40	E162	GMP	Powder from dehydrated edible beets
Canthaxanthin	73.75	E161g	< 30 mg/lb	Used for foods and also chicken feed to color flesh and eggs
Caramel	73.85	E150a-d	GMP	Heat treatment of specified carbohydrates and reagents
β-Apo-8'-carotenal	73.90	E160e	< 15 mg/lb	
β-Carotene	73.95	E160a	GMP	Synthetic or natural
Cochineal extract; Carmine	73.100	E120	GMP	Ethanol extract of *Dactylopius coccus costa* concentrated solution; pasteurized Carmine is the aluminum lake
Toasted partially deffated cooked cotton seed flour	73.140	none	GMP	Manufacturing processing defined
Fruit juice	73.250	none	GMP	Expressed from mature, fresh, edible fruit or water infusion of dried fruit
Vegetable juice	73.260	none	GMP	Expressed from mature, fresh, edible vegetable or water infusion of dried vegetable
Carrot oil	73.300	none	GMP	Hexane extraction of edible carrots (*Daucus carota* L.); vacuum distillation
Paprika	73.340	E160c	GMP	Ground, dried pod of *Capsicum annum* L.
Paprika oleoresin	73.345	E160c	GMP	Flavor and color extracted from *Capsicum annum* L. using approved solvents
Riboflavin	73.450	E101	GMP	
Saffron	73.500	E164	GMP	Dried stigma of *Crocus sativus* L.

Table 2.5.....continued

Color Additive	CFR #	EEC #	Level	Restrictions
Grape color Extract	73.169	E163?	GMP	Nonbeverage food use; specified processing
Grape skin extract	73.170	E163?	GMP	Manufacturing process given; use in specified beverages and beverages bases
Synthetic iron oxide	73.200	E172	< 0.1% < 0.25%	Sausage casings Dog and cat food
Dried algae meal	73.275	none	GMP	Obtained from dried Spongiococcum; Use for chicken feed
Tagetes meal extract (aztec marigold)	73.295	none	GMP	Chicken feed; Hexane extract of flower petals from *Tagetes erecta* L.
Corn endosperm oil	73.315	none	GMP	Chicken feed only; isopropyl alcohol and hexane extraction from the gluten of yellow corn grain
Titanium dioxide	73.575	E171	< 1%	Synthetically prepared and free from other substances
Turmeric	73.600	E100	GMP	Ground rhizome of *Curcuma longa* L.
Turmeric oleoresin	73.615	E100	GMP	Flavor and color extracted from *Curcuma longa* L. with one of 8 listed solvents
Astaxanthin	73.35	none	< 80 ppm	In feed to color salmonids
Ultramarine blue	73.50	none	< 0.5%	Manufacturing process given; salt for animal feed only
Canthaxanthin	73.75	E161g	4.41 mg/kg	Broiler chicken feed
Ferrous Gluconate	73.160	none	GMP	Use for ripe olives only

Limitations of uncertified colorants as compared to artificial dyes include their lower tinctorial strength, higher variability in pigment content and color shade, lower stability, likeliness to introduce undesirable flavors and odors, trace metals, insecticides, herbicides, bacteria, and higher cost (Marmion, 1991; Spears, 1988). Furthermore,

except for beets and anthocyanins, most of the other natural colorants are oil soluble and require chemical modifications or the use of carriers and stabilizers for dispersion in foods (Spears, 1988).

As a result of toxicological studies, safety issues and legislative action, the number of artificial dyes suitable for foods has decreased (Spears, 1988; Noonan, 1985). Norway has removed all artificial food dyes from their permitted list, while Japan has submitted record number of patent applications regarding natural coloring sources (Spears, 1988). There is a growing world market for natural alternatives to synthetic dyes because of a "healthy eating" marketing approach by the food industry and as a consequence of consumer preferences (Lauro, 1991; Spears, 1988). Consumer pressure for more food products incorporating natural ingredients has led to the research for new potential natural sources of colorants.

ANTHOCYANINS AS FOOD COLORANTS

Anthocyanins are widely distributed among flowers, fruits, roots, cereals, and vegetables. These water soluble compounds are responsible for the attractive blue, purple, violet, magenta, red and orange color of a wide range plant species and their products (Jackman and Smith, 1996).

Anthocyanins are more prominent in fruits and berries than in other plant organs (Timberlake and Bridle, 1982). The major sources of anthocyanins belong to the families Vitaceae (grapes) and Rosaceae (cherry, plum, raspberry, strawberry, peach, apple, etc.). Other families containing anthocyanins include the Ericaceae (blueberry, cranberry), Saxifragaceae (black and red currants), Caprifoliaceae (elderberry), Solanaceae (potato, huckleberry) and Cruciferae (radish, cabbage) (Jackman and Smith, 1996; Timberlake and Bridle, 1982). The anthocyanin content (Table 2.6) varies between different plant sources, as well as among varieties and maturity stages. Also, environmental factors such as light, temperature, fertilization, and water supply will affect the anthocyanin accumulation (Mazza and Miniati, 1993).

Table 2.6: Total anthocyanin content in some common fruits and vegetables

Source	Pigment content (mg/100 g fresh weight)	Reference
Apples (Scugog)	10	a
Bilberries	300-320	a
Blackberries	83-326	a
Black currants	130-400	b
Blueberries	25-495	a
Red Cabbage	25	b
Black Chokeberries	560	c
Cherries	4-450	c
Cranberries	60-200	b
Elderberry	450	c
Grapes	6-600	a
Kiwi	100	c
Red Onions	7-21	a
Plum	2-25	b
Red Radishes	11-60	d
Black Raspberries	300-400	b
Red Raspberries	20-60	a
Strawberries	15-35	b
Tradescantia pallida (leaves)	120	e

a: Data from Mazza and Miniati, 1993; b: Data from Timberlake, 1989; c: Data reported by Schafhalter et al., 1996; d: Data from Giusti and Wrolstad, 1998; e: Data reported by Shi et al., 1992.

The most significant function of anthocyanins in plants is their ability to impart color to the plant, playing an important role in attracting insects and birds for pollination purposes and animals for seed dissemination (Jackman and Smith, 1996; Brouillard, 1983). In leaves, anthocyanins act as a light screen against the damaging UV radiation (Mazza and Miniati, 1993). The accumulation of anthocyanins at wound or injury sites

in plants suggests a role in defense against viral and/or microbial infection. Also, they have been shown to participate in biological oxidations and enzyme inhibition (Jackman and Smith, 1996).

Large amounts of anthocyanins have been consumed for thousands of years without any apparent adverse effects on human health (Timberalke, 1988; Brouillard, 1982). On the contrary, anthocyanin pigments have been shown to have beneficial therapeutical properties including enhancement of sight acuteness (Timberlake and Henry, 1988), antioxidant capacity (Wang et al., 1997; Tamura and Yamagami, 1994; Rice-Evans et al, 1996), treatment of various blood circulation disorders resulting from capillary fragility (Timberlake and Henry, 1988), vaso-protective and anti-flammatory properties (Lietti et al., 1976), inhibition of platelet aggregation (Morazzoni and Magistretti, 1986), maintainment of normal vascular permeability (Timberalke and Henry, 1988), controlling diabetes, antineoplastic and chemoprotective agents (Kamei et al., 1995; Karaivanova et al., 1990), radiation-protective agents (Akhmadieva et al., 1993), and possibly others due to their diverse action on various enzymes and metabolic processes (Wang et al., 1997).

Preparations of anthocyanin-based extracts from wild blueberries and European bilberries (*Vaccinium myrtillus*) are marketed as potent phytochemicals (Timberlake, 1988).

Anthocyanins are potential sources of natural colorants because of improved stability of new anthocyanin-containing sources due to acylation and/or intermolecular co-pigmentation, their water solubility, and their health benefits. Anthocyanin concentrates obtained by physical means and using water as extracting solvent are permitted as food colorants under the category of fruit (21 CFR 73.250) or vegetable (21 CFR 73.260) juice color (Lipman, 1996). Several plant materials, including red fruits, vegetables, flowers, leaves, roots, and other storage organs have been suggested as sources of colorants (Francis, 1989). According to Jackman and Smith (1996), the best commercial sources of anthocyanins are those in which the pigment is a by product of the manufacture of other value-added products (grape skin extract), their pigment

yields are high and the materials are readily available (concord grapes), or the pigment source are obtained from inexpensive crops (red cabbage and bilberries).

Chemical Structure

Anthocyanins are part of the flavonoid group of compounds which are characterized by the $C_6C_3C_6$ carbon skeleton and same biosynthetic origin (Jackman and Smith, 1996). Although flavonoids are generally colorless, anthocyanins occur in the cell sap in chemical states strongly absorbing visible light (Brouillard, 1983). Model experiments, simulating the conditions under which anthocyanins exist in nature, have shown that anthocyanins are stable but in a colorless form. *In vivo* interactions of anthocyanins with other substances present in the vacuole (phenolic compounds, sugars, nucleic acids, amino acids, organic acids, mineral ions, etc.) may prevent color loss or even enhance its color (Brouillard, 1983).

Anthocyanins are glycosides of anthocyanidin (aglycon) chromophores, these being polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium (flavylium) salts (Jackman and Smith, 1996; Brouillard, 1982). There are only 6 major anthocyanidins found in nature (Figure 2.12) in spite of the great variety of plant colors (Goto, 1987). Anthocyanidins are rarely found in their free form in plant tissues and occur mainly in the glycosylated forms (Harborne, 1979). Under acidic conditions, the color of non- and mono-acylated anthocyanins is determined largely by substitution in the B-ring of the aglycon (Jackman and Smith, 1996). Mazza and Miniati (1993) reported that increased hydroxyl substitution on the B-ring results in a shift of the visible absorption maximum to longer wavelengths to yield a bluer hue (Figure 2.12).

The most common sugars substituted on the aglycon are glucose, rhamnose, xylose, galactose, arabinose, and fructose. They occur as monoglycosides, diglycosides (sophorose, rutinose, gentiobiose, laminaribiose) and triglycosides (Francis, 1989). The 3-hydroxyl is always replaced by a sugar which confers stability and solubility to the anthocyanin molecule.
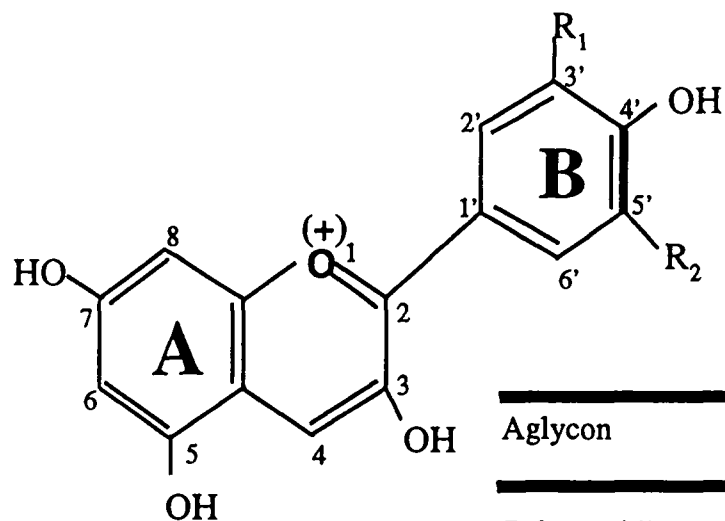
Aglycon	Substitution pattern		λ_{max} (nm)
	R1	R2	visible spectra
Pelargonidin	H	H	494 (orange)
Cyanidin	OH	H	506 (Orange-red)
Delphinidin	OH	OH	508 (Bluish-red)
Peonidin	OMe	H	506 (Orange-red)
Petunidin	OMe	OH	508 (Bluish-red)
Malvidin	OMe	OMe	510 (Bluish-red)

Figure 2.12: Structural and Spectral Characteristics of the Major Naturally Occuring Aglycones

When a second sugar is present, it is generally attached to the C-5 hydroxyl. Other glycosilation positions include the 7-, 3'- 4' and 5'- hydroxyl groups (Francis, 1989; Brouillard, 1982). Anthocyanins with five sugars (*Platycodon grandiflorum*) and six sugars (*Ipomoea purpura*) have been reported, being attached to the basic molecule with alternating sugar and acyl acid linkages (Francis, 1989). The nature of the sugar residue(s) appears to have a greater influence on anthocyanin stability than the nature of the aglycon, with decreasing stability reported for glycosyl moieties in the order glucose > galactose > arabinose (Jackman and Smith, 1996).

Increased glycosylation (3,5-glycosides, 3,7-glycosides) confers higher stability to the anthocyanin moiety (Mazza and Miniati, 1993). However, 3-monoglycosides have shown higher resistance to nucleophillic attack when compared to 3,5-diglucosides since higher glycosylation patterns enhances the electrophillic character of C-4 and results in larger amounts of hemiketal at equilibrium (Figueiredo et al., 1996; Dangles et al., 1993).

The sugar residues are often acylated with aromatic acids including *p*-coumaric, caffeic, ferulic, sinapic, gallic or *p*-hydroxybenzoic acids, and/or aliphatic acids such as malonic, acetic, malic, succinic or oxalic acids (Jackman and Smith, 1996). Acyl substituents are commonly bound to the C-3 sugar, esterified to the 6-OH or less frequently to the 4-OH group of the sugars. However, anthocyanins containing rather complicated acylation patterns attached on different sugar moieties have been reported (Odake et al., 1992; Lu et al., 1992; Shi et al., 1992c; Goto, 1987). Acylated anthocyanins follow a nonlinear degradation rate, probably due to the folding of the acyl molecules protecting the C-2 of the aglycon (Shi et al., 1992d). Acylation has an important stabilizing effect on anthocyanins via intramolecular copigmentation and will be covered later.

Methoxylation of anthocyanidins are found at the C-3' and C-5' positions and, less frequently, at positions C-7 and C-5. No natural anthocyanidin has been reported where glycosylation or methylation occurs at all C-5, C-7 and C-4' positions since a free hydroxyl group at any of those positions is essential for the formation of a quinonoidal

(anhydro) base structure (Jackman and Smith, 1996; Brouillard, 1982). The quinonoidal base is largely responsible for flower and fruit pigmentation under the conditions (weakly acidic or neutral aqueous solutions, pH 2.5-7.5) found in cell vacuoles, and is generated from the flavylium structure by loss of the acidic hydroxyl hydrogen (Brouillard, 1983). Ionized quinonoidal bases (pH>6) give rise to large bathochromic and hyperchromic shifts which contributes to a diversity colors (Mazza and Miniati, 1993). Structural transformations between the flavylium cation and the quinonoidal base, self-association and copigmentation are of great importance to explain pigmentation and color stabilization effects *in vivo*.

Glycosylation and acylation in the B-ring of anthocyanins have been reported in flowers such as *Lobelia erinus* (Yoshitama, 1977), *Tradescantia pallida* (Shi et al., 1992c), *Zebrina pendula* (Idaka, 1987a), cinerarin - *Senecio cruentus* (Goto et al., 1984) and gentiodelphin - *Gentiana makino* (Hosokawa et al., 1997; Goto et al., 1982). These anthocyanins have an extra absorption band at 560-600 nm and/or 600-640 nm in weakly acidic or neutral pH solutions, being highly colored at pH values above 4 where conventional anthocyanins are nearly colorless (Francis, 1992). Sugar substitution in the B-ring together with complex acylation appears to favor the existence of a 4'-keto form which is responsible for the extra band at higher wavelengths (Francis, 1989). B-ring substituted anthocyanins are remarkably stable to pH changes due to its ability to prevent addition of nucleophiles to the pyrylium ring and inhibiting the formation of a pseudobase or a chalcone (Brouillard, 1981).

Factors Affecting the Stability of Anthocyanins

Factors involved in anthocyanin degradation

A limitation of the use of anthocyanins as natural colorants is their reduced stability to several processing, formulation and storage conditions which restricts their applications to food products. Jackman and Smith (1996), Francis (1989), Mazza and

Miniati (1983) and Markakis (1982) have covered the major factors involved in anthocyanin stability.

The structural transformations of anthocyanins (Figure 2.13) due to changes in pH and interaction with water species (H^+, OH^- and H_2O) are fundamental to their color, stability, reactivity and spectral properties (Jackman and Smith, 1996, Brouillard, 1982). Under acidic conditions (pH<2), the anthocyanins exist primarily in the form of flavylium cation (AH^+), a rapid proton loss occurs as the pH is raised to yield the quinonoidal form (A). Further hydration of the flavylium cation gives the colorless carbinol pseudobase (B) or chalcone (C) forms (Mazza and Miniati, 1983). The relative concentration of the different anthocyanin species at equilibrium are dependent on the equilibrium constants controlling the acid-base proton transfer, hydration of the pyrylium nucleus, and ring-chain tautomeric reactions (Jackman and Smith, 1996; Brouillard, 1982). A comprehensive review of the structural transformations of anthocyanins is presented by Brouillard (1982).

Temperature and light exposure markedly influences the rate of degradation of anthocyanins, which generally follows first order kinetics (Attoe and Von Elbe, 1981). Increasing temperatures leads to the formation of a chalcone (C) and its subsequent cleavage yields carboxylic acids (from the B-ring) and carboxyaldehydes (from the A-ring) by proton transfer and rearrangements in the acidic aqueous medium (Piffaut et al., 1994). Further reactions results in the formation of complex brown polymeric compounds known as melanoidin pigments. The rate of thermal degradation of anthocyanins depend on the anthocyanin aglycon, its sugar moiety and presence of acylation (Figueiredo et al., 1996; Adams, 1973; Hrazdina et al., 1970). Photochemical degradation yields similar final products as for thermal degradation but through a different kinetic pathway involving the excitation of the flavylium cation (Furtado et al., 1993; Macarone et al., 1987). Light causes an increase in the rates at which anthocyanins undergo thermal degradation and a drastic decrease in the activation energies of the anthocyanins (Jackman and Smith, 1996; Attoe and Von Elbe, 1981).
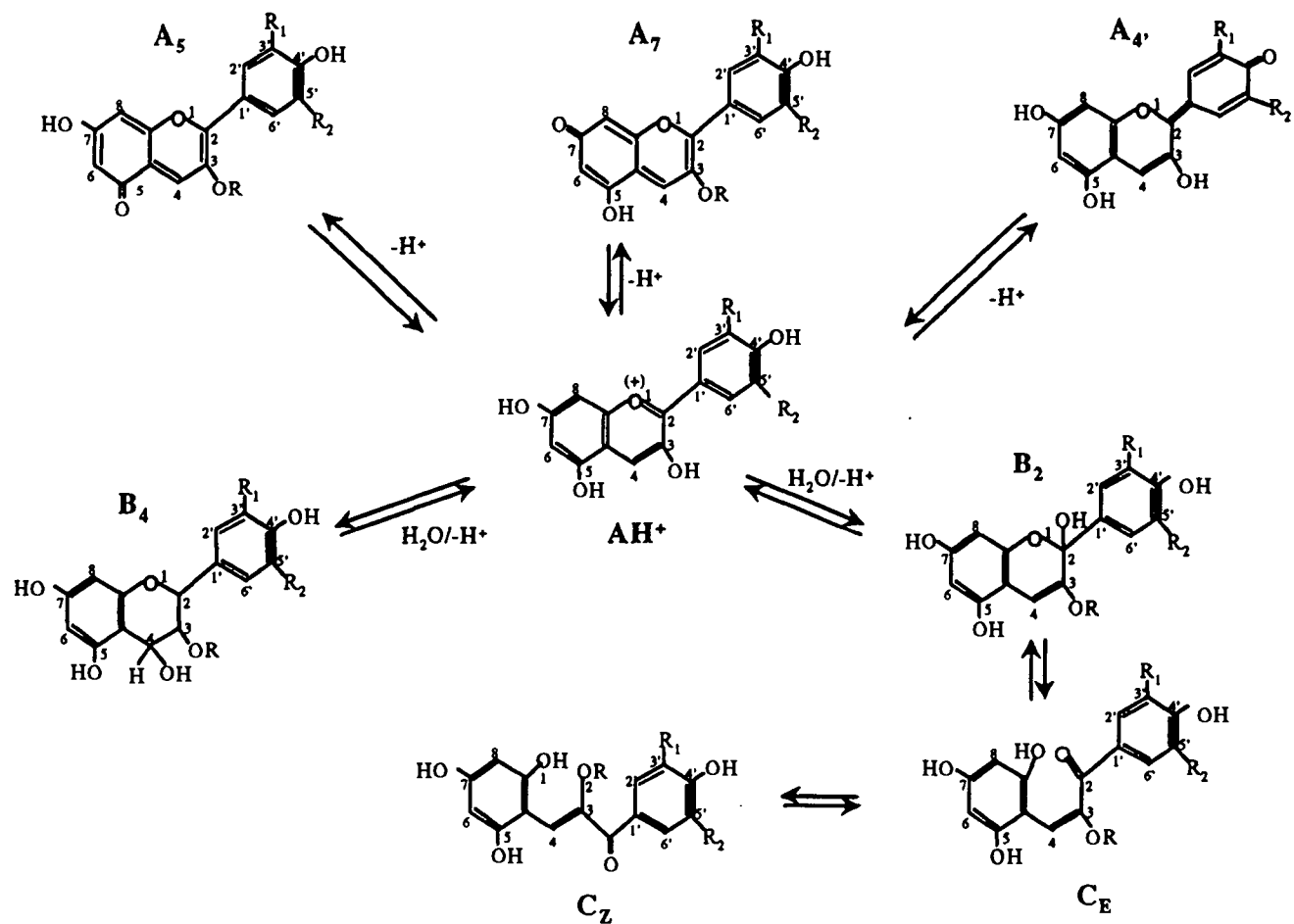
Figure 2.13: Structural Transformations of Anthocyanins in Water

Attoe and Von Elbe (1981) reported that molecular oxygen was necessary for significant photocatalyzed destruction of anthocyanins. Improved light stability has been reported upon addition of copigments such as rutin, gallic acid, tannic acid, flavones and flavonols (Francis, 1989).

Ascorbic acid-induced destruction of anthocyanins results from the indirect formation of H_2O_2 during aerobic oxidation of ascorbic acid. Anthocyanin bleaching occurs from the nucleophillic attack at C-2 position by H_2O_2 and subsequent cleavage of the pyrylium ring to yield a chalcone which undergoes further degradation reactions to produce polymeric compounds (Jackman and Smith, 1996). Another proposed mechanism for ascorbic acid degradation of anthocyanins is through its direct condensation with the pigments (Poei-Langston and Wrolstad, 1981; Jurd, 1972). SO_2 also has a decolorizing action on anthocyanins resulting in the formation of a colorless C-4 adduct. The reaction is reversible if the solution is acidified rapidly (Jackman and Smith, 1996).

Other factors involved in anthocyanin degradation are the presence of enzymes (glycosidases, polyphenol oxidases and peroxidases), metals, sugars and their degradation products, and acetaldehyde condensation.

Factors that improve pigment stability

Research involving the development of anthocyanin-containing food colorants has led to the discovery of a new group of anthocyanin molecules with complex patterns of glycosylation and acylation that exhibit remarkable stability to pH changes, heat treatment and light exposure (Dangles et al., 1993; Francis, 1992; Murai and Wilkins, 1990). Stabilization (Figure 2.14) has been attributed to intramolecular and intermolecular copigmentation, self-association, metal complexing and presence of inorganic salts (Goto, 1987; Brouillard, 1983). Increased anthocyanin concentration results in intensified color and enhanced pigment stability through the phenomena of intermolecular copigmentation and self-association (Mazza and Miniati, 1983).

Intermolecular copigmentation

Intermolecular copigmentation is a phenomenon that plays an important role in the expression of a wide range of brilliant colors by anthocyanins (Davies and Mazza, 1993). Copigmentation produces an increase in color intensity (hyperchromic effect) and a bathochromic shift of the maximum absorbance (Davies and Mazza, 1993; Mazza and Brouillard, 1990; Goto and Kondo, 1991; Asen et al., 1972). Molecules acting as copigments include flavonoids, polyphenols, alkaloids, amino acids and organic acids (Escribano-Bailón et al., 1996; Davies and Mazza, 1993; Hoshino et al., 1980; Asen et al., 1972).

The copigment can interact with the flavylium ion and the neutral anhydro base (Asen et al., 1972), enhancing the stability of anthocyanins by reducing the production of the carbinol pseudobase and stabilizing the quinonoidal base (Mazza and Brouillard, 1990; Brouillard, 1982; Williams and Hrazdina, 1979).

Goto et al. (1979) proposed that copigmentation in aqueous solutions arises from hydrophobic stacking between the aromatic nuclei of anthocyanins and flavone, which could be stabilized further by hydrophilic sugar moieties covering them (Goto, 1987). This mechanism provides good protection against water nucleophillic addition and subsequent color loss (Brouillard, 1982). Escribano-Bail\n et al. (1996) reported the formation of oligomeric structures containing two flavylium and two catechin residues connected through C-6/C-6 and C-8/C-8 linkages, and considered these products as an initial stage in a complex polymerization process leading to pigment precipitation in aged wines.

Copigmentation is affected by the structure and concentration of the anthocyanin and copigment, pH, temperature and ionic strength of the solvent (Davies and Mazza, 1993; Mazza and Brouillard, 1990; Asen et al., 1972). The effectiveness of the copigments depend on differences in stereochemistry or configuration required to bring the copigment into proximity with the pyrylium nucleus and differences in equilibrium constant for the complexation reaction (pK) between copigments (Davies and Mazza,

Intermolecular stacking Co-pigmentation

Intramolecular stacking Sandwich type

Intermolecular stacking Self-association

Anthocyanin   Flavone (co-pigment)   Aromatic acid   Sugar
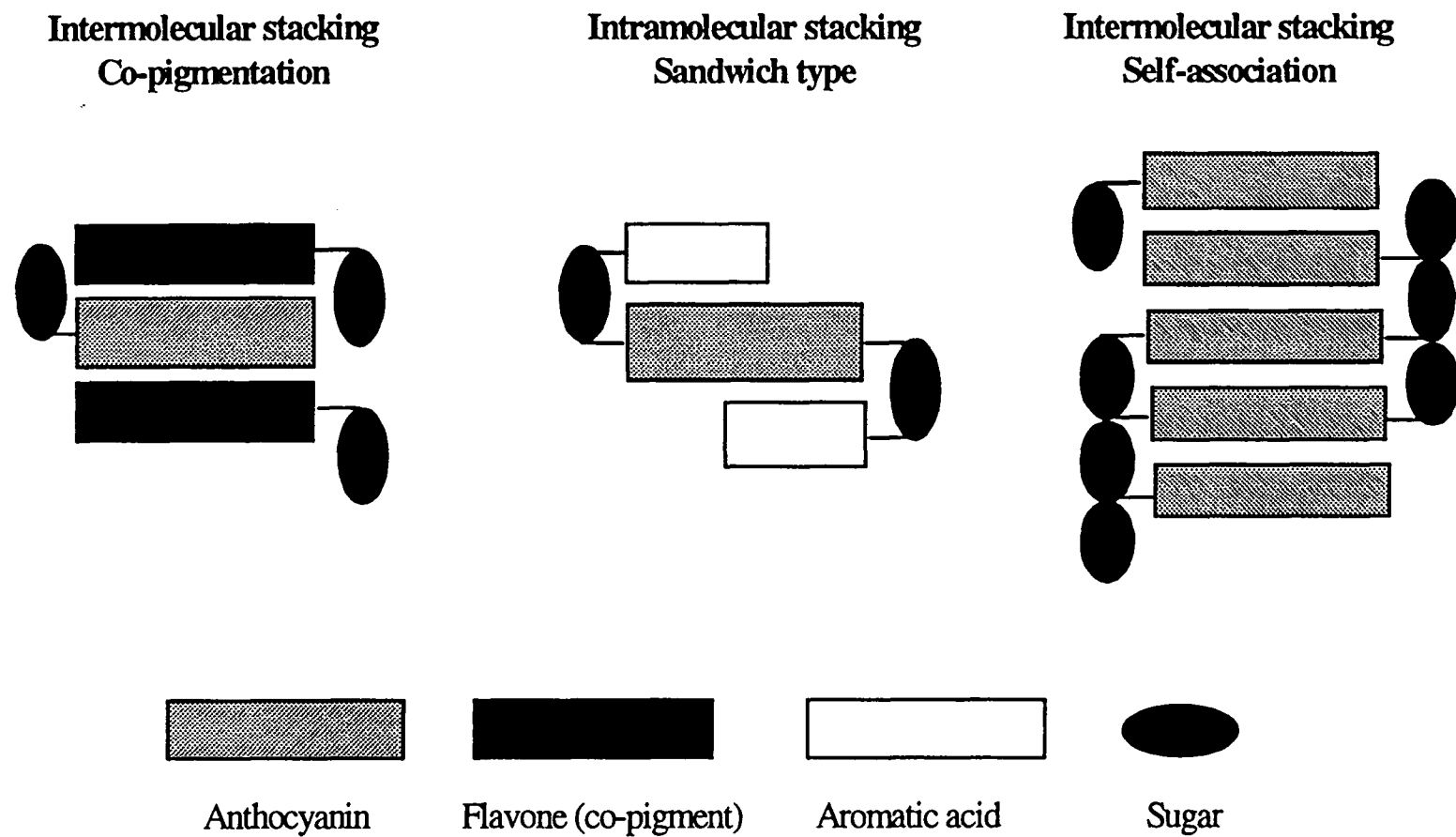
Figure 2.14:Stabilization Mechanisms of Anthocyanins

1993; Brouillard et al., 1989). Anthocyanins form copigment complexes with flavonoids and other compounds at pH ranging from 2.0 to neutrality, with maximum complex formation found at pH's 3.0 to 4.5 depending on the anthocyanin structure (Williams and Hrazdina, 1979; Asen et al., 1972). The presence of acyl groups on the anthocyanin molecule increases the strength of the complex formed and suppresses its dissociation, even in dilute solution (Hoshino et al., 1980). Monoacylated anthocyanins show a more efficient intermolecular copigmentation than unacylated ones (Brouillard, 1982). Neither a glycosyl residue nor a hydroxyl group are necessary for intermolecular copigmentation to take place (Brouillard, 1983; Goto, 1987).

Extracts from plant materials such as rosemary (*Rosmarinus officinalis* L.), peppermint (*Mentha X piperita*) and sage (*Salvia officinalis*) are rich in compounds, possibly flavonoid glucuronides, that stabilize and enhance the color of anthocyanin solutions (Lenoble et al., 1996). Addition of rosemary extract improved the thermal stability (at 40°C) and decreased the light degradation of different anthocyanin colorants (grape skin, elderberry, hibiscus and red cabbage), however a negative effect on the stability of red cabbage colorants at higher temperatures (75°C) was observed (Lenoble et al., 1996).

Intramolecular copigmentation

Intramolecular copigmentation is responsible for the improved color stability of polyacylated molecules under mildly acidic conditions as compared to 3-glycosides and 3,5-glycosides (Figueiredo et al., 1996). The planar aromatic residues of the acyl groups stack with the positively charged pyrylium nucleus, preventing the addition of nucleophiles, especially water, to the C-2 and C-4 positions of the anthocyanin and thus diminishing the formation of the pseudobase (Goto and Kondo, 1991; Goto, 1987; Brouillard, 1981). The extent of the hydration reaction is reduced, and since the proton transfer reactions apparently remain unaffected by the stacking process, the stability of the chromophores greatly increases (Brouillard, 1983). Intramolecular association does

not totally prevent the formation of the colorless forms, but it leads to the existence of colored solutions through a more extended pH range (Figueiredo et al., 1996).

The intramolecular interaction will be stronger for anthocyanins with longer and more linear chains which allows a greater flexibility for the folding of the planar ring(s) of the aromatic acids over the planar pyrylium ring and thus allowing the existence of an intramolecular complex through the formation of π-π hydrophobic interactions between the two parts of the molecule (Figueiredo et al., 1996).

The factors involved in the stacking process include the position of attachment of the acyl group to the sugar, the length and structure of the acyl group, and the structure of the sugar (Brouillard, 1983). Monoacylated anthocyanins do not show such a high stability of their neutral and ionized quinonoidal bases since only one side of the pyrylium ring can be protected against the nucleophillic attack of water. However, some weak intermolecular effect may still occur (Bouillard, 1983). Intramolecular copigmentation improves the thermal stability of anthocyanins because of a folded conformation of the pigments would diminish the extent of chalcone formation (Figueiredo et al., 1996).

Intramolecular copigmentation is more effcient in stabilizing the anthocyanin molecule than intermolecular copigmentation because of its entropic advantage, do not have to bring together molecules separated in solution (Brouillard, 1983). Furthermore, when intramolecular effect occurs, little or no intermolecular copigmentation can take place (Brouillard, 1983). Asen et al. (1977) reported that the color stability of morning glory (HBA) anthocyanins was not affected by addition of rutin or isoquercitin and that the color of HBA flowers is due to the anthocyanin structure and pH changes during maturation.

Self-association

Self-association occurs when the color intensity of the anthocyanin increases more than linearly with an increase in pigment concentration (Mazza and Miniati, 1993). Asen et al. (1972) reported that increasing the concentration of a cyanidin 3,5-

diglucoside solution (pH 3.16) from 10^{-4} to 10^{-2} M resulted in a 300 fold increase in the visible absorbance and a hypsochromic shift at λ_{max} of 5 nm, suggesting self-association of the flavylium ions. The stacking phenomenon is attributed to the hydrophobic interaction between aromatic nucleus of anthocyanins (Goto, 1987).

Anthocyanin chromophores can interact strongly with each other, irrespective of the mutual electrostatic repulsion. The stability of the red color of the flavylium form results from the reduction in the hydration constant (K_h) with the increase of self-association, preventing nucleophillic attack of water molecules (Hoshino, 1992). The smaller K_h, the higher the stability. Self-association occurs in concentrated flavylium cations and neutral quinonoidal bases, with at least two anthocyanin chromophores stacked vertically in a left-handed screw axis (Hoshino, 1992; Hoshino et al., 1981, 1982).

Increase in hydroxylation or methoxylation in the B-ring of the anthocyanin leads to the enhancement of the attraction between chromophores and increased strength of self-association, thus malvidin $\approx$ petunin $>$ delphinidin $\approx$ peonidin $>$ cyanidin $>$ pelargonidin (Hoshino, 1992). The 4'-OH and a 5-glycosyl are essential structural elements for the self-association process to occur (Brouillard, 1983).

Metal complexing

Metal complexing is responsible for the blue coloration of several flowers such as Commelinin and protocyanin (Goto, 1987). Although blue coloration can also be produced under alkaline conditions, it fades rapidly. The deep blue petals of *Commelina communis* consists of two molecules of an anthocyanin, malonylawobanin (M), a flavone (flavocommelin, F) and magnesium, its color and stability are explained in terms of a co-ordinated complex between two magnesium atoms and six molecules (M_6F_6) of each flavonoid (Goto et al., 1986; Hayashi and Takeda, 1970). Goto et al. (1979) proposed that the flavonoids in commelinin are stacked in parallel by hydrophobic interactions between the aromatic rings of both components, and that the magnesium ion was not an essential component. However, Takeda et al. (1994) showed that the yield of the blue

pigment was proportional to the amount of magnesium and the stable blue complex was not obtained without this element. Similar blue complexes can be formed by the use of manganese, cobalt, nickel, zinc and cadmium instead of magnesium (Takeda et al., 1994; Kondo et al., 1992).The blue flower-color development and the stability of the color can be explained by metal complexation of anthocyanin and the intermolecular hydrophobic association (Kondo et al., 1992).

Another metallo-anthocyanin is protocyanin which is obtained from the cornflower, *Centaurea cyanus*, and is composed of succinylcyanidin, a malonylflavone and Fe and Mg ions [(succinylcyanidin:malonylflavone)$_6$.Fe.Mg]$^{-12}$].

Anthocyanin quinonoidal bases as well as flavylium ions are strongly stabilized and do not form colorless pseudobases by hydration when dissolved in concentrated aqueous solutions of some neutral salts such as magnesium chloride and sodium chloride (Goto, 1987). It was suggested that stabilization in the sodium chloride solution may be due to promotion of self-association of anthocyanins, whereas magnesium chloride may reduce the concentration of free water by hydration of the magnesium ions (Hoshino et al., 1981).

Potential Sources of Anthocyanin-based Colorants

A number of potential food plants have been suggested as commercial sources of anthocyanin-based colorants, however their use have been limited by pigment stability, availability of raw material and economic considerations (Jackman and Smith, 1996).

By-products of the red-grape processing industry are important sources of commercial anthocyanins for use in foods because red grapes are produced in large quantities, have high pigment content and are inexpensive. The most important source is an extract of grape skins which is a by-product of wine manufacture, marketed under the trade name of Enocolor or Enociana (Jackman and Smith, 1996; Francis, 1989). The final product is bluish-red color and is available in liquid or powder forms. Some limitations include the presence of SO_2 (concern about allergic reactions) and natural pectins, sugars and other carbohydrates which can undergo Maillard browning during

processing (LaBell, 1993). Color extracted from Concord grape skins (*Vitis labrusca*) do not present these limitations making it more stable in storage and processing, and displays bright red hues comparable to Red # 40 in many food applications (LaBell, 1993). The presence of 3,5 diglucosides in Concord grape anthocyanins improves their resistance towards acid hydrolysis as compared to 3-monoglycosides from wine grapes (*Vitis vinifera*) (Markakis, 1982).

Sunflower-hull anthocyanin extracts have been petitioned for approval as natural food colorant to the FDA (Hettiarachchy, 1992). The dry sunflower hull pigments are stable at room temperature and can be successfully processed throughout a year (Wiesenborn et al., 1994). The pigments are cyanidin-3-glycosides (Wiesenborn et al., 1991), showing best stability at pH 3.0 and greater thermal stability when the pigments were extracted with SO_2 (Mok and Hettiarachchy, 1991). Improved extraction with SO_2 has been reported for grape pomace and hibiscus calyces (Pouget et al., 1990; Palamidis and Markakis, 1975). The bench-scale processing to derive a sunflower-hull extract has been proposed by Wiesenborn et al. (1994, 1991). Sunflower-hull pigments have been proposed as a by-product of sunflower oil processing but the purple-hulled trait is not yet found in commercial high-oil sunflower genotypes (Wiesenborn et al., 1994).

Francis (1989) provides a complete review on the colorants obtained from grapes and other plant sources.

Additional highly pigmented fruits have been suggested as sources of anthocyanin colorants, including extracts of cranberry press cake (Sapers et al., 1983; Clydesdale et al., 1979; Chiriboga and Francis, 1970), blueberries (Francis, 1985), black chokeberries (Kraemer-Schafhalter et al., 1996), elderberries (Bronnum-Hansen and Flink, 1986), Hibiscus calyces (Pouget et al., 1990), black currents (Rosa, 1973a,b), purple corn (Nakatani et al., 1979) among others. However, the anthocyanin composition of these conventional sources involve mainly mono- and di-glycosides which provides limited stability against hydration and pH changes (Brouillard et al., 1989).

Acylation of the anthocyanin molecule improve their stability through intramolecular and intermolecular copigmentation, and self-association reactions. Anthocyanins from red cabbage (*Brassica oleracea*) contains cyanidin 3-sophoroside-5-glucosides acylated with 1 or 2 molecules of cinnamic acids (Idaka et al., 1987b) and have shown excellent stability to heat and light exposure, and does not react with proteins (LaBell, 1990; Murai and Wilkins, 1990). The red cabbage color is pH dependent, being deep blue/red at pH 3.0 or below and becomes more blue in tone as the pH increases (Murai and Wilkins, 1990). Red cabbage extract is an approved natural food colorants and is commercially available for different food applications.

Acylated pelargonidin derivatives extracted from red radishes impart red color to maraschino cherries extremely close to that of FD&C Red # 40 at pH 3.5, with excellent stability (half-live of c.a. 30 wks) at room temperature and light exposure (Giusti and Wrolstad, 1996b). The major anthocyanin pigments of red radish cultivars have been identified as pelargonidin-3-sophoroside-5-glucoside acylated with malonic acid, and either *p*-coumaric and/or ferulic acids (Giusti and Wrolstad, 1996a; Giusti et al., 1998). Acylation shifts the orange-red hue of pelargonidin to an intense red color and also imparts resistance to acid hydrolysis (Giusti and Wrolstad, 1996a). However, this product would require the elimination of undesirable aromas derived from the glucosinolates in radish (Kucza, 1996).

Other potential sources of acylated anthocyanins include purple sweet potatoes (*Ipomoea batatas*) with the major anthocyanin being 3-caffeylferulylsophoroside-5-glucoside of peonidin and cyanidin (Odake et al., 1992; Shi et al., 1992a). Bassa and Francis (1987) showed that sweet potato pigments had improved stability as compared to enocyanin, but only slightly better than blackberries (cyanidin-3-glucoside).

Anthocyanins from the blue petals of morning glory, *Ipomoea tricolor* cv. Heavenly Blue (HBA), have been identified as peonidin-3-sophoroside-5-glucoside derivatives with complex glycosylation and acylation on the sophoroside moiety, containing 3 additional molecules of glucose and 3 molecules of caffeic acid (Goto, 1987). The complex anthocyanin structure of HBA provides high stability during storage

(Teh and Francis, 1988). Goto (1987) reported the folded conformation of HBA and that 2 aromatic acyl groups were stacked parallel with the anthocyanin nucleus, preventing the HBA molecule from reacting with water to form the pseudobase and therefore stabilizing the chromophore.

The leaves of *Tradescantia pallida* contain a high content of anthocyanins (Table 2.6) and their use for food colorants has been suggested (Shi et al., 1992d). The structure of its major anthocyanins are cyanidin 3,7,3'-triglucoside with one molecule of ferulic on each sugar with or without an extra terminal glucose (Shi et al., 1992c). Excellent stability of anthocyanins from tradescantia as compared to commercial grape skin extract, blackberries, concord grapes, and red cabbage has been reported (Baublis et al., 1994; Shi et al., 1992d), probably due to the poly-acylation and B-ring substitution that protects the molecule from hydration and fading through intramolecular copigmentation (Baublis et al., 1994). Tradescantia anthocyanins degrade 29 times slower than those from red cabbage in nonsugar drinks (Shi et al., 1992b).

Another B-ring substituted anthocyanin are isolated from the flowers of *Zebrina pendula*, which have been characterized as cyanidin 3,7,3'-triglucoside acylated with caffeic and ferulic acids (Idaka, 1987a). Brouillard (1981) reported the exceptional color stability of zebrina near neutrality due to the total absence of formation of colorless pseudobase or chalcone, and was attributed to intramolecular copigmentation. However, Teh and Francis (1988) reported that zebrina showed similar stability as cyanidin-3-glucoside during storage at pH 2.8.

These complex polyacylated and B-ring substituted anthocyanins offer promise as commercial colorants however, since none of these sources are recognized as normal food plants they would probably require FDA clearance before approval (Francis, 1989).

REFERENCES

Adams, J.B. 1973. Thermal degradation of anthocyanins with particular reference to the 3-glycosides of cyanidin. I. In acidified aqueous solution at 100/C. J. Sci. Food Chem. 24:747-762.

Akhmadieva, A.; Zaichkina, S.I.; Ruzieva, S.I. and Ganassi, E.E. 1993. The protective action of a natural preparation of anthocyan (pelargonidin-3,5-diglucoside). Radiobiologia 33:433-435.

Asen, S.; Stewart, R.N. and Norris, K.H. 1972. Co-pigmentation of anthocyanins in plant tissues and its effect on color. Phytochem. 11:1139-1144.

Asen, S.; Stewart, R.N. and Norris, K.H. 1977. Anthocyanin and pH involved in the color of "Heavenly Blue" morning glory. Phytochem. 16:1118-1119.

Attoe, E.L. and Von Elbe, J.H. 1981. Photochemical degradation of betanine and selected anthocyanins. J. Food Sci. 46:1934-1937.

Bassa, I.A. and Francis, F.J. 1987. Stability of anthocyanins from sweet potatoes in model beverages. J. Food Sci. 52: 1753-1754.

Baublis, A.; Spomer, A. and Berber-Jimenez, M.D. 1994. Anthocyanin pigments: Comparison of extract stability. J. Food Sci. 59:1219-1223.

Bronnun-Hansen, K. and Flink, J.M. 1986. Anthocyanin colorants from Elderberry *(Sambucus nigra* L.) IV. Further studies on production of liquid extracts, concentrates and freeze dried powders. J. Food Technol. 21:605-614.

Broulliard, R. 1981. Origin of the exceptional colour stability of *Zebrina* anthocyanin. Phytochem. 20: 143-145.

Brouillard, R. 1982. Chemical structure of anthocyanins. In *Anthocyanins as food colors*. P. Markakis Ed. Academic Press. NY.

Brouillard, R. 1983. The *in vivo* expression of anthocyanin colour in plants. Phytochem. 22: 1311-1323.

Brouillard, R.; Mazza, G.; Saad, Z.; Albrecht-Gary, M. and Cheminat, A. 1989. The copigment reaction of anthocyanins: A microprobe for the structural study of aqueous solutions. J. Am. Chem. Soc. 111:2604-2610.

Chiriboga, C.D. and Francis, F.J. 1970. An anthocyanin recovery system from cranberry pomace. J. Am. Soc. Hort. 95:233-236.

Clydesdale, F.M.; Main, J.H. and Francis, J. 1979. Cranberry pigments as colorants for beverages and gelatin desserts. J. Food Prot. 42:196-201.

Damon, G.E. and Janssen, W.F. 1973. Additives for eye appeal. FDA Consumer. July-August.

Dangles, O., Saito, N. and Brouillard, R. 1993. Anthocyanin intramolecular copigment effect. Phytochem. 34:119-124.

Davies, A.J. and Mazza, G. 1993. Copigmentation of simple and acylated anthocyanins with colorless phenolic compounds. J. Agric. Food Chem. 41:716-720.

Dziezak, J.D. 1987. Applications of food colorants. Food Technol. 41(4):78-88.

Escribano-Bailon, T.; Dangles, O. and Brouillard, R. 1996. Coupling reactions between flavylium ions and catechin. Phytochem. 41:1583-1592.

FDA. 1993. Food Color Facts. Food and Drug Administration (FDA) - International Food Information Council Foundation (IFICF) Brochure. Washington, D.C.

Figueiredo, P.; Elhabiri, M.; Toki, K.; Saito, N.; Dangles, O. and Brouillard, R. 1996. New aspects of anthocyanin complexation. Intramolecular copigmentation as a means for color loss?. Phytochem. 41:301-308.

Francis, F.J. 1985. Blueberries as a colorant ingredient in food products. J. Food Sci. 50:754-756.

Francis, F.J. 1987. Lesser-known food colorants. Food Technol 41:62-68.

Francis, F.J. 1989. Food colorants: anthocyanins. Crit. Rev. Food Sci. and Nutr. 28: 273-314.

Francis, F.J. 1992. A new group of food colorants. Trends Food Sci. Technol. 3: 27-31.

Furtado, P.; Figueiredo, P.; Cheves das Neves, H. and Pina, F. 1993. Photochemical and thermal degradation of anthocyanidins. J. Photochem. Photobiol. A: Chem. 775:113-118.

Giusti, M.M. and Wrolstad, R.E. 1996a. Characterization of red radish anthocyanins. J. Food Sci. 61(2): 322-326.

Giusti, M.M. and Wrolstad, R.E. 1996b. Radish anthocyanin extract as a natural red colorant for maraschino cherries. J. Food Sci. 61: 688-694.

Giusti, M. M.; RodrRguez-Saona, L. E.; Baggett J. R.; Reed, G. L.; Durst, R. W. and Wrolstad R. E. 1998. Anthocyanin Pigment Composition of Red Radish Cultivars as Potential Food Colorants. J. Food Sci. 63: 219-224

Goto, T. 1987. Structure, stability and color variation of natural anthocyanins. Prog. Chem. Org. Nat. Prod. 52:113-158.

Goto, T.; Hoshino, T. and Takase, S. 1979. A proposed structure of commelinin, a sky-blue anthocyanin complex obtained from the flower petals of *commelina*. Tetrahedron Lett. 31:2905-2908.

Goto, T.; Kondo, T.; Imagawa, H. and Miura, I. 1981. Heavenly Blue anthocyanin II. Trans-4-O-(6-O-(trans-3-O-(α-D-glucopyranosyl)caffeyl)-α-D-glucopyranosyl)-caffeic acid, a novel component in Heavenly Blue anthocyanin. Tetrahedron Lett. 22:3213-3216.

Goto, T.; Kondo, T.; Tamura, H. and Imagawa, H. 1982. Structure of gentiodelphin, an acylated anthocyanin isolated from *Gentiana makinoi*, that is stable in dilute aqueous solution. Tetrahedron Lett. 23:3695-3698.

Goto, T.; Kondo, T.; Kawai, T. and Tamura, H. 1984. Structure of Cinerarin, a tetraacylated anthocyanin isolated from the blue garden cineraria, *Senecio cruentus*. Tetrahedron Lett. 25:6021-6024.

Goto, T.; Tamura, H.; Kawai, T.; Hoshino, N.; Harada, N. and Kondo, T. 1986. Chemistry of metalloanthocyanins. Annals New York Acad. Sci. 471:155-173.

Goto, T. and Kondo, T. 1991. Structure and molecular stacking of anthocyanins - Flower color variation. Angew. Chem. Int. Ed. Engl. 30:17-33.

Hallagan, J.B. 1991. The use of certified food color additives in the United States. Cereal Foods World. 36:945-948.

Hayashi, K. and Takeda, K. 1970. Further purification and component analysis of commelinin showing the presence of magnesium in this blue complex molecule. Studies on anthocyanins LXII. Proc. Japan Acad. 46:535-540.

Hettiarachchy, N. 1992. Natural red colorant from sunflower seeds could replace red dye No 40. Industrial Bioprocessing. 14(9):2.

Hoshino, T. 1992. Self-association of flavylium cations of anthocyanidin 3,5-diglucosides studied by circular dichroism and ^{1}H NMR. Phytochem. 31:647-653.

Hoshino, T.; Matsumoto, U. and Goto, T. 1980. The stabilizing effect of the acyl group on the co-pigmentation of acylated anthocyanins with c-glucosylflavones. Phytochem. 19:663-667.

Hoshino, T.; Matsumoto, U. and Goto, T. 1981. Self-association of some anthocyanins in neutral aqueous solution. Phytochem. 20:1971-1976.

Hoshino, T.; Matsumoto, U.; Goto, T. and Harada, N. 1982. Evidence for the self-association of anthocyanins IV: PMR Spectroscopic evidence for the vertical stacking of anthocyanin molecules. Tetrahedron Lett. 23:433-436.

Hosokawa, K., Fukunaga, Y., Fukushi, E. and Kawabata, J. 1995. Acylated anthocyanins from red *Hyacinthus orientalis*. Phytochem. 39:1437-1441.

Hosokawa, K. Fukushi, E. Kawabata, J. Fujii, C. Ito, T. and Yamamura, S. 1997. Seven acylated anthocyanins in blue flowers of Gentiana. Phytochem. 45:167-171.

Hrazdina, G.; Borzell, A. J. and Robinson, W. B. 1970. Studies on the stability of the anthocyanin-3,5-diglucosides. [WINE]. Amer. J. Enol. Vitic. 21:201-204.

Hrazdina, G., Iredale, H. and Mattick, L.R. 1977. Anthocyanin composition of *Brassica oleracea* cv. Red Danish. Phytochem. 16: 297-299.

Idaka, E..; Ogawa, T. and Kondo, T. 1987a. Isolation of highly acylated anthocyanins from Commelinaceae plants, Zebrina pendula, Rhoeo spathacea and Setcreasea purpurea. Agric. Biol. Chem. 51:2215-2220.

Idaka, E., Suzuki, K., Yamakita, H., Ogawa, T., Kondo, T. and Goto, T. 1987b. Structure of monoacylated anthocyanins isolated from red cabbage, *Brassica oleracea*. Chem. Lett. pp 145-148.

Jackman R.L. and Smith J.L. 1996. Anthocyanins and Betalains. Ch. 8 In *Natural Food Colorants*. 2nd Ed. G.A.F.Hendry and J.D. Eds. Houghton Blackie A&P Great Britain.

Jurd, L. 1966. The formation of metal and co-pigment complexes of cyanidin 3-glucoside. Phytochem. 5:1263-1271.

Jurd, L. 1972. Some advances in the chemistry od anthocyanin-type plant pigments. In *The chemistry of plant pigments*. Chichester, Co. Academic Press. NY

Kamei, H.; Kojima, T.; Hasegawa, M.; Koide, T.; Umeda, T.; Yukawa, T. and Terabe, K. 1995. Suppression of tumor cell growth by anthocyanins in vitro. Cancer Invest. 13(6):590-594.

Karaivanova, M.; Drenska, D. and Ovcharov, R. 1990. A modification of the toxic effects of platinum complexes with anthocyans. Eksp. Med. Morfol. 29(2):19-24.

Kondo, T. Yoshida, K. Nakagawa, A. Kawai, T. Tamura, H. Goto, T. 1992. Structural basis of blue colour development in flower petals from Commelina communis. Nature. 358:515-518.

Kramer-Schafhalter, A.; Fuchs, H.; Silhar, S.S.; Kovac, M. and Pfannhauser, W. 1996. Process consideration for extraction from black chokeberry. In *The second international symposium on natural colorants INF/COL II*. The Hereld Organization. Acapulco, Mexico.

Kucza, M. M. 1996. Analysis of flavor precursors in radish and radish color extracts. M.S. thesis. Department of Food Science and Technology. Oregon St. Univer. Corvallis, OR.

LaBell, F. 1990. Technology diversifies use of natural colors. New cabbage coloring extends stability. Food Processing. 51(4):69-70.

LaBell, F. 1993. Grapes provide brilliant red color. Food Processing. 54(6): 88-89.

Lauro, G.J. 1991. A primer on natural colors. Am. Assoc. Cereal Chemists 36(11): 949-953.

Lenoble, R.; Richheimer, S.L.; Bank, V.R.; and Bailey, D.T. 1996. Enhanced stability and improved color of anthocyanins by the use of natural extracts. In *The second international symposium on natural colorants INF/COL II*. The Hereld Organization. Acapulco, Mexico.

Lietti, A.; Cristoni, A. and Picci, M. 1976. Studies on Vaccinium myrtillus anthocyanosides. I. Vasoprotective and antiinflamatory activity. Arzneim-Forsch. - 26(5):829-832.

Lipman, A.L. 1996. Current regulations for certification exempt color additives in the USA. In *The second international symposium on natural colorants INF/COL II*. The Hereld Organization. Acapulco, Mexico.

Lu, T.S.; Saito, N.; Yokoi, M.; Shigihara, A. and Honda, T. 1992. Acylated peonidin glycosides in the violet-blue cultivars of *Pharbis nil*. Phytochem. 31:659-663.

Maccarone, E.; Ferrigno, V.; Longo,M.L. and Rapisarda, R. 1987. Effects of light on anthocyanins. Kinetics and photodegradation products in acidic aqueous solution. Ann. Chim. (Rome) 77:409-508.

Markakis, P. 1992. Stability of anthocyanins in foods. Chp. 6. In *Anthocyanins as food colors*. P. Markakis Ed. Academic Press. NY.

Marmion, D.M. 1991. Handbook of U.S. colorants: Foods, drugs, cosmetics and medical devises. 3rd Edition. John Wiley and Sons, Inc. NY.

Mazza, G. and Broillard, R. 1990. The mechanism of co-pigmentation of anthocyanins in aqueous solutions. Phytochem. 29:1097-1102.

Mazza, G. and Miniati, E. 1993. Introduction. Chp. 1. In *Anthocyanins in fruits, vegetables, and grains*. G. Mazza and E. Miniati Eds. CRC Press, Boca Raton, FL.

Meggos, H.N. 1994. Effective utilization of food colors. Food Technol. 48(1):112.

Mok, C. and Hettiarachchy, N.S. 1991. Heat stability of sunflower-hull anthocyanin pigment. J. Food Sci. 56:553-555.

Morazzoni, P. and Magistretti, M.J. 1986. Effects of Vaccinium myrtillus anthocyanosides on prostacyclin-like ativity in rat arterial tissue. Fitoterapia 57 (1):11-14.

Murai, K. and Wilkins, D. 1990. Natural red color derived from red cabbage. Food Technol. 44:131

Nakatani, N.; Fukuda, H. and Fuwa, H. 1979. Major anthocyanin in Bolivian purple corn (*Zea mays* L.). Agric. Biol. Chem. 43:389.

Newsome, R.L. 1986. Food Colors. Food Technol. 40(7):49-56.

Noonan, J.E. 1972. Color additives in Food. In *CRC Handbook of Food Additives*. Chp. 14. 2nd Edition. CRC Press. USA.

Noonan, J. E. 1985. An analysis of the factors affecting the future of FD&C colors. Creal Foods World. 30:265-267.

Odake, K.; Terahara, N.; Saito, N.; Toki, K. and Honda, T. 1992. Chemical structures of two anthocyanins from purple sweet potato, *Ipomoea batatas*. Phytochem. 31:2127-2130.

Palamidis, N. and Markakis, P. 1975. Stability of grape anthocyanin in carbonated beverage. J. Food Sci. 40:1047-1049.

Piffaut, B.; Kader, M.; Girardin, M. and Metche, M. 1994. Comparative degradation pathways of malvidin 3,5-diglucoside after enzymatic and thermal treatments. Food Chem. 50:115-120.

Poei-Langston, M.S. and Wrolstad, R.E. 1981. Color degradation in an ascorbic acid - anthocyanin - flavonol model system. J. Food Sci. 46:1218-1222.

Pouget, M.P.; Lejeune, B.; Vennat, B. and Pourrat, A. 1990. Extraction, analysis and study of the stability of Hibiscus anthocyanins. Lebensm. -Wiss. U. -Technol. 23:103-105.

Rice-Evans, C.; Miller, N.J. and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol. Med. 20(7):933-956.

Rosa, J. 1973. Stability of anthocyanin pigment concentrates obtained from black current press cake. Ii. Studies on the rate of pigment destruction during storage. PR Inst. Lab. Badaw. Przem. Spozyw. 23:447-462.

Rosa, J. 1973. Stability of anthocyanin dye concentrates obtained from black current press-cake. I. Production and storage on concentrates as well as possibilities of application. PR Inst. Lab. Badaw. Przem. Spozyw. 23:269-300.

Rumore, M.M.; Strauss, S. and Kothari, A.B. 1992. Regulatory aspects of color additives. Pharm. Technol. 16(3):68-82.

Sapers, G.M.; Jones, S.B. and Maher, G.T. 1983. Factors affecting the recovery of juice and anthocyanin from cranberries. J. Am. Soc. Hort. Sci. 108:246.

Shi, Z., Bassa, I.A., Gabriel, S.L. and Francis, F.J. 1992a. Anthocyanin pigments of sweet potatoes -Ipomoea batatas. J. Food Sci. 57: 755-757, 770.

Shi, Z.; Francis, F.J. and Daun, H. 1992b. Quantitative comparison of the stability of anthocyanins from Brassica oleracea and Tradescantia pallida in non-sugar drink model and protein model systems. J. Food Sci. 57:768-770.

Shi, Z., Lin, M. and Francis, F.J. 1992c. Anthocyanins of Tradescantia pallida. Potential food colorants. J. Food Sci. 57: 761-765.

Shi, Z., Lin, M. and Francis, F.J. 1992d. Stability of anthocyanins from Tradescantia pallida. J. Food Sci. 57: 758-759.

Sorensen, E.J. 1992. Speciality potatoes. American Vegetable Grower. Jan. 36-39.

Spears, K. 1988. Developments in food colourings: the natural alternative. TIBTECH 6:283-288.

Takeda, K. Yanagisawa, M. Kifune, T. Kinoshita, T. Timberlake, C.F. 1994. A blue pigment complex in flowers of *Salvia patens*. Phytochem. 35:1167-1169.

Tamura, H. and Yamagami, A. 1994. Antioxidative activity of mono-acylated anthocyanins isolated from Muscat Bailey A grape. J. Agric. Food Chem. 42:1612-1615.

Teh, L.S. and Francis, F.J. 1988. Stability of anthocyanins from *Zebrina pendula* and *Ipomoea tricolor* in a model beverage. J. Food Sci 53: 1580-1581.

Timberalke, C.F. and Bridle, P. 1982. Distribution of anthocyanins in food plants. Chp. 5. In *Anthocyanins as food colors*. P. Markakis Ed. Academic Press. NY.

Timberlake, C.F., 1988. The biological properties of anthocyanin compounds. NATCOL quarterly bulletin.

Timberlake, C.F. and Henry, B.S. 1988. Anthocyanins as natural food colorants. Prog. Clin. Biol. Res. 280:107-121.

Wang, H.; Cao, G. and Prior, R.L. 1997. Oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem. 45:304-309.

Wiessenborn, D.; Golz, J.; Hanzel, J.; Helgeson, D.; Hettiarachchy, N.; Holm, E. and Lindley, J. 1991. Red food colorant extract derived from purple-hulled sunflower. North Dakota Farm Res. 49:19-21.

Wiessenborn, D.; Zbikowski, Z.; Glowacki, A. and Hettiarachchy, N. 1994. Bench-scale processing of red-colored extracts from sunflower hulls. Trans. ASAE. 37:515-520.

Williams, M. and Hrazdina, G. 1979. Anthocyanins as food colorants: Effect of pH on the formation of anthocyanin-rutin complexes. J. Food Sci. 44:66-68.

Yoshitama, K. 1977. An acylated delphinidin 3-rutinoside-5,3',5'-triglucoside from *Lobelia erinus*. Phytochem. 16:1857-1858.

CHAPTER 3

INFLUENCE OF POTATO COMPOSITION ON CHIP COLOR QUALITY

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ABSTRACT

Potato constituents were monitored to evaluate their contribution to potato chip color. Five chipping potato varieties: Snowden, AC Ptarmigan, FL 1625, FL 1815 and ND2471-8 were evaluated. Ascorbic acid, sugars, phenolic acids, and amino acids were determined and quantified by High Performance Liquid Chromatography (HPLC) and the color of potato chips was measured by both CIELab Hunter-ColorQuest and Agtron instruments. Composition and chip color varied among the different varieties. AC Ptarmigan and ND2471-8 produced the darkest chip color (on average $L^* = 49.0$, chroma= 19.5, and hue angle= 62.9) compared with FL 1815 ($L^* = 58.4$, chroma= 28.3 and hue angle= 75.7). Reducing sugar concentration did not completely explain or predict color quality when it was present in low concentrations (ca. < 60 mg/100g). Other reactants present in the potato slices played an important role in the final color quality of potato chips. Multiple correlation analysis showed negative association of ascorbic acid (r= -0.7), fructose (r= -0.7), a chlorogenic acid isomer (r= -0.7), glucose (r= -0.7) and glutamine (r= -0.5) with potato chip color. Sucrose, chlorogenic acid and asparagine were poor estimators of chip color quality.

INTRODUCTION

A major problem confronting the potato chip industry is the maintenance of satisfactory color (Kadam et al., 1991). Excessive browning during frying produces an undesirable color and unacceptable bitter taste (Roe et al., 1990). The flavor and color of potato chips is due to products of the Maillard reaction (Shallenberger et al., 1959; Smith, 1987) which results in the formation of brown melanoidin pigments from reactions involving compounds with amino and carbonyl groups (Eskin, 1990; Danehy, 1986; Feeney and Whitaker, 1982).

Reducing sugar (glucose and fructose) and sucrose levels have been used to predict the suitability of materials for potato chip processing. Reducing sugars are normally the limiting factor in color development (Roe et al., 1990; Sowokinos et al.,

1987; Marquez and Añon, 1986). Sucrose may enter the Maillard reaction due to hydrolysis during frying (Leszkowiat et al., 1990; Shallenberger et al., 1959); however, the role of sucrose in potato chip color is only marginal (Roe and Faulks, 1991). While sugar levels play an important role in color development of potato chips, they are not the only constituents involved in the browning reaction. Different varieties with similar sugar levels can yield chips with quite different color characteristics (Habib and Brown, 1956). Several other potato constituents participate in nonenzymatic reactions. Amino acids (lysine, glycine, glutamine and arginine) have been identified as a major component responsible for color development in fried potatoes (Khanbari and Thompson, 1993; Roe and Faulks, 1991). Browning may result also from the non-enzymatic autoxidation of polyphenolic compounds, favored by alkaline pH (Cilliers and Singleton, 1989; Singleton, 1987) and ascorbic acid reacting with amino acids during frying (Smith, 1987). A better understanding of the level of participation of potato constituents such as ascorbic acid, amino acids, and phenolic acids along with sugars in the non-enzymatic browning reaction would favor the color optimization of potato chips. This information will be useful for developing better quality control methods, optimizing storage regimes and developing of new varieties.

In this study we selected five chipping potato varieties and measured the concentration of those compounds which may play an important role in potato chip browning, the major objective being to determine whether compositional differences can account for variation in color quality among varieties.

MATERIALS AND METHODS

Plant Material

Ten chipping potato varieties were screened for sugar content, and from those, 5 varieties with different levels of sugars and chip color were selected. Potato seed tubers FL 1625, FL 1815, ND2471-8, AC Ptarmigan and Snowden were planted in 4 replicated plots and grown under simulated commercial conditions at the Oregon State University

Vegetable Research Farm during the 1994 growing season. Tubers were analyzed after 6 weeks of storage at 10°C.

Color Measurements

Five randomly selected tubers were cut radially into halves, and one half of each tuber was sliced (4 mm thick). Eight slices from each tuber were fried in partially hydrogenated canola oil for 4-5 minutes at 180°C. Doneness was determined by the absence of bubbles in the frying oil. The potato chips obtained were crushed into fine pieces. Hunter L^* a^* b^* values were determined using a Hunter CT1100 ColorQuest colorimeter (HunterLab, Hunter Associates Laboratories Inc., Reston, VA). The color measurements were made using the reflectance specular included mode, illuminant C and 10° observer angle in a 5 cm pathlength optic glass cell. Chroma (c) and hue angle were calculated. Agtron units were determined using an Agtron E-10 colorimeter (Fillper Magnuson, Reno, NV) with a red filter, calibrated to read zero with a black disk and 90 with a white disk.

Compositional Analysis

Five randomly selected tubers were cut into small cubes, frozen in liquid nitrogen and stored at -20°C until analyzed. Each compositional analysis was performed in duplicate.

Preparation of Potato Extracts

Fifty grams of frozen potato tissue were blended with 100 mL ethanol (95%) for 1 min using a Waring blendor. Internal standard (1 mL) containing mannitol (80 mg), epicatechin (2.5 mg) and α- aminobutyric acid (ABA)(40 mg) was added to the resulting slurry. The slurry was mixed, filtered through Whatman No 1 paper and the residue was rinsed twice with 50 mL ethanol (80%). The ethanol was evaporated at 40EC using a rotary evaporator (Rotavapor R., Buchi, Switzerland) and taken to a volume of 25 mL

with deionized distilled water. The extract was centrifuged (1610 x g) for 10 min using a clinical centrifuge (International Equipment Company, Mass., USA) and the supernatant was collected and stored at -20°C for phenolic acid, sugar and amino acid analysis.

Sugar Analysis

Sample preparation

The sugar composition of the potato varieties was determined using the procedure described by Spanos and Wrolstad (Spanos and Wrolstad, 1987). Five mL of the potato extract was passed through a C_{18}-Sep-Pak cartridge (previously activated with methanol and rinsed with water). The eluate was passed through a 1.8 mL BioRex-5 anion exchange resin (Bio-Rad Lab., Hercules, CA), filtered through a 0.45 μm millipore filter type A and injected into the HPLC.

HPLC analysis of sugars

Equipment: A High Performance Liquid Chromatograph (Varian LC 5020), equipped with a column heater, Varian Refractive Index detector (Varian Instrument Group, Walnut Creek, CA), an LCI-100 Perkin Elmer Laboratory computing Integrator and a Beckman 501 autosampler with a 50 μL loop were used. Column: 30 x 0.78 cm I.D. Aminex Carbohydrate HPX-87 fitted with a 4 x 0.46 cm Carbo C micro guard column (Bio-Rad Lab., Hercules, CA) at 87°C. Mobile phase: 0.2 mg/mL $Ca(NO_3)_2$ run isocratically at a flow rate of 0.7 mL/min. The sugar standard curve was constructed using 4 concentrations of sucrose, glucose and fructose (0.5, 1, 2.5 and 5 mg/mL) and mannitol was used as an internal standard at a concentration of 4 mg/mL. Each standard solution was prepared by diluting stock solutions of sucrose (50 mg/mL), glucose (50 mg/mL), fructose (50 mg/mL) and mannitol (80 mg/mL) (Sigma Chemical Co., St. Louis, MO).

Phenolic Acid Analysis

<u>Sample preparation</u>

Spanos and Wrolstad (1990) described the methodology for phenolic acid isolation, separation and quantification. The phenolic constituents in 10 mL of potato extract were concentrated by adsorption on a C_{18} Sep-Pak cartridge (Waters Assoc., Millford, MA) and eluted with methanol. The methanol was evaporated and the phenolic compounds were re-dissolved in 2 mL of deionized water, filtered through a 0.45 μm millipore filter type A and injected into the HPLC.

<u>HPLC analysis of phenolic acids</u>

Equipment: A High Performance Liquid Chromatograph Perkin-Elmer Series 400, equipped with a Hewlett-Packard 1040A photodiode array detector, Gateway 2000 P5-90 computer with a Hewlett-Packard $HPLC^{2D}$ ChemStation software and a Beckman 501 autosampler with a 50 μL loop was used. Column: 25 x 0.46 cm I.D. Supelcosil LC-18 column (Supelco Inc., Bellefonte, PA) fitted with a 1 x 0.46 cm Spherisorb ODS-2 micro guard cartridge (Alltech, Deerfield, IL). Mobile phase: solvent A: 0.07M KH_2PO_4 adjusted to pH 2.5 with phosphoric acid; solvent B: methanol. The program used a linear gradient from 15% B to 35% B in 25 min, from 35% to 45% B in 10 min, from 45 to 65% B in 5 min and isocratic conditions with 65% B for 5 min, at a flow rate of 1 mL/min, with a total run time of 45 min. The effluent was monitored at 280 and 320 nm and the spectra were collected for all peaks. The phenolic acids used as standards were purchased from Sigma (Sigma Chemical Co., St. Louis, MO). The standard curves was constructed using three different concentrations of tryptophan (15, 35, and 75 μg/mL), chlorogenic acid (0.1, 0.2, 0.4 and mg/mL), caffeic acid (0.1, 0.2 and 0.4 mg/mL) and epicatechin as internal standard at a concentration of 0.4 mg/mL. Each solution was prepared by diluting stock solutions of tryptophan (1 mg/mL), tyrosine (0.5 mg/mL), chlorogenic acid (2 mg/mL), caffeic acid (2 mg/mL) and

epicatechin (2 mg/mL). Chlorogenic acid isomers were prepared following the procedure described by Nagels et al. (1980).

Mass Spectroscopy

Unknown phenolic and chlorogenic acids were isolated using a semi-prep HPLC system. A 25 x 2.12 cm I.D. Supelcosil PLC-18 column (Supelco Inc., Bellefonte, PA) was used and the separation conditions were the same as described previously. Liquid Chromatography Mass Spectrometry (LCMS) was performed using a SCIEX API III Plus triple-quadrupole mass spectrometer (Thornhill, Ontario, Canada) equipped with an atmospheric pressure chemical ionization system. High performance liquid chromatography was performed on a Perkin Elmer Model 400 equipment using a 10 x 0.22 cm I.D. Spherisorb ODS-2 column. Mobile phase: solvent A: 0.1% trifluoroacetic acid (TFA) in water and solvent B: 0.1% TFA in acetonitrile. The program used a linear gradient from 10% to 60% B in 30 min. A chlorogenic acid standard (Sigma Chemical Co., St. Louis, MO) was also used.

Free Amino Acid Analysis

Sample preparation

The free amino acids in the potato extract (4 mL) were bound to a cation exchange SP Sephadex C-25 (Sigma Chemical Co., St. Louis, MO), eluted from the column with 0.2 M ammonium sulfate and the fraction collected. One hundred FL of this fraction was derivatized using phenylisothiocyanate (PITC) following the procedure described by Hagen et al. (1993). The derivatized samples were diluted with 2 mL deionized distilled water, filtered through a 0.45 μm millipore filter type HA and injected into the HPLC.

HPLC analysis of amino acids

The same equipment described for phenolic acid analysis was used. Separations were carried out using coupled columns, a 25 x 0.46 cm I.D. Spherisorb ODS-2 (Alltech Associates, Deerfield, IL) and 15 x 0.39 cm I.D. Pico-Tag (Waters Chrom. Division, Milford, MA), fitted with a 1 x 0.46 cm Spherisorb ODS-2 micro guard cartridge (Alltech Associates, Deerfield, IL), at room temperature. Solvents used were A: 0.14 M sodium acetate with 0.5 mL/L triethylamine (TEA) adjusted to pH 6.0 with glacial acetic acid, and B: 60% acetonitrile in distilled water. The program used isocratic 15% B for 10 min, linear gradient 15-50% B for 20 min followed by a 50-100% B gradient for 10 min, and holding with 100% B for 5 min, at a flow rate of 1 mL/min. The effluent was monitored at 254 nm. The standard curves was prepared using 4 concentrations (10, 40, 60 and 80 μg/mL) of aspartic acid, glutamic acid, asparagine, glutamine, histidine, alanine, phenylalanine, tryptophan, cysteine and lysine; ABA was used as internal standard (100 μg/mL). Each solution was prepared from a stock solution containing 0.4 mg/mL of each amino acid (Sigma Chemical Co., St. Louis, MO).

Total Ascorbic Acid

Sample preparation

Ascorbic acid was determined by the method proposed by Sapers et al. (1990). Thirty grams of potato tissue were blended with a solution containing 30 mL 2.5% metaphosphoric acid and 60 mL acetonitrile:0.05M KH_2PO_4 (75:25). The homogenate was filtered through a Whatman No. 1 paper, passed through a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA) and filtered through a 0.45 μm Millipore filter type HV (Millipore Corp., Bedford, MA).

HPLC analysis of ascorbic acid

The same equipment described for the phenolic acid analysis was used. Column: a 25 x 0.46 cm I.D. Econosphere NH_2 (Alltech, Deerfield, PA). Mobile phase:

Acetonitrile:0.05M KH_2PO_4 (75:25) with 1 g/L dithiothreitol (DTT) (Sigma Chemical Co., St. Louis, MO), run isocratically at a flow rate of 1 mL/min. The effluent was monitored at 254 nm and the spectra were recorded for all peaks. An ascorbic acid standard curve was prepared using solutions containing 25, 50, 75 and 100 μg/mL, prepared from a stock solution containing 200 μg/mL ascorbic acid (Sigma Chemical Co., St. Louis, MO).

Sensory Analysis

The sensory characteristics of the potato chips obtained from the 5 potato varieties and a commercial sample were analyzed using Difference from Control and Ranking tests. Crushed potato chips (ca 25g) were presented to a total of 43 panelists in small containers. Each container was coded with a 3-digit number and the order of presentation was randomized among panelists. A 9-point difference from control (1 = no difference to 9 = extremely different) test was performed. Each panelist was served 7 samples: one control (commercial sample), labeled as Control, and a set of the six coded samples (a blind control was included). The panelists were asked to rate the color of the samples as compared to that of the control. A Ranking test using a 6-point scale (1 = like the most and 6 = like the least) was also performed. Six samples (including the control) were ranked according to the panelist preference for the chip color.

Statistical Analysis

Analysis of variance was used to analyze the data as a complete randomized block (variety and plot) design with one missing unit. Significant differences among means of the different potato varieties were determined by multiple comparison test (LSD). The closeness of a linear relationship between 2 variables was determined by multiple correlation. The ranking test was analyzed using the Friedman test, a non-parametric test based on the evaluation of rank sums for each sample. Statistical analyses were performed using Statgraphics 5.0 software (Manugistics, Inc., Rockville, MD).

RESULTS AND DISCUSSION

Potato Composition

Preliminary screening of ten potato varieties for sugar composition and chip color characteristics (data not presented) was done to choose potato varieties with contrasting chipping qualities. Five varieties were selected for detailed compositional analysis which encompassed the range for sugar content and color development. The low concentration of sugars in some of the potato varieties analyzed, and the presence of several interfering peaks in our potato extract necessitated an extensive sample clean-up using a C_{18} Sep-Pak cartridge to remove non polar compounds and an anion exchange resin (BioRex 5) treatment to adsorb acids. Figure 3.1 shows the final sugar HPLC profile. Table 3.1 shows the sugar and ascorbic acid content of the potato varieties evaluated on a fresh weight basis.

Table 3.1: Ascorbic acid and sugar content of potato tubers (mg/100g fresh weight)

Potato cultivar	Ascorbic acid		Sucrose		Glucose		Fructose	
FL-1625	18.58	b	88.51	a	26.51	b	27.84	b
	(0.57)		(13.89)		(2.16)		(1.53)	
FL-1815	11.98	a	81.49	a	15.73	a	16.55	a
	(2.53)		(4.39)		(3.75)		(1.31)	
ND2471-8	23.38	c	288.78	b	96.85	d	63.01	d
	(3.40)		(50.04)		(8.00)		(3.18)	
Ptarmigan	23.37	c	77.03	a	63.41	c	53.55	c
	(1.56)		(11.94)		(8.25)		(9.06)	
Snowden	19.94	b	99.81	a	24.81	b	24.45	b
	(2.09)		(10.89)		(2.56)		(1.67)	

In parenthesis are presented the standard deviation and the different letters indicate differences among means (p-value < 0.01). The values reported are mean responses from four replications.
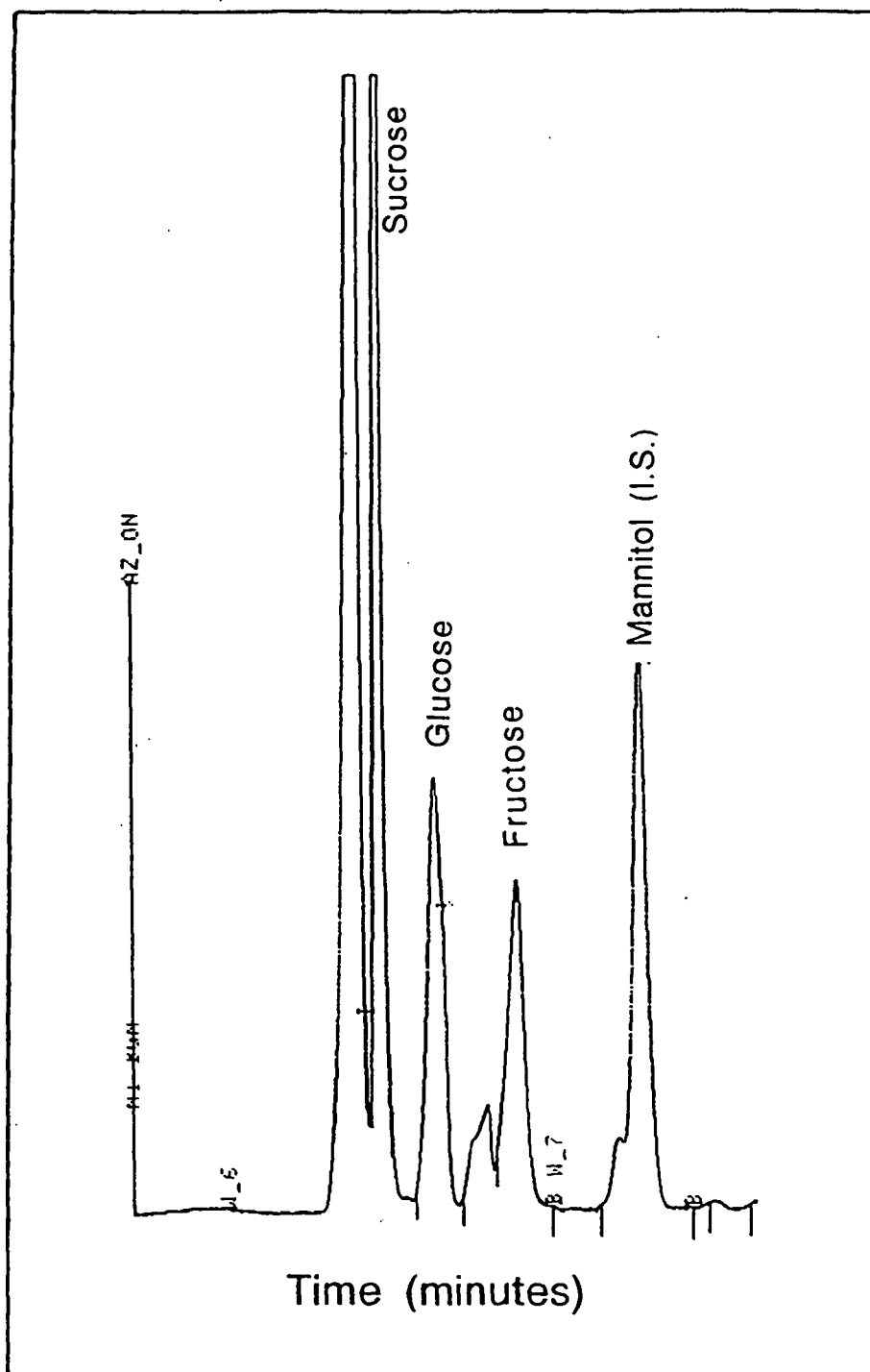
Figure 3.1: HPLC separation of sugars from potato tubers. Column: 30 x 0.78 cm I.D. Aminex Carbohydrate HPX-87 at 87°C. Mobile phase: 0.2 mg/mL Ca(NO₃)₂ run isocratically at a flow rate of 0.7 mL/min.

The sugar and ascorbic acid content were significantly different among varieties (p-value < 0.01). The sucrose content obtained for FL 1815, FL 1625, Snowden and AC Ptarmigan varieties averaged 87 mg/100 g. The concentration of sucrose in ND2471-8 was approximately three times higher than that found in the other varieties (289 mg/100 g).

The ratio of glucose to fructose was roughly 1:1 in most varieties. ND2471-8 and AC Ptarmigan had the highest reducing sugar content, followed by Snowden and FL 1625, whereas FL 1815 had the lowest reducing sugar content. The sucrose and glucose content found in these varieties were within the range reported by Sinha et al. (1992) for 10 potato varieties after harvest.

Very good separation and resolution of ascorbic acid was obtained by the chromatographic method (Fig. 3.2). The method was simple and allowed rapid identification and quantitation of ascorbic acid as reported by Sapers et al. (1990). However, the extract was not stable over time resulting in a 15% decrease in ascorbic acid content after 24 hr at 4°C. Rapid analysis of the samples and the use of reducing agents such as DTT in the mobile phase were important for reliable results. The mean ascorbic acid content in the potato varieties studied ranged from 12.0 to 23.4 mg/100 g. Sugar and ascorbic acid content in potatoes is highly variable and depends on factors such as variety, temperature, pre-conditioning, handling of the tubers, storage temperature and storage duration (McCay et al., 1987; Talburt et al., 1987; Sieczka and Matta, 1986; Linnemann et al. 1985; Habib and Brown, 1956).

The HPLC separation of the phenolic acids in potato tubers (Figure 3.3) shows that the major phenolic acid present was chlorogenic acid (5-O-caffeoylquinic acid). By HPLC analysis, we identified the 3- and 4-O-caffeoylquinic acid isomers (neochlorogenic and cryptochlorogenic acids, respectively).

The UV spectrum of an additional unidentified peak was also very similar to chlorogenic acid. Mass spectrometry showed that the unidentified phenolic acid produced the molecular ion (355.2) and the same mass fragments of 163.2 (base peak) and 145.2 as the chlorogenic acid standard; the latter ions being produced by

dehydration of the caffeoyl portion (M^+= 181.2) of chlorogenic acid. Nagels et al. (1980) reported 4 different esters of caffeoylquinic acid, the 3-, 4- and 5-O-caffeoylquinic acid obtained from chlorogenic acid and 1-O-caffeoylquinic acid synthesized from 1-(3',4'-dicarboethoxy caffeoyl) acetonequinide. This information strongly suggests that the unidentified phenolic acid is the 1-O-caffeoylquinic acid (CHL-1) isomer of chlorogenic acid.
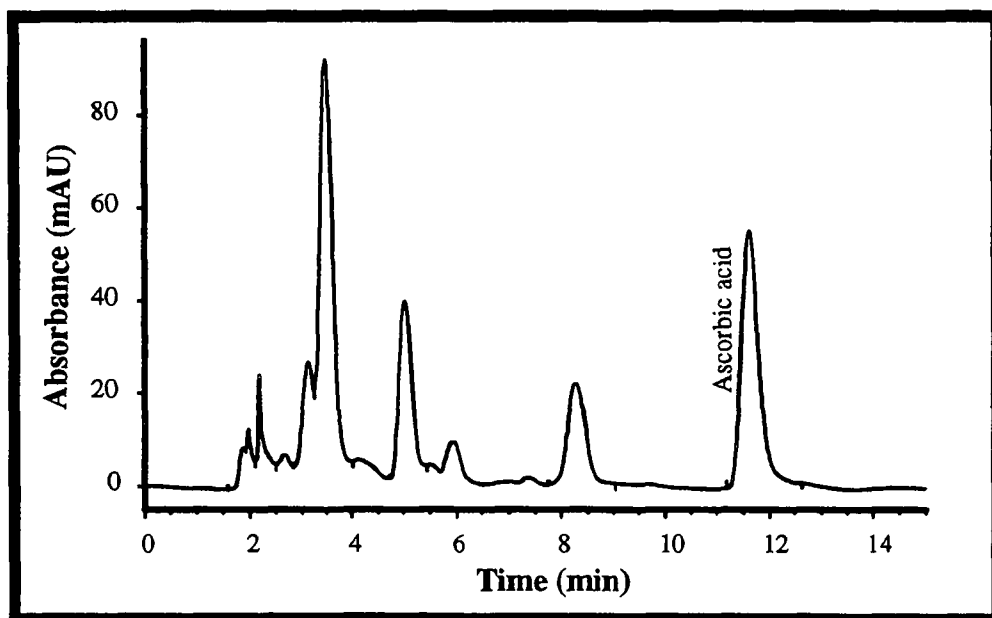
Figure 3.2: HPLC separation of ascorbic acid from potato tubers. Column: 25 x 0.46 cm I.D. Econosphere NH_2. Mobile phase: Acetonitrile:0.05M KH_2PO_4 (75:25) with 1 g/L dithiothreitol (DTT) run isocratically at a flow rate of 1 mL/min.
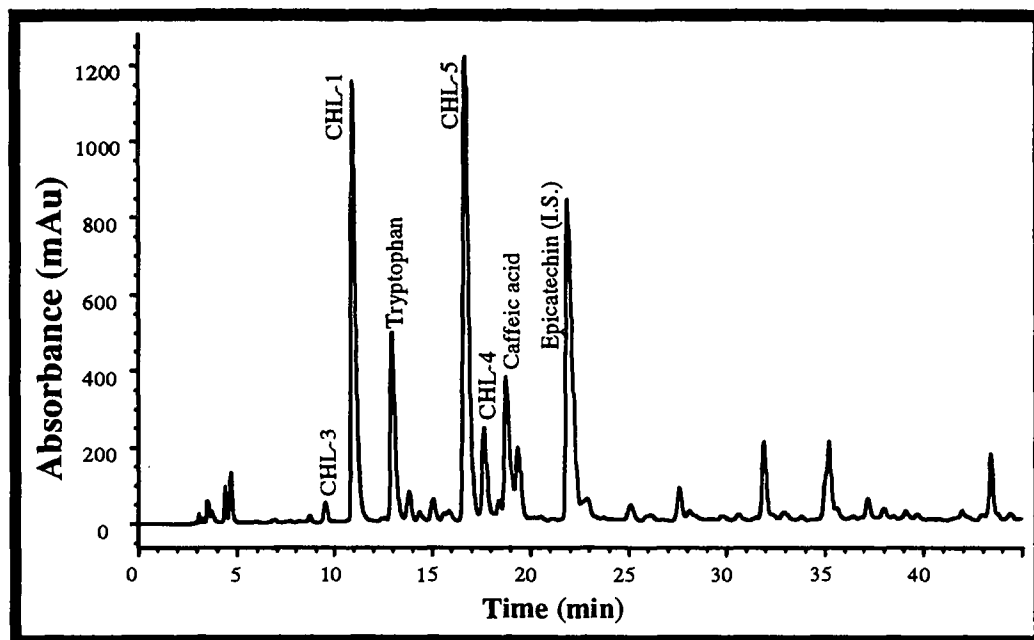
Figure 3.3: HPLC separation of phenolic acids from potato tubers. Column: 25 x 0.46 cm I.D. Supelcosil LC-18 column. Mobile phase: A: 0.07M KH_2PO_4 (pH 2.5) and B: methanol. The program used a gradient: 15% B to 35% B in 25 min, 35% to 45% B in 10 min, 45 to 65% B in 5 min and isocratic conditions with 65% B for 5 min, at a flow rate of 1 mL/min, with a total run time of 45 min.

Total chlorogenic acid (chlorogenic acid and its isomers) varied among varieties (p-value < 0.01) and ranged from 7.2 to 16.8 mg/100 g which accounted for more than 95% of the total phenolic acid content (Table 3.2). Accumulation of polyphenols in potato tubers (especially in the skin) have been reported during wound healing from mechanical damage and when exposed to light (Ramamurthy et al., 1992; Zucker, 1963).

Tryptophan (Table 3.3), an aromatic amino acid, adsorbed to the C_{18} Sep-Pak cartridge with the phenolic acids and was quantified under these conditions. Figure 3.4 shows an HPLC profile for the potato amino acids.

Table 3.2: Phenolic acid content of potato tubers (mg/100g fresh weight)

Potato cultivar	CHL-1 (*)	CHL-3 (**)	CHL-4 (***)	CHL-5 (****)	Total Chlorogenic	Caffeic acid
FL-1625	1.08 b	0.31 a	2.27 b	5.25 a	8.96 a,b	0.07 a
	(0.27)	(0.06)	(0.41)	(1.39)	(1.53)	(0.05)
FL-1815	1.10 b	0.19 a	0.84 a	5.02 a	7.17 a	0.84 b
	(0.39)	(0.06)	(0.25)	(1.46)	(1.52)	(0.28)
ND2471-8	6.17 d	0.36 a	2.00 b	8.25	16.81 c	2.00 c
	(0.28)	(0.10)	(0.34)	(0.97)	(1.30)	(0.49)
Ptarmigan	2.62 c	0.34 a	1.50 b	8.05 a,b	12.54 b,c	1.45 c
	(1.78)	(0.08)	(0.33)	(2.98)	(3.28)	(0.44)
Snowden	0.39 a	0.31 a	1.73 b	11.84 b	14.30 b,c	1.73 c
	(0.10)	(0.05)	(0.36)	(4.70)	(5.08)	(0.57)

*CHL-1 corresponds to 1-O-caffeoylquinic acid; **CHL-3 corresponds to 3-O-caffeoylquinic acid (neochlorogenic acid); ***CHL-4 corresponds to 4-O-caffeoylquinic acid (cryptochlorogenic acid); ****CHL-5 corresponds to 5-O-caffeoylquinic acid (chlorogenic acid). Total chlorogenic represent the sum of concentrations of all caffeoylquinic acid isomers. In parenthesis are presented the standard deviation and different letters corresponds to significant differences among means (p-value < 0.01).

Asparagine and glutamine were the major amino acids present and accounted on average for 54 % of the total peak area, while the 9 amino acids identified (Fig. 3.4) represented ca. 70 % of the total peak area. The free ammonia present in all samples resulted from the ammonium sulfate used to elute the free amino acids. Peaks that eluted between 17 and 20 min showed significant areas but were not identified. Arginine and alanine eluted within that time range but good resolution was not obtained, and the response obtained among and within varieties for those peaks were highly variable. The amino acid content of potato tubers is presented in Table 3.3.

The average asparagine content was 300 mg/100 g with no significant differences among varieties (p-value 0.15). The glutamine content depended on the variety (p-value < 0.01) and ranged from 123 to 278 mg/100 g. Glutamic acid was also

an important amino acid present in similar concentration in all varieties (p-value 0.13) with an average content of 44 mg/100 g. The basic amino acids lysine and histidine were identified; however, we found high variability in response within varieties, as evidenced by the high standard deviations shown in Table 3.3.

Table 3.3: Free amino acid content of potato tubers (mg/100g fresh weight)

Potato cultivar	ASN	GLN	VAL	ASP	GLU	PHE	TRP	HIS	LYS
FL-1625	276.4 a	123.1 a	10.3 a	15.9 a	52.7 a	9.3 a	1.2 a	7.1	11.0
	(21.6)	(16.7)	(3.3)	(11.9)	(19.0)	(1.3)	(0.3)	(5.7)	(6.5)
FL-1815	342.4 a	179.8 b,c	17.6 a	12.5 a	38.4 a	32.1 b	2.5 b,c	14.7	14.1
	(39.6)	(18.0)	(4.4)	(6.3)	(11.9)	(3.4)	(0.6)	(8.3)	(6.2)
ND2471-8	283.2 a	220.2 c	18.1 a	14.7 a	49.2 a	19.3 a	1.5 a,b	21.5	21.9
	(85.2)	(46.2)	(5.6)	(7.4)	(9.1)	(11.3)	(0.4)	(18.2	(17.9
Ptarmigan	350.4 a	277.8 d	40.3 b	19.3 a	34.2 a	31.0 b	3.1 c	21.8	33.3
	(51.8)	(33.4)	(10.3)	(14.5)	(17.5)	(3.5)	(2.0)	(1.3)	(2.4)
Snowden	250.5 a	151.5 a,b	11.8 a	16.1 a	47.9 a	19.9 a	1.8 a,b	18.8	17.1
	(32.6)	(15.3)	(2.1)	(3.7)	(9.7)	(5.0)	(0.9)	(9.9)	(6.8)

In parenthesis are presented the standard deviations and the different letters corresponds to significant differences among means (p-value < 0.01).

Figure 3.4: HPLC separation of amino acids from potato tubers. Columns: 25 x 0.46 cm I.D. Spherisorb ODS-2 coupled with a 15 x 0.39 cm I.D. Pico-Tag. Solvents: A: 0.14 M sodium acetate with 0.5 mL/L Triethylamine (TEA) pH 6.0 and B: 60% acetonitrile in deionized distilled water. The elution program was, at a flow rate of 1 mL/min, 10 min isocratic at 15% B, 15-50% B for 20 min, 50-100% gradient with B for 10 min, and holding with 100% B for 5 min (total run time of 45 min).

Potato Chip Color

The color attributes of chips are summarized in Table 3.4. Colors are expressed in terms of three attributes: hue (color itself), lightness, and saturation or chroma (Anonymous, 1993). The chip color produced by the different potato varieties were significantly different (p-value < 0.01). Lighter chips (higher L* values) were obtained with FL 1825, Snowden and FL 1625. The color of the chips obtained with the different potato varieties were in the orange-red to yellow range according to the CIELAB hue sequence (McGuire, 1992) which defines red-purple as 0°, yellow as 90, bluish-green as 180° and blue as 270°. In general, the potato varieties FL 1815, and Snowden showed the higher levels of yellowness (hue angle 75°), while AC Ptarmigan and ND2471-8 showed higher levels of redness (hue angle 63°). The more intense chip color (high

chroma, c) was obtained with FL 1815 (c=28); less color intensity was obtained for the

other varieties, with Snowden and FL 1625 giving intermediate chroma values (c=26),

and AC Ptarmigan and ND2471-8 producing the least intense color (c=19).

Table 3.4: Color measurements (Hunter CIELab and Agtron) of potato chips obtained
from different potato cultivars

Potato Variety	L*	A*	b*	Chroma	Hue (Degree	Agtron value
FL-1625	56.69 **b**	7.48 **a,d**	24.89 **b**	26.00 **b**	73.27 **b**	36.30 **a,b**
	(0.92)	(0.34)	(0.94)	(0.19)	(1.69)	(4.05)
FL-1815	58.43 **b**	6.86 **b,c**	27.37 **c**	28.26 **c**	75.69 **b**	41.33 **b**
	(0.72)	(0.82)	(0.94)	(0.93)	(0.93)	(1.96)
ND2471-8	49.89 **a**	8.33 **d**	16.69 **a**	18.69 **a**	63.11 **a**	27.70 **a**
	(0.95)	(0.28)	(2.21)	(2.20)	(3.68)	(5.57)
Ptarmigan	49.35 **a**	9.62 **e**	18.09 **a**	20.21 **a**	62.60 **a**	27.37 **a**
	(0.95)	(0.13)	(1.03)	(2.94)	(2.98)	(1.52)
Snowden	57.22 **b**	6.33 **b**	23.83 **b**	24.67 **b**	75.09 **b**	38.10 **a,b**
	(2.60)	(0.88)	(0.76)	(0.55)	(2.34)	(1.84)
Commercial Sample	68.70 **c**	2.68 **a**	26.09 **d**	26.25 **b**	84.19 **c**	61.00 **c**
	(0.91)	(0.03)	(0.81)	(0.77)	(0.16)	(0.15)

In parenthesis are presented the standard deviations and the different letters represent
significant differences among means (p-value < 0.01).

The results from the sensory evaluation of chip color are presented in Table 3.5.
The difference from control test showed that variety had a significant effect (p-value

< 0.01) on the chip color, and the panelists found all samples significantly different from

each other. The magnitude of the chip color differences ranged from moderately (FL

1815) to extremely different (AC Ptarmigan and ND2471-8) compared to the

commercial sample. We found that potato varieties (Snowden and FL 1625) that did

not show significant differences in color with the Hunter ColorQuest were rated as

different by the panelists. FL 1815 and Snowden, varieties with similar L* and hue angle

values, but different chroma, were considered different in color by the panelists. This shows that potato chips differing only in one color dimension (L*, chroma or hue angle) gave different visual characteristics.

Table 3.5: Difference from control and ranking tests for six potato chip samples

Potato cultivar	Difference from control test		Ranking test	
Commercial	1.27	a	1.68	a
sample	(0.55)		(1.17)	
FL-1625	6.68	d	3.68	b
	(1.06)		(0.88)	
FL-1815	4.63	b	2.07	a
	(1.24)		(0.72)	
ND2471-8	8.68	f	5.85	d
	(0.57)		(0.57)	
Ptarmigan	7.95	e	4.76	c
	(0.84)		(0.83)	
Snowden	5.49	c	3.05	b
	(1.23)		(1.22)	
LSD	0.305		0.790	
p-value	<0.01		<0.01	

In parenthesis are presented the standard deviations and the different letters represent significant differences among means (p-value < 0.01). The values reported are mean responses for 43 observations.

A consumer test (Ranking) was performed to compare the performance of the chips obtained with the different varieties against a commercial brand. Significant differences (p-value < 0.01) were found among all the treatments. The panelists ranked the samples, based on color, in the following order: most liked were the commercial brand and FL 1815, followed by Snowden, FL 1625, and least liked were AC Ptarmigan and ND2471-8. Even though FL 1815 and the control (commercial sample) had different color, the panelists preferred both samples equally.

Influence of Composition of Tubers on Color Development

The correlation coefficients (r) between potato chip color and sugars, ascorbic acid, major phenolic acids and amino acids are presented in Table 3.6. The correlation coefficients obtained for L^*, chroma and hue angle were very close, and for discussion purposes we are reporting the averages. A very good correlation (r= 0.95) between Agtron units and the CIELAB measurements was obtained (Table 3.6). The sucrose content showed some correlation (r= -0.5) with potato color, however, Ptarmigan produced the darkest chips with one of the lowest sucrose levels. The reducing sugars, fructose and glucose, showed high negative correlation (r= -0.7) with color. Darker chip colors were obtained with AC Ptarmigan and ND2471-8, varieties which showed the higher reducing sugar contents. The small contribution of sucrose and the important role of reducing sugars in potato chip color found agrees with the results reported by Pritchard and Adam (1994), Marquez and Añon (1986) and Mazza (1983). A maximum tolerable level of sucrose and glucose of 100 and 35 mg/100 g respectively, has been suggested for acceptable potato chip color (Sowokinos and Preston, 1988). We found that the potato chip color did not completely depend on the reducing sugar content in varieties with low reducing sugar content (< 60 mg/100 g). Although reducing sugar content may explain most of the color development, some potato varieties show considerable variation with this association (Habib and Brown, 1956).

In this study we found that varieties (Snowden and FL 1815) with different reducing sugar content produced chips with similar color attributes (L* and hue angle), while varieties with similar reducing sugar content (FL 1625 and Snowden) were rated as different by the panelists based on chip color.

Some other compounds that might explain the extent of non-enzymatic browning of potato chips are amino acids, ascorbic acid and phenolic acids. Amino acids are important substrates in the Maillard reaction; however, their participation in chip color has been reported to be only marginal since their concentration is rarely the limiting factor (Marquez and Añon, 1986).

Table 3.6: Correlation coefficients between color and composition for tubers of five potato cultivars

	L*	a*	b*	Chroma (c)	Hue angle	Agtron
Ascorbic	-0.71	0.52	-0.80	-0.80	-0.73	-0.81
	(0.001)	(0.021)	(0.000)	(0.000)	(0.001)	(0.000)
Sucrose	-0.51	0.20	-0.46	-0.49	-0.46	-0.56
	(0.028)	(0.419)	(0.047)	(0.034)	(0.048)	(0.012)
Fructose	-0.70	0.52	-0.74	-0.74	-0.71	-0.77
	(0.001)	(0.022)	(0.000)	(0.000)	(0.001)	(0.000)
Glucose	-0.64	0.34	-0.70	-0.71	-0.62	-0.68
	(0.003)	(0.161)	(0.001)	(0.001)	(0.004)	(0.002)
Reducing sugars	-0.71	0.48	-0.79	-0.79	-0.71	-0.81
	(0.001)	(0.002)	(0.000)	(0.000)	(0.001)	(0.000)
CHL-1	-0.68	0.51	-0.66	-0.65	-0.68	-0.75
	(0.001)	(0.002)	(0.002)	(0.003)	(0.002)	(0.000)
Chlorogenic acid	0.07	-0.18	-0.16	-0.19	0.02	0.09
	(0.777)	(0.449)	(0.523)	(0.427)	(0.922)	(0.721)
Total Chlorogenic	-0.46	0.15	-0.53	-0.56	-0.37	-0.51
	(0.045)	(0.553)	(0.020)	(0.014)	(0.116)	(0.027)
Asparagine	0.10	0.24	0.24	0.29	0.02	0.12
	(0.678)	(0.330)	(0.322)	(0.225)	(0.938)	(0.628)
Glutamine	-0.49	0.58	-0.42	-0.38	-0.54	-0.49
	(0.035)	(0.010)	(0.075)	(0.110)	(0.018)	(0.033)
Agtron	0.95	-0.75	0.95	0.93	0.95	1.00
	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)

In parenthesis are presented the p-values. The coefficients reported were determined ignoring varieties and plots (19 observations).

Our finding that glutamine (r= -0.5) correlated with color of potato chips agrees with the results obtained by Khanbari and Thompson (1993) who found that glutamine had an important role in fry color development at low reducing sugar concentrations and that arginine had a smaller effect compared to glutamine. We could not quantify arginine in our samples but we do not dismiss its presence. Asparagine has been reported to decrease the gray color intensity in model systems (Khanbari and Thompson, 1993); however, a very low correlation (r= 0.02 for hue angle) with chip color was obtained in this study. Lysine, also found in all tubers, has been reported to play an important role in browning of potato chips (Roe and Faulks, 1991).

Ascorbic acid will react with amino acids during frying and produce dark color in model systems (Smith, 1987); however, Mazza (1983) reported poor correlation between ascorbic acid content and color development in potato chips. We found a very good correlation (r= 0.7) between ascorbic acid levels and potato chip color.

Ascorbic acid concentration in the potato tubers was close to those of glucose and fructose in some varieties and might be enough to cause darkening of potato chips. Phenolic compounds, such as caffeic acid, can undergo nonenzymatic oxidation, favored by alkaline pH and temperature, and generate brown pigments (Cilliers and Singleton, 1989). Chlorogenic acid, the major phenolic acid present in all potato varieties studied, showed poor correlation (r= 0.02 for hue angle) with chip color; however, the CHL-1 showed significant correlation (r= -0.7) with color development. Snowden, a variety that produced good chip color, had intermediate amounts of reducing sugars and ascorbic acid, but had the lowest content of CHL-1.

A high correlation (r> 0.6) between reducing sugars, ascorbic acid, CHL-1 and glutamine was obtained (Table 3.7). Varieties with the highest reducing sugar content also had the highest concentration of ascorbic acid (ND2471-8 and AC Ptarmigan); while FL 1815 showed the lowest reducing sugar and ascorbic acid content. Similar results were obtained for CHL-1 and glutamine. Due to the high correlation among different potato constituents, we could not assign the individual effect of each component on the final potato chip color.

Table 3.7: Correlation coefficients among compositional attributes of five potato cultivars

	Sucrose	Fructose	Glucose	RS	CHL-1	CHL-5	Total CHL	ASN	GLN
Ascorbic	0.46	0.72	0.66	0.77	0.56	0.30	0.59	-0.26	0.42
	(0.05)	(<0.01)	(<0.01)	(<0.01)	(0.01)	(0.22)	(<0.0	(0.29	(0.08)
Sucrose		0.63	0.86	0.67	0.82	0.16	0.57	-0.29	0.13
		(<0.01)	(<0.01)	(<0.01)	(<0.01	(0.53)	(0.01	(0.23	(0.59)
Fructose			0.79	0.97	0.85	0.12	0.54	-0.08	0.71
			(<0.01)	(<0.01)	(<0.01	(0.62)	(0.02	(0.73	(<0.01)
Glucose				0.93	0.84	0.23	0.63	-0.10	0.43
				(<0.01)	(<0.01	(0.34)	(<0.0	(0.68	(0.07)
RS					0.69	0.25	0.28	-0.16	0.54
					(<0.01	(0.31)	(0.26	(0.50	(0.02)
CHL-1						0.02	0.54	0.06	0.58
						(0.92)	(0.02	(0.82	(0.01)
CHL-5							0.85	-0.40	0.02
							(<0.0	(0.09	(0.94)
Total CHL								-0.36	-0.27
								(0.13	(0.26)
ASN									0.53
									(0.02)

In parenthesis are presented p-values. The correlation coefficients were determined ignoring varieties and plots (19 observations)

CONCLUSIONS

We found that sucrose concentration is not a reliable estimator of color quality in potato chips. Reducing sugars, ascorbic acid, phenolic acids and glutamine were highly correlated with potato chip color. Further studies are needed to determine their individual role in the development of color. Varieties with different concentrations of reducing sugars produced chips with similar color attributes suggesting that reducing

sugar content alone does not explain or predict color quality. At low reducing sugar content (<60 mg/100g), other reactants appeared to play a more important role in the final color quality of potato chips.

REFERENCES

Anonymous. 1993. Precise color communication. Minolta Camera Co.

Cilliers J.J.L. and Singleton V.L. 1989. Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. J. Agric. Food Chem. 37:890-896.

Danehy, J.P. 1986. Maillard reactions: Nonenzymatic browning in food systems with special reference to the development of flavor. Adv. Food Res. 30:77-138.

Eskin, N.M. 1990. Biochemistry of food processing: Browning reactions in foods. In *Biochemistry of foods*. Second edition. Academic Press Inc. USA.

Feeney, R.E. and Whitaker, J.R. 1982. The Maillard reaction and its prevention. In *Food Protein Deterioration*. Am. Chem. Soc. 201-647.

Habib, A.T. and Brown, H.D. 1956. Factors influencing the color of potato chips. Food Technol. 10(7):332-336.

Hagen, S.; Augustin, J.; Grings, E. and Tassinari, P. 1993. Pre-column phenylisothiocyanate derivatization and liquid chromatography of free amino acids in biological samples. Food Chem. 46:319-323.

Kadam, S.S.; Dhumal, S.S. and Jambhale, N.D. 1991. Structure, nutritional composition, and quality. In "Potato: processing, production, and products". D.K. Salunkhe, S.S. Kadam and S.J. Jadhav Eds. CRC Press. USA.

Khanbari, O.S. and Thompson, A.K. 1993. Effects of amino acids and glucose on the fry colour of potato crisps. Potato Res. 36:359-364.

Leszkowiat, M.J.; Baricello, V.; Yada, R.Y.; Coffin, R.H.; Lougheed, E.C. and Stanley, D.W. 1990. Contribution of sucrose to nonenzymatic browning in potato chips. J Food Sci. 55(1):281-282.

Linnemann, A.R.; Van Es, A. and Hartmans, K.J. 1985. Changes in the content of L-ascorbic acid, glucose, fructose, sucrose and total glycoalkaloids in potatoes (cv. Bintje) stored at 7, 16 and 28EC. Potato Res. 28:271-278.

Marquez, G. and Añon, M.C. 1986. Influence of reducing sugars and amino acids in the color development of fried potatoes. J. Food Sci. 51(1):157-160.

Mazza, G. 1983. Correlations between quality parameters of potatoes during growth and long-term storage. Am. Potato J. 60:145-159.

McCay, C.M.; McCay, J.B. and Smith, O. 1987. The nutritive value of potatoes. In *Potato processing*. Fourth edition. Van Nostrand Reinhold Company Inc. USA.

McGuire, R.G. 1992. Reporting of objective color measurements. HortSci. 27(12):1254-1255.

Nagels, L.; Van Dongen, W.; De Brucker, J. and De Pooter, H. 1980. High-Performance liquid chromatographic separation of naturally occurring esters of phenolic acids. J. Chrom. 187:181-187.

Pritchard, M.K. and Adam, L.R. 1994. Relationships between fry color and sugar concentration in stored Russet Burbank and Shepody potatoes. Am. Potato J. 71:59-68.

Ramamurthy, M.S.; Maiti, B.; Thomas, P. and Nair, P.M. 1992. High-Performance liquid chromatography determination of phenolic acids in potato tubers (*Solanum tuberosum*) during wound healing. J. Agric. Food Chem. 40:569-572.

Roe, M.A.; Faulks, R.M. and Belsten, J.L. 1990. Role of reducing sugars and amino acids in fry colour of chips from potatoes grown under different nitrogen regimes. J. Sci. Food Agric. 52: 207-214.

Roe, M.A. and Faulks, R.M. 1991. Color development in a model system during frying: Role of individual amino acids and sugars. J. Food Sci. 56(6):1711-1713.

Sapers, G.M.; Douglas, F.W.; Ziolowski, M.A.; R.L. Miller and Hicks, K.B. 1990. Determination of ascorbic acid, dehydroascorbic acid and ascorbic acid-2-phosphate in infiltrated apple and potato tissue by high performance liquid chromatography. J. Chrom. 503:431-436.

Shallenberger, R.S., Smith, O. and Treadway, R.H. 1959. Role of the sugars in the browning reaction in potato chips. J. Agric. Food Chem. 7(4):274-277.

Sieczka, J.B. and Maatta, C. 1986. The effects of handling on chip color and sugar content of potato tubers. Am. Potato J. 63:363-372.

Singleton, V.L. 1987. Oxygen with phenols and related reactions in musts, wines and model systems: Observations and practical implications. Am. J. Enol. Vitic. 38:69-77.

Sinha, N.K.; Cash, J.N. and Chase, R.W. 1992. Differences in sugars, chip color, specific gravity and yield of selected potato varieties grown in Michigan. Am. Potato J. 69:385-389.

Smith, O. 1987. Potato chips. In *Potato processing*. Fourth edition. Van Nostrand Reinhold Company Inc. USA.

Sowokinos, J.R.; Orr, P.H.; Knoper, J.A. and Varns, J.L. 1987. Influence of potato storage and handling stress on sugars, chip quality and integrity of the starch (amyloplast) membrane. Am. Potato J. 64:213-226.

Sowokinos, J.R. and Preston, D.A. 1988. Maintenance of potato processing quality by chemical maturity monitoring (CMM). Station Bulletin 586. Minnesota Agricultural Experiment Station. University of Minnesota.

Spanos, G.A. and Wrolstad, R.E. 1987. Anthocyanin pigment, nonvolatile acids and sugar composition of red raspberry juice. Assoc. Off Anal. Chem. 70:712-722.

Spanos, G.A. and Wrolstad, R.E. 1990. Influence of variety, maturity, processing and storage on the phenolic composition of pear juice. J. Agric. Food Chem. 38:817-824.

Talburt, W.F.; Schwimmer, S. and Burr, H.K. 1987. Structure and chemical composition of the potato tuber. In *Potato processing*. Fourth edition. Van Nostrand Reinhold.

Zucker, M. 1963. Influence of light on synthesis of protein and of chlorogenic acid in potato tuber tissues. Plant Physiol. 38:575-580.

ACKNOWLEDGEMENTS

CHAPTER 4

MODELING THE CONTRIBUTION OF SUGARS, ASCORBIC ACID, CHLOROGENIC ACID AND AMINO ACIDS TO NON-ENZYMATIC BROWNING OF POTATO CHIPS

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ABSTRACT

The contribution of potato constituents to color development in fried chips was evaluated using model systems. Soluble constituents of potato slices were removed using water and ethanol. Leached slices were infiltrated with pH 6.2 solutions containing sucrose, reducing sugars (glucose and fructose), ascorbic, chlorogenic and amino acids (asparagine and glutamine) in quantities typical for potatoes. Infiltrated slice composition was evaluated by HPLC. Slices were fried and the color measured. Reducing sugars showed linear association with L* and hue angle and quadratic relationship with chroma of chips. Ascorbic acid affected chip color at low reducing sugar levels (40 mg/100g tuber); sucrose also contributed to chip color development.

INTRODUCTION

Potato growers supplying raw product to potato chip processors sustain considerable losses when their products are out-of-grade. A major quality defect is excessive browning during frying, which produces an unacceptable color and bitter taste (Roe et al., 1990). The industry uses reducing sugar levels to predict potato tuber suitability for chip processing since they are often an indicator for color development (Roe et al., 1990; Sowokinos and Preston, 1988; Marquez and Añon, 1986).

Although reducing sugar levels may explain most of the color development, some cultivars show considerable variation in this relationship (Habib and Brown, 1956). Reducing sugars did not completely explain color development in cultivars with reducing sugar content < 60 mg/100g (Rodriguez-Saona and Wrolstad, 1997). Amino acids are important substrates in the Maillard reaction; however, their participation in chip color has been reported to be marginal since their concentration is rarely a limiting factor (Marquez and Añon, 1986). Other compounds that might be related to the extent of non-enzymatic browning of potato chips are ascorbic and phenolic acids. A high correlation between color development in potato chips and reducing sugars, ascorbic acid, a chlorogenic acid isomer and glutamine has been reported (Rodriguez-Saona and

Wrolstad, 1997); however, individual effects of components on final color were not determined. Ascorbic acid reacts with amino acids during frying and produces dark color in model systems (Smith, 1987); however, Mazza (1983) reported a low correlation between ascorbic acid content and color development in potato chips. Phenolic compounds, such as caffeic acid, can undergo non-enzymatic oxidation, favored by alkaline pH and temperature, and produce quinones that may react further with amino groups (Kalyanaraman et al., 1987; Wang et al., 1985) or polymerize (Takizawa et al., 1985) and generate brown pigments (Cilliers and Singleton, 1989). Sucrose can be important in chip color development through hydrolysis during frying (Leszkowiat et al., 1990; Mazza, 1983; Shallenberger et al., 1959). However, low correlations between sucrose levels and potato chip color have been reported (Rodriguez-Saona and Wrolstad, 1997; Pritchard and Adam, 1994; Marquez and Añon, 1986), and the effect of sucrose has been described as marginal (Roe and Faulks, 1991).

Our objective was to determine the individual contribution of ascorbic acid, amino acids (glutamine and asparagine), phenolics (chlorogenic acid), sucrose and reducing sugars to development of potato chip color by carefully controlling their concentrations through use of model systems.

MATERIALS AND METHODS

Preparation of Potato Chips

Russet Gold potato tubers were obtained from the local market (Cub Foods, Corvallis, OR) washed, sliced (4 mm thick) and immediately placed in cold water to prevent browning. The soluble components of potato slices were removed using the procedure described by Khanbari and Thompson (1993). Potato slices were placed in hot distilled water (60°C) for 40 min, drained, and transferred to a heated 50% ethanol solution (45-50°C) for 40 min. The slices were washed with cold distilled water several times and stored in distilled water at 1°C. The effectiveness of leaching was confirmed by HPLC analysis and the absence of chip color development when fried.

Leached potato slices were placed in beakers with 300 mL phosphate buffer solution containing different concentrations of sugars, chlorogenic acid, ascorbic acid and amino acids (asparagine and glutamine), according to the model under investigation. All reagents were obtained from Sigma Chemical Co, St. Louis, MO. The potato slices were infiltrated under vacuum for 1 hr at room temperature using a controlled environment oven (National Appliance Co., Portland, OR) to improve the absorption and equilibration of components by the slices. After infiltration slices were drained and their surfaces were dried with paper tissues before frying and analysis.

Infiltrated slices (50g) were fried in fresh partially hydrogenated canola oil for 3 min at 180°C using an automatic heat control cooker/fryer (Sears, Roebuck and Co., Albany, OR) and drained by placing the chips for a few minutes on paper towels.

Model Systems

Model 1: Preliminary study. Leached potato slices (60g) were infiltrated with 0.1M phosphate buffer (pH 7.0) solutions containing fixed concentrations of reducing sugars (1.25 mg/mL each), sucrose (2.25 mg/mL), L-ascorbic acid (1.25 mg/mL), L-glutamine (2.0 mg/mL), L-asparagine (3.5 g/mL) and chlorogenic acid (0.05 mg/mL). Additional 60g samples of leached slices were infiltrated in the same way with all but one of the components. We eliminated one component at a time to determine which components were contributing to color development. The order of the treatments was randomized and the experiment was duplicated.

Model 2: Solutions of 0.2M phosphate buffer (pH 6.2) containing different concentrations of reducing sugars (0.45, 0.85, 1.70, 3.40 and 6.80 mg/mL), ascorbic acid (0.05, 0.07, 0.15, 0.3 and 0.45 mg/mL) and amino acids (5, 7, 10, 13 and 15 mg/mL) were used to infiltrate leached potato slices (100g). The treatments were arranged as a 3 factor central composite design with a total of 15 different treatment combinations with 3 replications at the center point. The order of the treatments was randomized and the experiment was duplicated.

Model 3: Solutions of 0.2M phosphate buffer (pH 6.2) containing different concentrations of ascorbic acid (0.35, 0.70, and 1.40 mg/mL), chlorogenic acid (0.07, 0.1 and 0.4 mg/mL), and fixed concentrations of reducing sugars (0.70 mg/mL), and glutamine (2.7 mg/mL) were used to infiltrate the leached potato slices (100g). A 3x3 randomized factorial design was used, the order of the treatments was randomized and the experiment was done in triplicate.

Model 4: Solutions of 0.2M phosphate buffer (pH 6.2) containing different concentrations of sucrose (0.65, 1.30, and 2.60 mg/mL) and a fixed concentration of glutamine (2.7 mg/mL) were used to infiltrate the slices (100g), and were compared with a solution containing reducing sugars (0.70 mg/mL) instead of sucrose. The experiment was done in triplicate and the order of treatments was randomized.

Color Measurements

Potato chips were crushed into small pieces and placed in an optical glass cell (13 cm dia x 5 cm ht, Hunter Associates Lab. Inc., Reston, VA) forming a layer 2 cm deep. Hunter CIE L* (lightness), chroma (saturation) and hue angle (color itself), were determined using a HunterLab 45/0 ColorQuest colorimeter (Hunter Associates Laboratories Inc., Reston, VA). Measurements were made using Reflectance Specular included mode, Illuminant C and 10° observer angle.

Compositional Analysis

Potato slices extract

A sample of 25 g of infiltrated slices was blended with 100 mL ethanol (95%) for 1 min using a Waring Blendor (Dynamics Corporation of America, New Hartford, CT). One mL of a solution containing mannitol (40 mg/mL), epicatechin (0.25 mg/mL) and α-aminobutyric acid (ABA, 30 mg/mL) was added to the resulting slurry as an internal standard and the mixture was filtered through Whatman No 4 paper. The solid residue was rinsed twice with 50 mL ethanol (80%) and the eluate was added to the previous

filtrate. The solution was placed in a rotovapor (Buchi, Switzerland) at 40°C to evaporate ethanol and taken to a volume of 10 mL with deionized distilled water. The extract was stored at -20°C until analyzed.

Fried chips extract

Composition after frying was evaluated for chips infiltrated with different levels of ascorbic acid and amino acids, and chips from models 1 and 4. Fried chips (20g) were blended with 100 mL acetone to extract all soluble components, and filtered through Whatman No 4 paper. The residue was washed with 50 mL 70% acetone. The filtrate was placed in a separatory funnel with 2 volumes of chloroform and stored overnight at 1°C. Water soluble components were concentrated in the top aqueous portion, while lipids and other non water soluble compounds remained in the acetone/chloroform portion. The aqueous portion was collected, and placed in a Buchi rotovapor at 40°C until all residual acetone was evaporated. The aqueous extract was made up to a known volume with distilled water.

Sugar Analysis

Sample preparation

Five mL of potato extract were passed through an activated C_{18}-Sep-Pak cartridge and the eluate was passed through a 1.5 mL BioRex-5 anion exchange resin (Bio-Rad Laboratories, Hercules, CA). The solution was filtered through a 0.45 μm millipore filter type HA (Millipore Corp., Bedford, MA) and analyzed by HPLC (Rodriguez-Saona and Wrolstad, 1997).

HPLC analysis of sugars

Equipment: A High Performance Liquid Chromatograph (Varian LC 5020), equipped with a column heater, Varian Refractive Index detector (Varian Instrument Group, Walnut Creek, CA), a Perkin Elmer LCI-100 Laboratory computing Integrator (Perkin

Elmer, Norwalk, CT) and a Beckman 501 autosampler (Beckman, Emmen, The Netherlands) with a 50 μL loop was used. Column: 30 x 0.78 cm I.D. Aminex Carbohydrate HPX-87C fitted with a 4 x 0.46 cm Carbo-C micro guard column (Bio-Rad Laboratories, Richmond, CA) at 87°C. Mobile phase: 0.2 mg/mL $Ca(NO_3)_2$ run isocratically at a flow rate of 0.7 mL/min. The sugar standard curve was constructed using 3 concentrations of sucrose, glucose and fructose (2.5, 1 and 0.5 mg/mL) and mannitol was the internal standard at 4 mg/mL. Each standard solution was prepared by diluting stock solutions of sucrose (25 mg/mL), glucose (25 mg/mL), fructose (25 mg/mL) and mannitol (40 mg/mL).

Free Amino Acid Analysis

Sample preparation

Potato slices extracts (15 FL) were derivatized with phenylisothiocyanate (PITC) following the procedure described by Hagen et al. (1993). Derivatized samples were diluted with 1 mL deionized distilled water, filtered through a 0.45 μm millipore filter type HA and injected into the HPLC.

HPLC analysis of amino acids

Equipment: A High Performance Liquid Chromatograph Perkin-Elmer Series 400 (Perkin Elmer, Norwalk, CT), equipped with a Hewlett-Packard 1040A photodiode array detector (Hewlett-Packard, Waldbronn, Germany), Gateway 2000 P5-90 computer with a Hewlett-Packard HPLC[2D] ChemStation software (Hewlett-Packard, Wilmington, DE) and a Beckman 501 autosampler (Beckman, Emmen, The Netherlands) with a 50 μL loop was used. The separations used coupled columns, a 25 x 0.46 cm I.D. Spherisorb ODS-2 (Alltech Associates, Deerfield, IL) and 15 x 0.39 cm I.D. Pico-Tag (Waters Chrom. Division, Milford, MA), fitted with a 1 x 0.46 cm Spherisorb ODS-2 micro guard cartridge. The solvents were A: 0.14 M sodium acetate with 0.5 mL/L triethylamine (TEA) adjusted to pH 6.0 with glacial acetic acid, and B:

60% acetonitrile in deionized distilled water. The elution program was 10 min isocratic at 15% B, 30 min linear gradient with 15-80% B, and 5 min at 80% B, using a flow rate of 1 mL/min. The effluent was monitored at 254 nm. An amino acid standard curve was prepared using asparagine and glutamine (40, 20 and 10 μg/mL) and α-aminobutyric acid as internal standard (20 μg/mL), prepared from a stock solution containing 0.4 mg/mL of each of the amino acids.

Total Ascorbic and Chlorogenic Acids

Sample preparation

Ascorbic and chlorogenic acids were determined using the method of Sapers et al. (1990). Tuber slices (25g) were blended with a solution containing 30 mL 2.5% metaphosphoric acid and 60 mL acetonitrile:0.05M KH_2PO_4 (75:25). The homogenate was filtered through Whatman No 4 paper and filtered through a 0.45 μm Millipore filter type HV (Millipore Corp., Bedford, MA).

HPLC analysis

The same equipment described for the amino acid analysis was used. Separation was carried out using a 25 x 0.46 cm I.D. Econosphere NH_2 (Alltech, Deerfield, PA) column with an amino guard column. Mobile phase: Acetonitrile:0.05 M KH_2PO_4 (75:25) with 1 g/L dithiothreitol (DTT) (Sigma Chemical Co., St. Louis, MO), run isocratically at a flow rate of 2 mL/min and the effluent was monitored at 254 nm. The standard curve was prepared using 100, 75, 50 and 25 μg/mL solutions of chlorogenic and ascorbic acids.

Statistical Analyses

The model systems were analyzed by Response Surface Analysis, ANOVA (Randomized Complete Block Design) and Regression, with significance defined at

p < 0.05. Significant differences among mean responses were determined by multiple comparison tests (LSD and Contrast). All statistical analyses were performed using SAS statistical software (SAS system for windows, release 6.11, SAS Institute, Inc., 1996).

RESULTS AND DISCUSSION

Contribution of Different Components to Potato Chip Color (Model 1)

In a preliminary study, the contribution of different potato tuber components to the color quality of fried chips was evaluated (Table 4.1). Tuber slices were infiltrated with different components under standardized conditions and their composition was determined by HPLC. Some variability in infiltration rate of different components into the slices and among different batches of infiltrated slices was observed. Therefore, for greater accuracy in all statistical analyses we used the true composition of slices as measured by HPLC rather than the equilibrium values of infiltrated solutions.

The leaching of potatoes resulted in slices that developed very little color (L*= 52.1, chroma= 16.9, and hue angle= 89.5) when fried, as compared to slices with the complete set of components (L*= 46.5, chroma= 39.8, and hue angle=68.5). Elimination of reducing sugars or amino acids had an effect on chroma (lower saturation) and hue angle (higher yellowness) of chips (p-value < 0.01). Reducing sugars had the most marked influence on lightness and their absence produced the brighter colors. Chips infiltrated with solutions without ascorbic acid showed higher mean values for L* (lighter color) and hue angle (lower redness) than those with the complete set of components, indicating a positive correlation between ascorbic acid content and potato chip color development. However, statistical analysis (contrast) showed that differences between means were not significant (p-value > 0.3) as compared to the complete set. Removal of sucrose and chlorogenic acid did not show any effect on color characteristics of fried chips (p-value > 0.5).

Table 4.1: Effects of tuber constituents on color development of fried chips

Model	pH	Color Indices		
		L*	Chroma	Hue angle
Complete set	6.88	46.5 a	39.8 a	68.5 a
		(1.4)	(0.9)	(0.5)
No Ascorbic acid	6.99	49.1 a	38.6 a	70.1 a
		(0.7)	(1.1)	(0.2)
No chlorogenic acid	6.88	45.5 a	40.3 a	67.2 a
		(1.6)	(0.6)	(0.1)
No amino acids	6.90	49.3 a	33.8 b	75.6 b
		(3.4)	(0.7)	(4.0)
No sucrose	6.88	45.5 a	39.0 a	68.6 a
		(0.5)	(0.3)	(0.4)
No reducing sugars	6.88	57.2 b	31.3 b	86.0 b
		(3.3)	(2.3)	(1.8)

Composition of Complete Set Model (mg/100g)

Reducing sugars	102 ± 24
Sucrose	75 ± 10
Chlorogenic acid	4 ± 1
Asparagine	229 ± 59
Glutamine	148 ± 39

Note: Values are averages from 2 replications. Standard deviations ( ). Different letters indicate significant differences among means (p-value < 0.01).

Solutions containing ascorbic acid had a lower pH (6.9) than the other sets (pH 7.0). Roe and Faulks (1991) reported that pH had an effect on rate and extent of chip browning, and this pH change, though small, may have influenced results. In all models thereafter we increased the buffer solution from 0.1M to 0.2M to increase the buffering capacity and prevent any effect of pH on rate of the Maillard reaction. The composition of 5 chipping potato cultivars (FL 1815, FL 1625, ND2476-8, AC Ptarmigan and Snowden) and its contribution to potato chip browning (Rodriguez-Saona and Wrolstad, 1997) has been reported. The average pH of those tubers was 6.2 ± 0.1, within the

values (5.5-6.5) reported for potato tubers (Burton, 1966). Therefore, the pH of our buffer solutions was modified to 6.2 for subsequent model systems.

Effect of Reducing Sugars, Ascorbic and Amino Acids on Chip Color (Model 2)

Effects of reducing sugar, ascorbic acid and amino acid content on color of fried chips were evaluated by infiltrating leached slices with different concentrations reported for potato cultivars (Rodriguez-Saona and Wrolstad, 1997; Sinha et al, 1992; Linnemann et al., 1985; Davies, 1977; Talley and Porter, 1970). Levels of reducing sugars and amino acids explained most of the variations in chip color (75 to 88%). Reducing sugar content showed a linear relationship with lightness (p-value < 0.01) and hue angle (p-value < 0.01) and a quadratic relationship with chroma (p-value < 0.01) of the fried slices (Fig. 4.1). With the regressions we found that an increase of 10 mg/100 g of reducing sugars, with other reactants constant, would cause a 1 unit decrease in mean L* and hue angle. The amino acid (asparagine and glutamine) content of the slices did not affect the L* or hue angle values of the chips. Amino acids had only minor effects (p-value of 0.03) on chip chroma (Fig. 4.2), however, this small p-value was due to a single point with an unusually large amount of glutamine. This confirmed findings that amino acid concentration was rarely the limiting factor in potato chip browning reactions (Roe et al., 1990; Marquez and Añon, 1986). Ascorbic acid did not affect (p-value > 0.43) chip quality at the concentrations evaluated. Also, there were no significant interaction effects between the components.
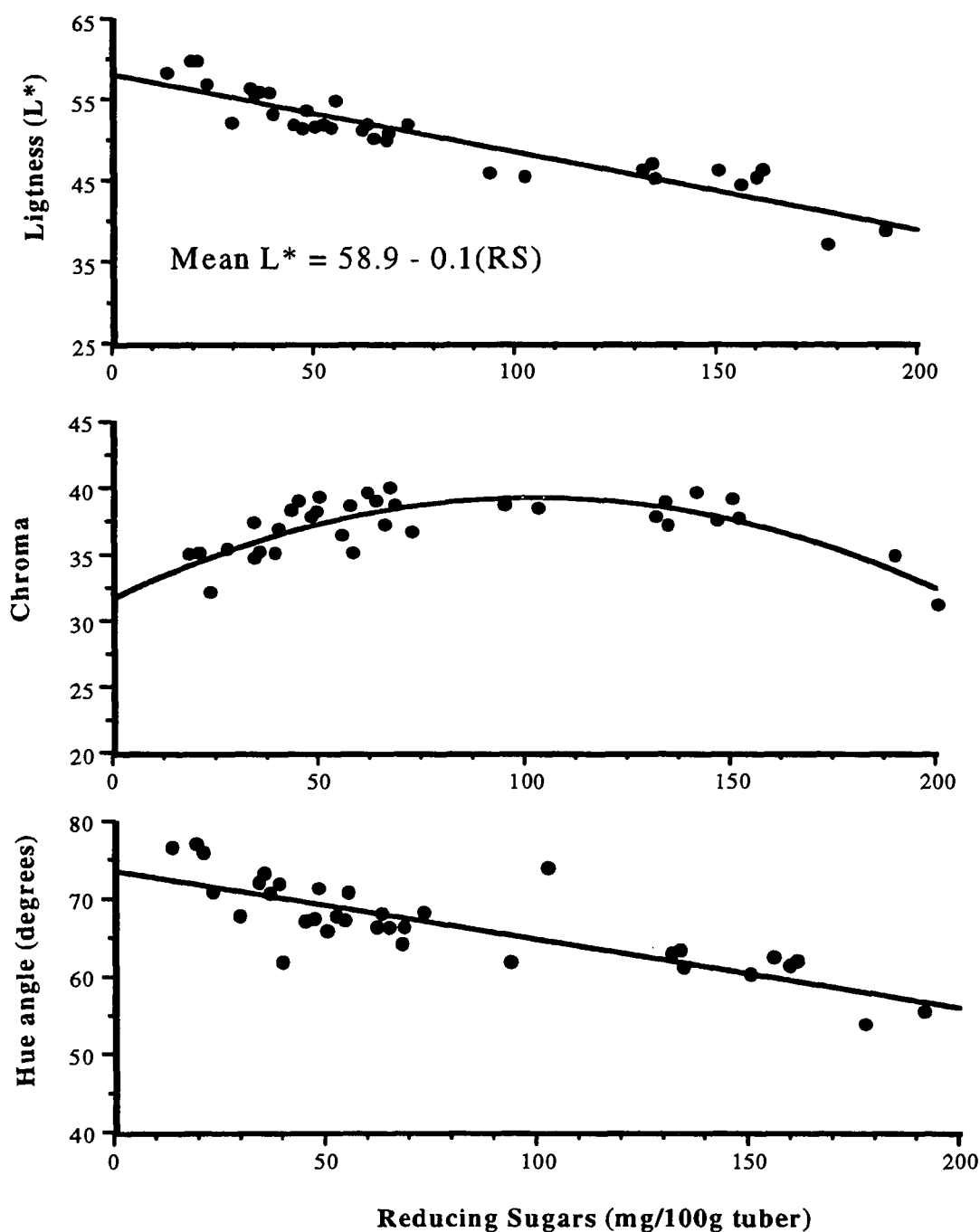
Figure 4.1. Effects of reducing sugar concentration on potato chip color, as suggested by Model 2. Lines shown represent the ordinary least squares fit, and for chip chroma ignore the effects of Gln. Included are the regression equations with variables at p-value < 0.01. The regression equation for mean chroma is **32.2 + 0.12(RS) + 0.005(Gln) - 0.0006(RS)^2** which is quadratic in RS for any fixed value of Gln. RS: Reducing sugars (glucose + fructose) at levels from 20-210 mg/100g. Gln: glutamine.
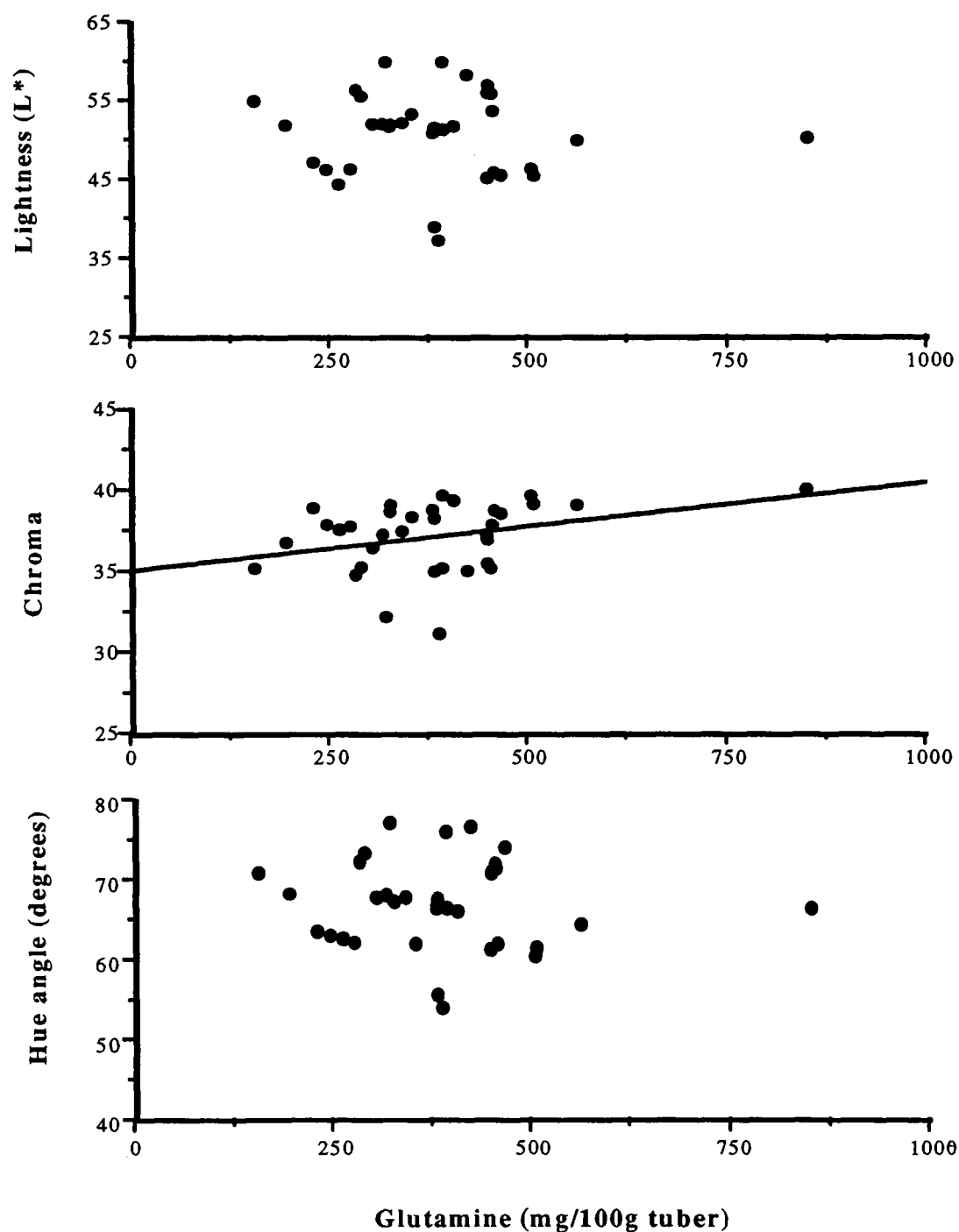
Figure 4.2. Effects of glutamine concentration on potato chip color, as suggested by Model 2. Lines shown represent the ordinary least squares fit. Included are the regression equations with variables at tp-value < 0.01. The regression equation for mean chroma is **32.2 + 0.12(RS) + 0.005(Gln) - 0.0006(RS)^2** which is linear in Gln for any fixed value of RS. Gln: Glutamine at levels from 200-850 mg/100g.

Changes in Potato Chip Composition with Frying

Frying of the tuber slices resulted in an average 56 ± 1.6% loss in weight and some changes in different components infiltrated into the slices (Table 4.2). Reducing sugars and glutamine were completely consumed during frying while an average of 18% ascorbic acid, 20% sucrose and 43% asparagine were recovered. Asparagine and glutamine are the major free amino acids in potato tubers and are generally present in comparable amounts but show very different browning potential (Khanbari and Thompson, 1993; Roe and Faulks, 1991).

Table 4.2: Effects of frying on composition of infiltrated potato slices

Compound	Infiltrated* (mg/100g)	Remaining after frying (mg/100g)	Recovery (%)
Ascorbic acid	61 ± 1	12 ± 3	19 ± 4
	37 ± 2	6 ± 0.7	16 ± 3
Sucrose	200 ± 9	29 ± 3	16 ± 1
	137 ± 5	26 ± 3	19 ± 2
	65 ± 5	16 ± 2	24 ± 2
Reducing sugars	75 ± 16	N.D.	N.D.
Asparagine	241 ± 20	116 ± 8	48 ± 7
	356 ± 24	134 ± 5	38 ± 1
Glutamine	235 ± 21	N.D.	N.D.

* Average concentration (mg) of different compounds in 100g of potato slices after 60 min infiltration under vacuum.

Asparagine content has been reported to have low correlation with chip color development (Rodriguez-Saona and Wrolstad, 1997) and to decrease the grey color intensity in model systems (Khanbari and Thompson, 1993). Glutamine has been described as an important amino acid in fried chip color development (Khanbari and

Thompson, 1993; Roe and Faulks, 1991). Asparagine is only partially involved during browning reactions and would probably have minor effects on chip color. Based on these results we decided to use only glutamine in subsequent model systems.

Effect of Ascorbic and Chlorogenic Acids on Chip Color at Low Reducing Sugar Levels (Model 3)

Ascorbic acid and chlorogenic acid are found in fresh potato tubers in concentrations from 8 to 30 mg/100g and 4.5 to 19 mg/100g, respectively (Rodriguez-Saona and Wrolstad, 1997; Dao and Friedman, 1992; Smith, 1968). The ability of ascorbic and phenolic acids (chlorogenic and caffeic) to undergo nonenzymatic oxidation in alkaline conditions and produce brown pigments has been reported in model systems (Monsalve et al., 1990; Cilliers and Singleton, 1989). However, the effects of ascorbic and phenolic acids in color development of fried chips have not been completely determined. Rodriguez-Saona and Wrolstad (1997) reported that reducing sugar content did not completely predict color quality when present at low (< 50 g/100g) concentrations. In Model 3 we evaluated the effects of ascorbic and chlorogenic acid contents at low reducing sugar concentrations (40 mg/100g). Results showed that ascorbic acid had an effect on chroma and hue angle of the potato chips (p-value < 0.01) while chlorogenic acid content did not influence color development (p-value > 0.12). The regression showed that ascorbic acid content explained 48% of the chroma and 60% of the hue angle even after accounting for the effects of reducing sugars. A linear response was found between ascorbic acid concentration and color attributes (chroma and hue angle) of the chips (Fig. 4.3). Potato slices with higher ascorbic acid contents resulted in chips with higher saturation (chroma) and increased redness (hue angle). Smith (1987), using a model system of filter paper disks, reported that ascorbic acid concentrations in potato tubers were usually not high enough to produce unacceptable chip darkening without reducing sugars. Our results show, however, that ascorbic acid concentration could be important in chip browning when reducing sugars are at low concentrations.
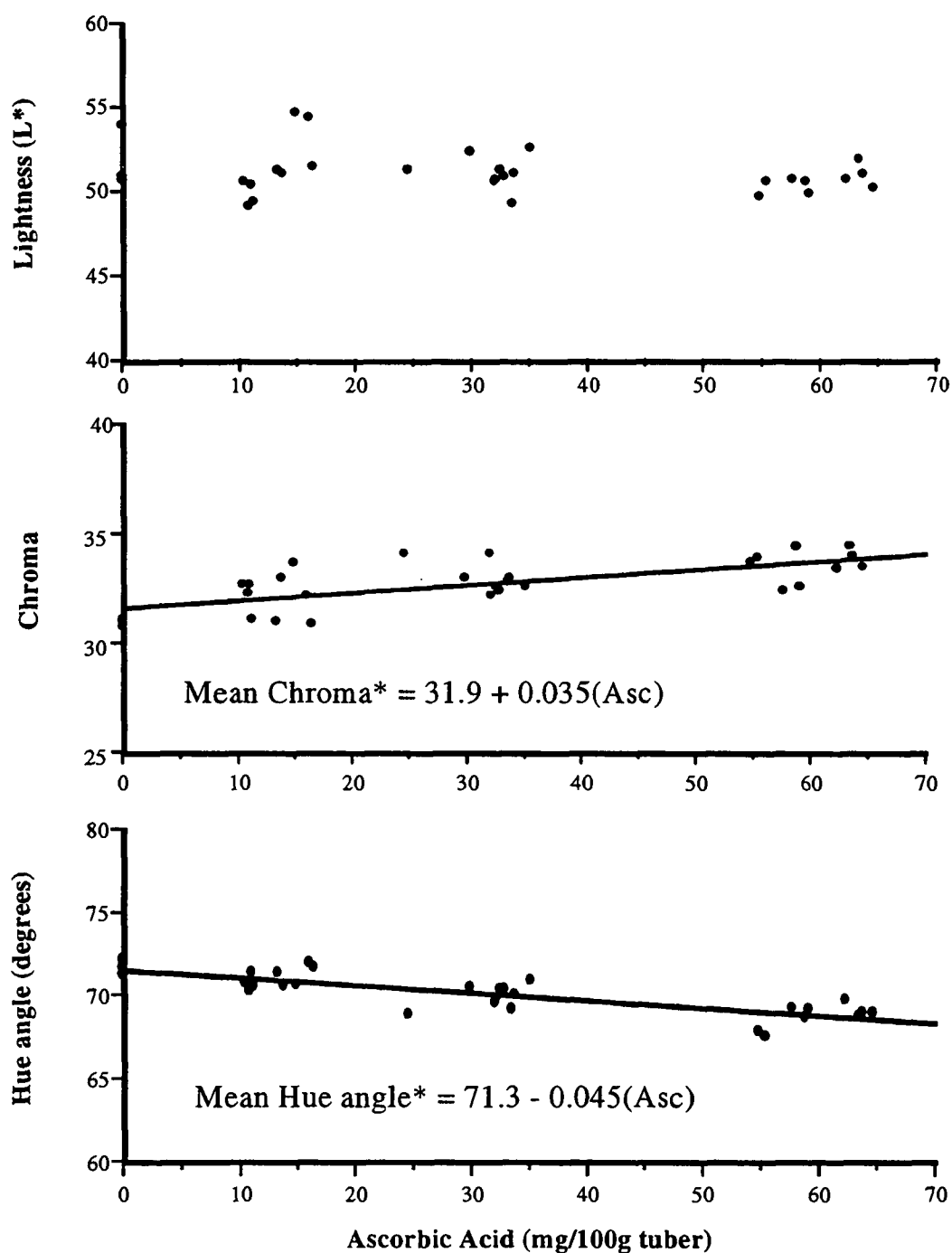
Figure 4.3. Effects of ascorbic acid on potato chip color at low reducing sugar concentration (40 mg/100g), as suggested by Model 3. Included are the regression equations with variables at p-value < 0.01. Asc: Ascorbic acid at levels from 15-60 mg/100g and Chl: Chlorogenic acid at levels from 5-35 mg/100g.

Effect of Sucrose Concentration in Chip Color (Model 4)

Potato slices were infiltrated with different concentrations of sucrose and with glutamine (250 mg/100g) to determine its participation in the browning reaction. A control treatment (no sugars added) and a treatment containing reducing sugars (40 mg/100g) and glutamine were also compared. Sucrose concentration showed an effect on chroma and hue angle of fried chip (p-value < 0.001) and the results also suggested some effect on L* (p-value= 0.04). Sucrose can participate in the browning reaction of potato chips through its hydrolysis during frying (Leszkowiat et al., 1990; Shallenberger et al., 1959), although sucrose content alone did not correlated highly with fry color (Rodriguez-Saona and Wrolstad, 1997; Roe and Faulks, 1991; Marquez and Añon, 1986). Our results (Fig. 4.4) showed a linear relationship between sucrose concentration and chip color. The model explained 82% of the variability in chroma and 85% in hue angle, after accounting for the effects of reducing sugars. According to the regression, an increase of 10 mg/100 g of sucrose, maintaining the other reactants constant, would result in a 0.2 unit decrease in mean hue angle and a 0.16 unit increase in mean chroma. However, when slices were infiltrated with low concentrations of reducing sugars (much lower than sucrose), we found more extensive chip browning with mean values for L* = 48.0 ± 2.2, chroma = 33.9 ± 1.0, and hue angle = 67.5 ± 1.2. Analysis of the fried chips (Table 4.2) showed that about 80% of sucrose was lost during frying and no glucose or fructose was detected. Shallenberger et al. (1959) reported that participation of sucrose in carbonyl-amino browning was contingent on sucrose hydrolysis, with subsequent reaction of glucose and fructose with amino acids.

Sucrose hydrolysis and production of melanoidin pigments would be increased by acidic and high temperature conditions (Leszkowiat et al.,1990) as well as frying time. Removal of water during frying could limit sucrose hydrolysis (Leszkowiat et al.,1990). There was a substantial loss of sucrose in our model and complete loss of reducing sugars. The amount of color generated, however, was not as much as that predicted from the quantity of reducing sugars generated.
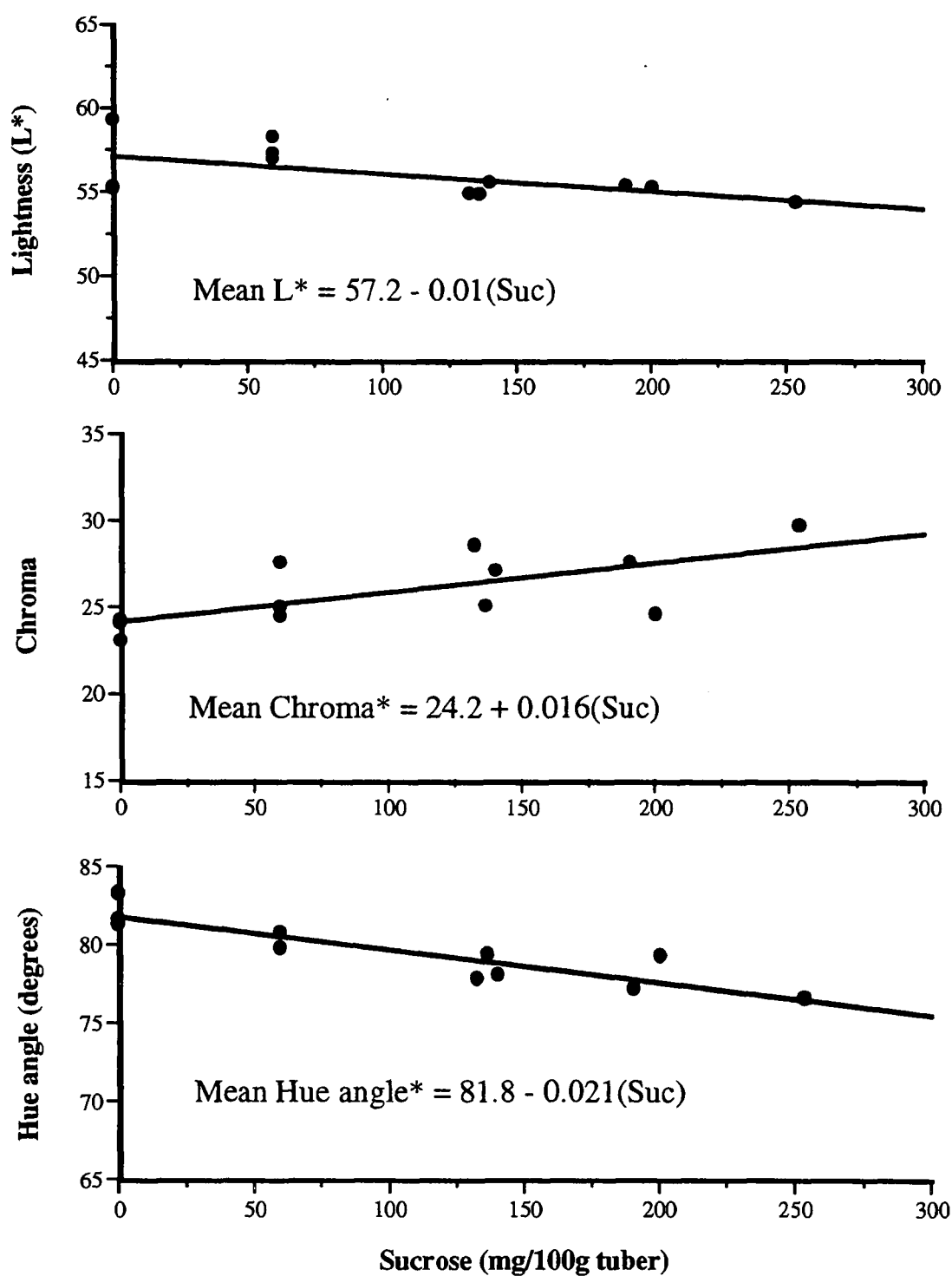
Figure 4.4. Sucrose contribution to potato chip color in the presence of glutamine (250 mg/100g), as suggested by Model 4. Included are the regression equations with variables at p-value < 0.01. Suc: Sucrose at levels from 60-215 mg/100g.

A possible explanation was that additional frying time might be required for color development from the intermediate (colorless) reaction products.

Observations

In Model 3, the lightness and chroma after frying of leached potatoes were L*= 53.1, chroma = 16.8, close to those values from Models 1 and 2. The hue angle with Model 3 (hue angle = 85.5) was 4 degrees lower than those from Model 1 and 2. The fried leached potatoes in model 4 had darker color (L*=49.0, chroma = 19.9 and hue angle = 82.5). These color differences might be due to differences in intrinsic characteristics of the tubers and could explain the differences in color among models for the chips with low reducing sugars (40 mg/100g).

The color of fried chips from five chipping potato cultivars has been evaluated (Rodriguez-Saona and Wrolstad, 1997), and a sensory panel rated preferences for the chips. Our model systems produced chips with color attributes similar to those reported for the chipping potato cultivars. Based on reported color preferences by panelists and results from our modeling systems we suggest that levels in the order of 40 mg/100g of reducing sugar, 400 mg/100g of glutamine, 16-20 mg/100g of ascorbic acid and 60-100 mg/100g of sucrose should provide acceptable potato chip color.

CONCLUSIONS

This study confirmed the major contribution of reducing sugar concentration to the development of chip color. Amino acid (glutamine) concentration affected the chroma of potato chips. Chlorogenic acid, the major phenolic acid in potato tubers, had no effect on color development. At low concentration of reducing sugars (40 mg/100g), ascorbic acid and sucrose concentration affected color quality (chroma and hue angle) of fried chips. Selection of chipping potato cultivars with low reducing sugars would not ensure low browning if other reactants such as ascorbic acid or sucrose were accumulated.

REFERENCES

Burton, W.G. 1966. *The Potato*. 2nd edition. Veenman, Wageningen.

Cilliers J.J.L. and Singleton V.L. 1989. Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. J. Agric. Food Chem. 37:890-896.

Dao, L. and Friedman, M. 1992. Chlorogenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry. J. Agric. Food Chem. 40:2152-2156.

Davies, A.M.C. 1977. The free amino acids of tubers of potato varieties grown in England and Ireland. Potato Res. 20:9-21.

Habib, A.T. and Brown, H.D. 1956. Factors influencing the color of potato chips. Food Technol. 10(7):332-336.

Hagen, S., Augustin, J.,Grings, E. and Tassinari, P. 1993. Pre-column phenylisothiocyanate derivatization and liquid chromatography of free amino acids in biological samples. Food Chem. 46:319-323.

Kalyanaraman, B., Premovic, P.I. and Sealy, R.C. 1987. Semiquinone anion radicals from addition of amino acids, peptides, and proteins to quinone derived from oxidation of catechols and catecholamines. J. Biol. Chem. 262:11080-11087.

Khanbari, O.S. and Thompson, A.K. 1993. Effects of amino acids and glucose on the fry colour of potato crisps. Potato Res. 36:359-364.

Leszkowiat, M.J., Baricello, V., Yada, R.Y., Coffin, R.H., Lougheed, E.C. and Stanley, D.W. 1990. Contribution of sucrose to nonenzymatic browning in potato chips. J. Food Sci. 55:281-282.

Linnemann, A.R., Van Es, A. and Hartmans, K.J. 1985. Changes in the content of L-ascorbic acid, glucose, fructose, sucrose and total glycoalkaloids in potatoes (cv. Bintje) stored at 7, 16 and 28 EC. Potato Res. 28:271-278.

Marquez, G. and Añon, M.C. 1986. Influence of reducing sugars and amino acids in the color development of fried potatoes. J. Food Sci. 51:157-160.

Mazza, G. 1983. Correlations between quality parameters of potatoes during growth and long-term storage. Am. Potato J. 60:145-159.

Monsalve, A., Powers, J.R. and Leung, H.K. 1990. Browning of dehydroascorbic acid and chlorogenic acid as a function of water activity. J. Food Sci. 55:1425-1428.

Pritchard, M.K. and Adam, L.R. 1994. Relationships between fry color and sugar concentration in stored Russet Burbank and Shepody potatoes. Am. Potato J. 71:59-68.

Rodriguez-Saona, L.E. and Wrolstad, R.E. 1997. Influence of potato composition on chip color quality. Am. Potato J. 72:87-106.

Roe, M.A., Faulks, R.M. and Belsten, J.L. 1990. Role of reducing sugars and amino acids in fry colour of chips from potatoes grown under different nitrogen regimes. J. Sci. Food Agric. 52:207-214.

Roe, M.A. and Faulks, R.M. 1991. Color development in a model system during frying: Role of individual amino acids and sugars. J. Food Sci. 56:1711-1713.

Sapers, G.M., Douglas, F.W., Ziolowski, M.A., R.L. Miller and Hicks, K.B. 1990. Determination of ascorbic acid, dehydroascorbic acid and ascorbic acid-2-phosphate in infiltrated apple and potato tissue by high performance liquid chromatography. J. Chrom. 503:431-436.

Shallenberger, R.S., Smith, O. and Treadway, R.H. 1959. Role of the sugars in the browning reaction in potato chips. J. Agric. Food Chem. 7(4):274-277.

Sinha, N.K., Cash, J.N. and Chase, R.W. 1992. Differences in sugars, chip color, specific gravity and yield of selected potato varieties grown in Michigan. Am. Potato J. 69:385-389.

Smith, O. 1968. Potatoes: Production, Storing, Processing. AVI publishing Co. Westport. CO.

Smith, O. 1987. Potato chips. In *Potato processing*. Fourth edition. Van Nostrand Reinhold Company Inc. USA.

Sowokinos, J.R. and Preston, D.A. 1988. Maintenance of potato processing quality by chemical maturity monitoring (CMM). Station Bulletin 586. Minnesota Agricultural Experiment Station. University of Minnesota.

Takizawa, Y., Munakata, T., Iwasa, Y. Suzuki, T. and Mitsuhashi, T. 1985. Novel oxidative coupling of monophenols in the system of cupric chloride-Oxygen-Alcohol. J. Org. Chem. 50:4383-4386.

Talley, E.A. and Porter, W.L. 1970. Chemical composition of potatoes. Relationship of the free amino acid concentrations to specific gravity and storage time. Am. Potato J. 47:214-224.

Wang, T.S.C., Chen, J. and Hsiang, W. 1985. Catalytic synthesis of humic acids containing various amino acids and dipeptides. Soil Sci. 140:3-10.

ACKNOWLEDGEMENTS

CHAPTER 5

ANTHOCYANIN PIGMENT COMPOSITION OF RED-FLESHED POTATOES

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ABSTRACT

Red potatoes (*Solanum tuberosum and S. stenotomum*) were evaluated as potential sources for natural red colorants. Qualitative anthocyanin composition, pigment content and phenolic composition were screened on 33 cultivars. Monomeric anthocyanin content, determined by pH differential, ranged from 2 to 40 mg/100g tuber fresh weight. Two breeding clones, NDOP5847-1 and NDC4069-4, showed anthocyanin content >35 mg/100g. Anthocyanin composition was characterized by HPLC, spectral analyses and mass spectroscopy (MS). All red potato samples showed similar pigment profiles, with pelargonidin-3-rutinoside-5-glucoside acylated with *p*-coumaric acid being the major anthocyanin (ca 70%). Anthocyanin content and profiles of epidermal tissue and flesh were compared for NDC4069-4. The presence of glycoalkaloids in color extracts was detected by MS. Some red-fleshed potatoes may be good potential sources of food colorants.

INTRODUCTION

There is an interest worldwide in the development of food colorants from natural sources (Francis, 1987; Lauro, 1991). Use of "natural" colorants has increased as a consequence of perceived consumer preference as well as legislative action which has continued the de-listing of approved artificial dyes (Garcia and Cruz-Remes, 1993).

Anthocyanins are considered as potential replacements for banned dyes because of their bright attractive colors and water solubility that allows their incorporation into aqueous food systems as well as possible health benefits (Mazza and Miniati, 1993). Acylation of the anthocyanin molecule improves pigment stability during processing and storage (Bassa and Francis, 1987; Teh and Francis, 1988; Murai and Wilkins, 1990; Giusti and Wrolstad, 1996a,b). This increased stability is chemically attributed to the stacking of the acyl groups with the pyrylium ring of the flavylium cation, thereby reducing the susceptibility of nucleophile attack of water and subsequent formation of a pseudobase or a chalcone (Broulliard, 1981; Brouillard, 1983). These findings have

encouraged research on acylated anthocyanin-based food colorants such as red cabbage extract (Hrazdina et al., 1977; Idaka et al., 1987; Murai and Wilkins, 1990) which is approved as a food colorant, *Tradescantia pallida* (Shi et al., 1992a,b), red radish (Giusti and Wrolstad, 1996a,b), and sweet potatoes (Bassa and Francis, 1987; Shi et al., 1992c).

Several anthocyanin-based natural colorants such as grape skin extract, grape colorant and red cabbage extract impart a purplish hue at pH > 3.5 (Kucza and Wrolstad, 1995). Carmine, a chelate of carminic acid (extracted from dried bodies of the insect *Dactylopius coccus costa)*, provides a red color in the pH 4 to 10, but precipitates from solution when the pH is < 3.5 (Henry, 1996). There is demand for a red colorant that can effectively substitute for synthetic colorants such as FD&C Red # 40 and FD&C Red # 3. Giusti and Wrolstad (1996b), reported that acylated pelargonidin derivatives extracted from red radish could impart red color to maraschino cherries extremely close to that of FD&C Red # 40 at pH 3.5. This product however would require the elimination of undesirable aromas derived from the glucosinolates in radish (Kucza, 1996).

Pink or purple-fleshed potato (*Solanum tuberosum*) cultivars are becoming popular in the US (Johnson, 1995), because of their color appeal, outstanding taste and "mashability" (Sorensen, 1992). There has been a consumer interest in potatoes with colored flesh for use in salads and novelty crisps, especially since color is retained after cooking or frying (Lewis et al., 1996). The pigments in colored potatoes have been identified as the 5-glucoside 3-rhamnosylglucoside derivatives of all the common anthocyanidins, monoacylated with *p*-coumaric or ferulic acids (Harborne, 1960; Sachse, 1973; Andersen et. al., 1991, Lewis, 1996).

The objectives of this study were to characterize and measure the concentration of anthocyanin pigments in red-fleshed potatoes as potential red food colorants. Different cultivars were evaluated in terms of total pigment content, the qualitative anthocyanin pattern and phenolic composition. Comparisons were also made between potato skin and flesh with respect to anthocyanin pigment and phenolic composition. We

also evaluated the color using potato anthocyanin extract as compared to FD&C Red # 40 and radish anthocyanin extracts to color a pH 3.5 solution.

MATERIALS AND METHODS

Plant Material

Red-fleshed potato (*Solanum tuberosum*) tubers were supplied by the USDA Agriculture Research Service at Prosser, WA (19 breeding clones); Oregon State University Crop Science Department at Corvallis, OR (6 breeding clones); Ronniger's Seed Potatoes (Moyie Springs, ID) supplied potato seed tubers (5 cultivars) that were planted at the Oregon State University Vegetable Research Farm in Corvallis (OR); Luis P. Rodriguez-Maurer (Lima, Peru) supplied 3 red potato cultivars from the specie *Solanum stenotomum*.

From the 33 cultivars used, 14 were evaluated for two consecutive plantings (supplied by the USDA Agriculture Research Service). Tubers obtained in the second planting (1996) were grown in greenhouse pots and were of smaller size (average wt 38g/tuber) as compared with tubers from the first planting (1995) that were grown in the field (average wt 125g/tuber).

Tubers were received fresh, with the exception of the Peruvian cultivars which were received frozen. Fresh tubers were washed with cold water upon arrival to eliminate extraneous matter and refrigerated at 4°C until analyzed. Frozen tubers were kept at -20°C until analyzed.

Standards

Anthocyanidin standards were prepared from the acid hydrolysis of strawberry (Kerr Concentrates, Inc., Salem, OR) and grape juice (Welch's, Westfield, NY) concentrates, as described by Wrolstad et al. (1995). Radish anthocyanin extract was prepared as described by Giusti and Wrolstad (1996a). Chlorogenic, caffeic, ferulic, and

p-coumaric acids were obtained from Sigma Chemical Co. (St. Louis, MO). Chlorogenic acid isomers were prepared as described by Nagels et al. (1980).

Pigment Extraction

Extractions followed the procedure described by Giusti and Wrolstad (1996a). Potato tubers (ca 50 g) were cut in slices (ca 2 cm thick), blanched at 100°C for 10 min, blended with 100 mL acetone and filtered on a Buchner funnel. The filter cake residue was re-extracted with aqueous acetone (30:70 v/v) until a clear solution was obtained. Filtrates were combined, shaken in a separatory funnel with chloroform (2:1 chloroform:acetone) and stored overnight at 1°C. The aqueous portion (top portion) was collected and placed on a Buchi rotovapor at 40°C until all residual acetone was evaporated. The aqueous extract was brought to a known volume with distilled water. Due to the limited sample size, measurements were made in duplicate and average values are reported. In the case of the potato tubers provided by the Oregon State University Crop Science Department we were able to replicate the pigment extractions.

Monomeric Anthocyanin Content

Monomeric anthocyanin content was determined using a pH-differential method (Wrolstad et al., 1982). A Shimadzu 300 UV spectrophotometer and 1 cm pathlength cells were used for spectral measurements at 420, 510 and 700 nm. Pigment content was calculated as Pelargonidin-3-glucoside (Pg-3-glu), using an extinction coefficient of 31,600 L cm^{-1} mol^{-1} and molecular weight (MW) of 433.2 g mol^{-1} (Wrolstad, 1976).

Anthocyanin Purification

The aqueous extract was passed through a C-18 mini-column (high load C-18 tube), 20 mL capacity and 5 g sorbent weight (Alltech Assoc., Inc., IL), previously activated with methanol followed by 0.01% aqueous HCl (Giusti and Wrolstad, 1996a). Anthocyanins and phenolic acids were adsorbed onto the mini-column; sugars and acids

were eluted with 2 volumes of 0.01% aqueous HCl and anthocyanins were recovered with methanol containing 0.01% HCl (v/v). The methanol was evaporated using a Buchi rotovapor at 40°C and pigments were dissolved in deionized water with 0.01% HCl.

Alkaline Hydrolysis of Anthocyanins

Purified pigment was saponified in a screw-cap test tube with 10 mL of 10% aqueous KOH for 15 min at room temperature (ca. 23°C) in the dark, as described by Hong and Wrolstad (1990). The solution was neutralized using 2N HCl, and the hydrolysate was purified using a C-18 Sep-Pak classic cartridge (Waters Assoc., Milford, MA), as described.

Acid Hydrolysis of Anthocyanins

Fifteen mL of 2N HCl was added to the purified saponified pigment in a screw-cap test tube, flushed with nitrogen and capped. The pigment was hydrolyzed for 60 min at 100°C, then cooled in an ice bath. The hydrolysate was purified using a C-18 Sep-Pak classic cartridge.

HPLC Analytical System

An analytical High Performance Liquid Chromatograph (HPLC) Perkin-Elmer Series 400, equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with a Hewlett-Packard HPLC^{2D} ChemStation software was used. **System I**: Polymer Labs PLRP-S column (5 micron) 250 x 4.6 mm ID (Polymer Labs, Amherst, MA), fitted with a Polymer Labs 15 x 4.6 mm ID guard column. Solvent A: 100% HPLC grade acetonitrile; B: 4% phosphoric acid (aqueous). **System II**: ODS C-18 column (5 micron), 250 x 4.6 mm ID (Poly LC Inc., Columbia, MD), fitted with a 10 x 4.6 mm ID Spherisorb ODS-2 micro guard column (Alltech, Deerfield, IL). Solvent A: 100% HPLC grade acetonitrile, B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water. **System III**: Supelcosil LC-18 column (5 micron) 250 x

4.6 mm ID (Supelco Inc., Bellefonte, PA), fitted with a 10 x 4.6 mm Spherisorb ODS-2 micro guard column (Alltech, Deerfield, IL). Solvent A: 100% HPLC grade methanol; B: 0.07M KH_2PO_4 (aqueous) adjusted to pH 2.5 with phosphoric acid. All analytical systems were run at 1 mL/min and an injection volume of 50μL. Solvents and samples were filtered through a 0.45 μm Millipore filter type HA (Millipore Corp., Bedford, MA).

HPLC Semi-preparative System

An analytical HPLC Perkin-Elmer Series 400 and a Semi-Preparative Dynamax Rainin Model SD-300 Liquid Chromatograph, equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with a Hewlett-Packard $HPLC^{2D}$ ChemStation software was used. **Columns and mobile phase:** Microsorb C-18 reversed phase column (5 micron) 250 x 21.4 mm ID (Rainin Instrument Co., Inc., Emeryville, CA), fitted with a 50 x 21.4 mm ID guard module (Rainin Instrument Co., Inc., Emeryville, CA). Solvents: same as System II. Flow rate: 20 mL/min. Injection volume: 1 mL. Solvents and samples were filtered through a 0.45 μm Millipore filter type HA (Millipore Corp., Bedford, MA).

Anthocyanin and Anthocyanidin Analyses

Potato anthocyanins were separated using System I. The program followed a linear gradient from 10 to 20% A in 25 min and isocratic conditions with 20% A for 5 min. Saponified anthocyanins and anthocyanidins were separated with System II. The program used was a linear gradient from 0 to 30% A in 30 min. Simultaneous detection at 520, 320 and 280 nm was used and the spectra were collected for all peaks. The acylating groups were identified by means of retention times at 320 nm and spectra as compared with pure standards.

Phenolic Acid Analyses

Phenolic acids from the potato extract were isolated using System III. The program used a linear gradient from 20% to 45% A in 30 min, from 45% to 70% A in 10 min and isocratic conditions with 70% for 5 min. Simultaneous detection was at 320 and 280 nm and the spectra were collected for all peaks. The identity of the acyl groups was confirmed by retention times and spectra of pure standards.

Isolation of Purified Pigments

Pigments were isolated using semi-preparative HPLC, the program followed a linear gradient from 10 to 18% A in 10 min. Simultaneous detection was done at 520, 320 and 280 nm. Purified anthocyanins were concentrated by adsorption on a C-18 Sep-Pak cartridge and re-dissolved in distilled water.

Mass Spectroscopy of Potato Anthocyanins

Low-resolution mass spectroscopy was performed using electrospray ionization (ESMS). The instrument was a Perkin-Elmer SCIEX API III+ Mass Spectrometer, equipped with an Ion Spray source (ISV=4700, orifice voltage of 80) and loop injection. Partially purified potato anthocyanin extract and HPLC purified anthocyanins were injected directly into the system.

Color Analyses

Citric acid (0.1M) solutions (pH 3.5) were colored with 15, 30 and 45 mg anthocyanin/100mL using potato (ND04069-4 breeding clone) and radish (cv Fuego) anthocyanin extracts with monomeric anthocyanin contents of 147 or 232 mg/100mL, respectively. Solutions were prepared in triplicate. The color was compared to a similar solution colored with FD&C Red # 40 (150 ppm). Colored solutions were placed in an optical glass cell (2.5 mm pathlength, Hellma, Borough Hall Station, NY) and Hunter CIE L* (lightness), chroma (saturation) and hue angle (color itself) were determined

using a HunterLab CT1100 ColorQuest colorimeter (Hunter Associates Laboratories Inc., Reston, VA). Color and Haze measurements were made using total transmittance, specular included mode, Illuminant C and 10° observer angle.

RESULTS AND DISCUSSION

Extraction of Potato Anthocyanins

Formation of brown pigments was observed during preparation and overnight storage (1°C) of some potato extracts. Anthocyanins can be degraded by enzyme systems in plant tissues such as glycosidases (anthocyanases), polyphenoloxidases (PPO) and peroxidases (Francis, 1989). Glycosidases cleave the sugar substituent to yield anthocyanidins, which are very unstable and subsequently degrade to colorless derivatives. PPO oxidizes ortho-diphenols to ortho-quinones to form brown polymers (Francis, 1989); PPO can react directly with anthocyanins; however, destruction mainly results from the secondary reaction between the quinones formed from oxidation of phenolic acids (e.g. chlorogenic acid) and anthocyanins (Peng and Markakis, 1963; Sakamura et al., 1965). Steam blanching of tuber slices at 100°C for 10 min before acetone/chloroform extraction prevented pigment degradation. Enzymatic destruction of anthocyanins after acetone treatment suggested the presence of an enzyme system resistant to denaturation or capable of rapid regeneration. Shi et al. (1992a) reported enzymatic degradation of anthocyanins from sweet potato (*Ipomoea batatas*), extracted using 1% acetic acid in methanol.

Screening Potato Cultivars for Pigment Content

The monomeric anthocyanin content (Table 5.1) of different potato cultivars ranged from 2.4 to 40.3 mg/100g tuber. The potato selections with highest pigment content were NDOP5847-1 and NDC4069-4, with monomeric anthocyanin content > 35 mg/100g.

Table 5.1: Monomeric anthocyanin content of red-fleshed potato varieties

Source	Sample	Monomeric Anthocyanin (mg/100g)tuber)	
		Field-grown	Greenhouse
a	NDC4069-4	34.8	
a	ND5538-2	18.4	
a	ND5849-1	10.5	
a	6236-1	12.8	
a	6236-2	10.9	
a	4239	9.0	
b	NDOP5847-1	40.3	25.5
b	NDOP5847-2	6.2	8.7
b	NDOP5847-3		10.4
b	NDOP5589-1	2.4	8.3
b	NDOP5589-2	5.1	
b	NDOP5538-1	16.9	10.6
b	NDOP5538-2	8.3	5.7
b	NDOP5538-3	13.6	7.6
b	NDOP5538-4	4.7	8.9
b	NDOP5538-5	11.1	8.4
b	NDOP5538-6	17.7	8.0
b	NDOP5538-7	20.6	10.5
b	NDOP5538-8	9.1	
b	NDOP5538-9	10.9	
b	NDOP5538-10	12.6	11.3
b	NDOP5538-11	13.9	
b	NDOP5538-12	15.8	13.1
b	NDOP5538-13	19.1	13.8
b	ND3261-5R	4.1	
c	Huckberry	8.6	
c	All Red	7.0	
c	Blossom	2.8	
c	Levitts Pink	3.7	
c	Cherry Jubilee	2.9	
d	Wayru	5.4	
d	Leque chaki	9.1	
d	Pokko lomocho	6.5	

a: supplied by A.R. Mosley and B.A. Charlton (OSU, OR); b: supplied by C.R. Brown (Prosser, WA); c: supplied by Ronniger's Seed Potatoes (ID); d: supplied by Luis Rodriguez Maurer (Peru).

Breeding clones and cultivars with the lowest pigment content were NDOP 5589-1, Cherry Jubilee and Blossom with levels between 2.4 and 3.0 mg/100g tuber.

Higher pigment content has been reported for purple potato cultivars such as Urenika, with average concentration of 183.6 and 507.8 mg/100g FW in the flesh and skin, respectively (Lewis, 1996). Through potato breeding there might be the potential to develop a red potato clone with pigment content similar to those of purple potatoes.

The pigment content of breeding clones NDC4069-4 and NDOP5847-1 was comparable to those reported for such commodities as red radish cv Fuego (30 mg/100g root), red cabbage (25 mg/100g) from which an approved "natural" colorant is obtained, strawberries (15-30 mg/100g), and red raspberry (30-40 mg/100g) (Giusti and Wrolstad, 1996a; Timberlake, 1988; Kuhnau, 1976). Grapes, black currant and other berries contained greater concentrations (60-400 mg/100g) of pigments (Kuhnau, 1976). However, since their major anthocyanins were non-acylated (Jackman and Smith, 1996) the stability of these pigments after extraction is a limiting factor.

Several breeding clones, supplied by the USDA Irrigated Agriculture Research and Extension Center (Prosser, WA), were evaluated during 2 consecutive plantings (Table 5.1). Breeding clones from the second planting (1996) showed a marked decrease (about 33%) in pigment content as compared with those from the previous season. Potatoes from the first planting were grown in the field, while those from the second planting were grown in greenhouse pots.

From the tuber size and weight, potatoes obtained from the second crop (1996) were immature, indicating that potatoes tend to accumulate anthocyanins with age. Tuber development and light exposure are important factors in anthocyanin accumulation in potatoes (Verma et al., 1972; Hung et al., 1997).

HPLC of Anthocyanins and Anthocyanidins

Initial characterization was done on cultivars Huckberry and All Red since they were available early in the season. Seven anthocyanins from red-fleshed potato tubers were separated by HPLC (Fig. 5.1). Peak 5 was the major anthocyanin and represented

ca. 70% of the total area, while peaks 1 and 6 represented ca. 6 and 14 % of the total area, respectively.
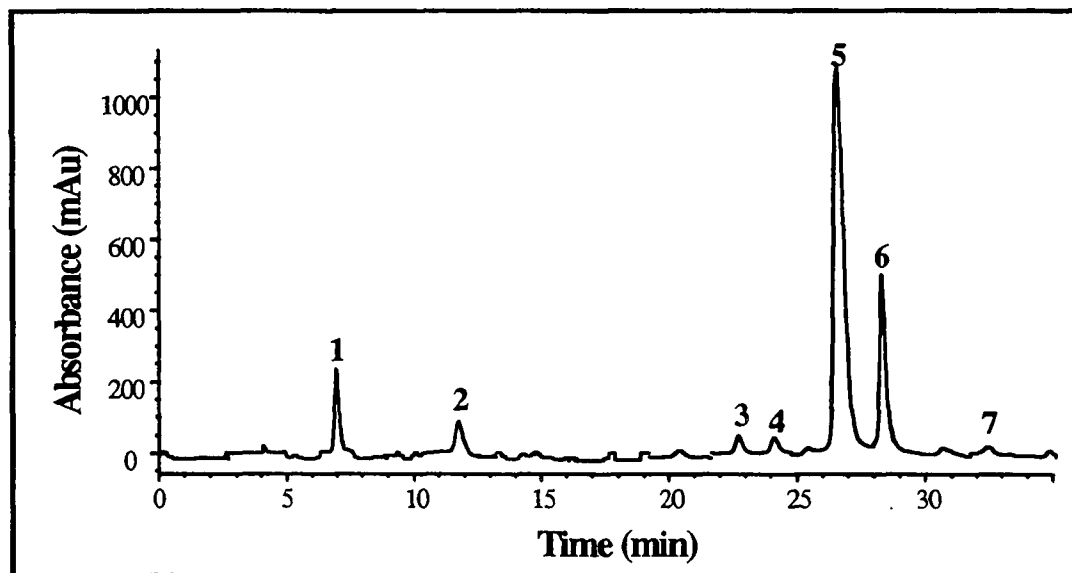
Figure 5.1. HPLC separation of red potato anthocyanins. Polymer Labs PLRP-S, 250 x 4.6 mm i.d. column. Solvent A: 100% acetonitrile, B: 4% phosphoric acid. Linear gradient from 10 to 20% A in 25 min and isocratic at 20% A for 5 min. Flow rate: 1 mL/min. Injection volume: 50µL. Pigments are: Pg-3-rut-5-glu (1), Pg-3-rut (2), unknown (3), Pg-3-rut-5-glu acylated with *p*-coumaric acid (4 & 5), Pg-3-rut-5-glu acylated with ferulic acid (6), and Pg-3-rut acylated with *p*-coumaric acid (7).

Spectral data provides important information about structural properties of anthocyanins (Hong and Wrolstad, 1990). All anthocyanin peaks showed a maximum absorbance between 495-505 nm (Fig. 5.2), characteristic of pelargonidin derivatives.

The glycosidic substitution pattern of the anthocyanins can be inferred by absorption in the 400-460 nm region since the 3-glycosides exhibit ratios of $E_{440}/E_{\lambda max}$ about 2 times greater than those for 3,5-diglycosides (Harborne, 1967). The $E_{440}/E_{\lambda max}$ ratios obtained for peaks 1, 4, 5 and 6 were 25% while peaks 2, 3 and 7 showed a $E_{440}/E_{\lambda max}$ of 45 - 60 %, providing evidence that the former pigments were 3,5-glycosides and the latter were 3-glycosides. Sachse (1973) reported $E_{440}/E_{\lambda max}$ ratios between 17 and 23% for different colored potatoes with 5-glycosylation patterns.
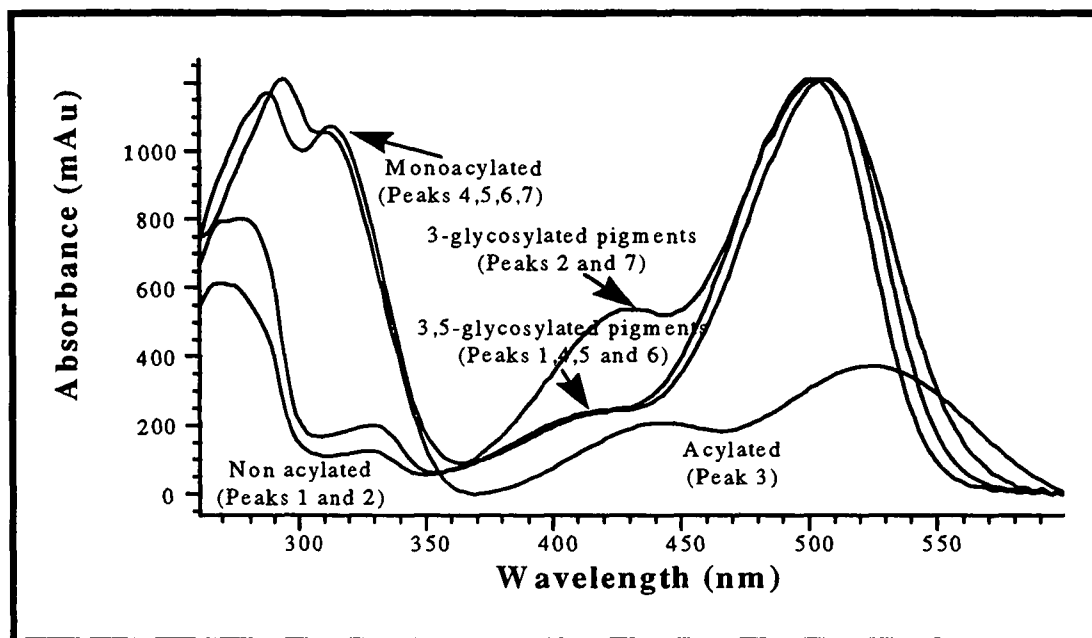
Figure 5.2. Spectral characteristics of potato anthocyanins, indicating glycosylation and acylating patterns.

The presence of acylation with hydroxylated aromatic organic acids is indicated by absorption in the 310 nm range (Harborne, 1958; Hong and Wrolstad, 1990). We found that peaks 4, 5, 6 and 7 showed high absorbance at 315 nm with $E_{\lambda max}(acyl)/E_{\lambda max}$ (visible) ratios of 65 to 95% which suggested mono-acylation of anthocyanins with cinnamic acids (Harborne, 1958; Sachse, 1973; Giusti and Wrolstad, 1996a). Peak 3 showed a $E_{\lambda max}(acyl)/E_{\lambda max}$ (visible) ratio of 280%, suggesting an anthocyanin with more than one acylating group.

Anthocyanin saponification (Fig. 5.3) yielded one major peak representing ca 87% of the total area, corresponding to peak 1 of the starting material (Fig. 5.1). Peak 2 from the original material was also detected after saponification. Both peaks showed a λ_{max} at 500 nm, while another small peak (Fig. 5.3) had a λ_{max} at 515 nm suggesting the presence of more than one anthocyanidin.
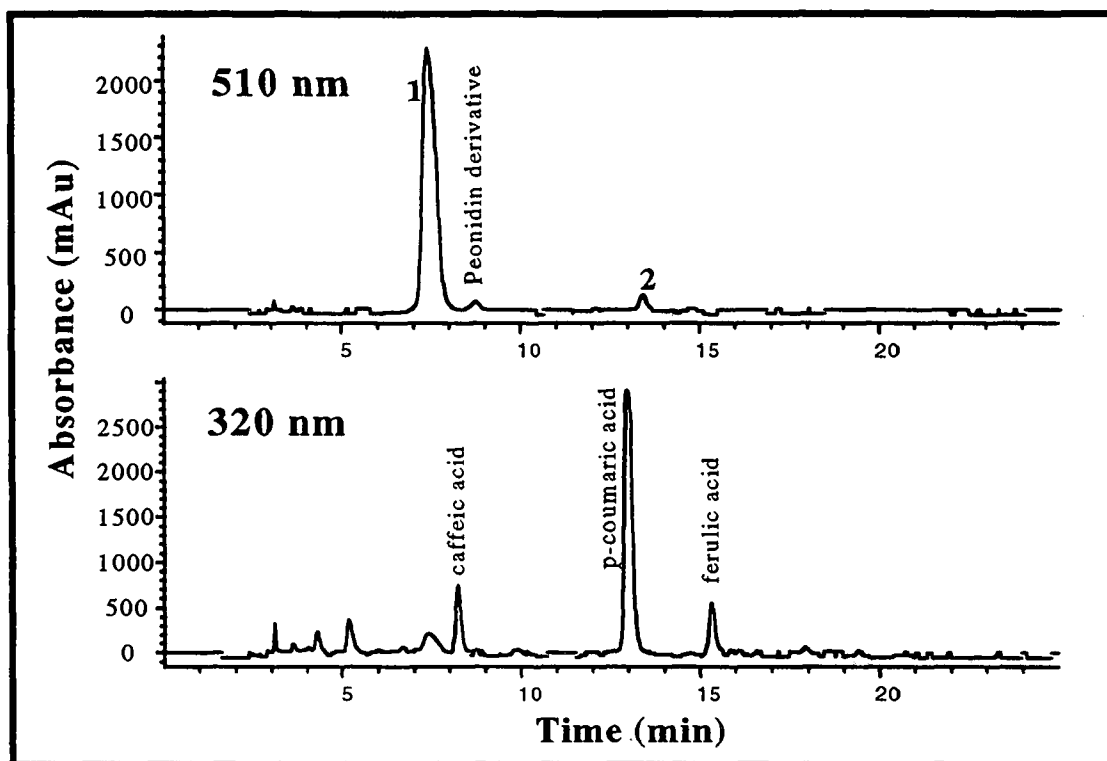
Figure 5.3: HPLC separation of saponified potato anthocyanin (510 nm) and acylating groups (320 nm). ODS C-18, 250 x 4.6 mm i.d. column. Solvent A: 100% acetonitrile; B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water. Linear gradient from 0 to 30% A in 30 min. Flow rate: 1 mL/min. Injection volume: 50μL.

Cinnamic acids (p-coumaric and ferulic) were identified as acylating substituents at 320 nm, with p-coumaric acid being the major acyl group (Fig. 5.3). Caffeic acid was also identified after pigment saponification. Treatment of the pigment extract with ethyl acetate to remove free phenolic acids prior to saponification confirmed that caffeic acid was derived from chlorogenic acid (caffeoylquinic acid) and not an anthocyanin acylating group.

The anthocyanidins were identified using hydrolyzed strawberry (pelargonidin) and grape (all remaining anthocyanidins). Acid hydrolysis showed pelargonidin as the major anthocyanidin as well as the presence of traces of peonidin. The presence of peonidin was confimed with an augmented sample (hydrolyzed potato and grape pigments).

Mass Spectroscopy

The presence of 3-rhamnoglucoside-5-glucoside derivatives of all major anthocyanidins have been reported in colored potato tubers (Harborne, 1960; Sachse, 1973; Andersen et al., 1991, Lewis, 1996) and other *Solanum* species (Price and Wrolstad, 1995). The mass spectroscopy (MS) analyses (Fig. 5.4) provided important information about the presence of charged molecules in the pigment extract. The HPLC results indicated that the major anthocyanins were 3,5-diglycosides acylated with cinnamic acids. The combined information from HPLC and MS allowed us to identify molecular ions as: MW 741 corresponded to pelargonidin 3-rhamnoglucoside-5-glucoside (Pg-3-rut-5-glu), MW 887 and 916 corresponding to Pg-3-rut-5-glu acylated with *p*-coumaric and ferulic acids, respectively. The MS also revealed the presence of 2 major molecular ions with masses of 852 and 866 which were later identified as the potato alkaloids α-chaconine and α-solanine, respectively.
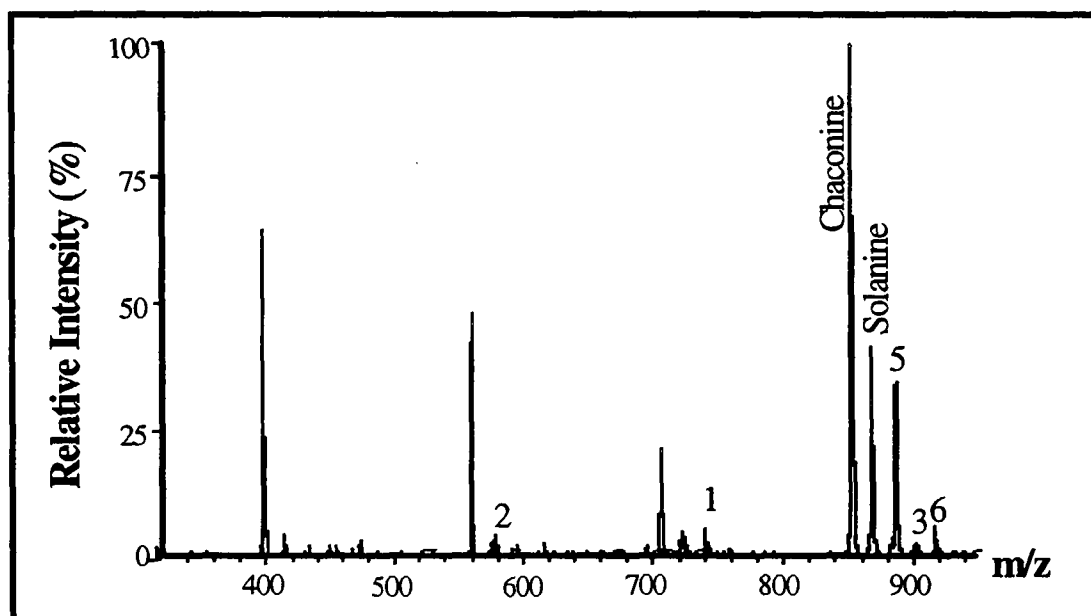
Figure 5.4: Mass spectroscopy of potato pigment extract. Molecular ions correspond to anthocyanins and alkaloids detected in the extract.

Peak Identification

Individual anthocyanins from breeding clone NDC4069-4 purified with semi-preparative HPLC and identified by ESMS and HPLC were: Pg-3-rut-5-glu (**Peak 1**, M⁺ of 741.6), Pg-3-rut (**Peak 2**, M⁺ of 578.8), Pg-3-rut-5-glu acylated with *p*-coumaric acid (**Peak 4 & 5**, M⁺ of 887.2), Pg-3-rut-5-glu acylated with ferulic acid (**Peak 6**, M⁺ of 916.6), and Pg-3-rut acylated with *p*-coumaric acid (**Peak 7**, M⁺ of 725.2). Hosokawa et. al. (1995) reported anthocyanins with identical formulae in *Hyacinthus orientalis* that were identified as *p*-coumaroyl isomers (*cis* and *trans*) of pelargonidin 3-O-(6-O-p-coumaroyl-ß-D-glucoside)-5-O-(4-O-malonyl-ß-D-glucoside). They also found malonic acid as an acyl substituent in two positions of the sugar moiety (positions 4 and 6). Peaks 4 and 5 showed the same chemical formula, which suggested these peaks corresponded to either geometric isomers (*cis* vs *trans*) or acyl substitutions in different positions of the sugar moiety.

Peak 3 (m/z of 903) was a very unusual pigment with a purplish hue and a λ max of 525 nm. We compared the hue angles of peaks 3 and 5 in pH 1.0 buffer diluted to the same chroma (10.2) and obtained hue angle values of 10.7° and 20.7°, respectively. Saponification of purified peak 3 yielded 2 anthocyanins with HPLC retentions and ESMS the same as Pg-3-rut-5-glu and Pg-3-rut, along with *p*-coumaric acid. Saponification resulted in a change in hue from red-purple (hue angle of 10°) to orange (hue angle of 20°). Dangles et. al. (1993), while investigating the intramolecular copigment effect in *Pharbitis nil.* anthocyanins, reported that polyacylation of a pelargonidin derivative would produce a bathochromic shift of the visible absorption maxima similar to those caused by intermolecular copigmentation. Identification of peak 3 was incomplete, but intermolecular copigmentation and acylation may account for the 525 nm λ max of this minor pigment.

Lewis (1996) characterized the major pigments in the potato cultivar Red Flesh as Pg-3-(*p*-coumaroyl-rut)-5-glu and Pn-3-(*p*-coumaroyl-rut)-5-glu. In the samples we evaluated, only traces of peonidin derivatives were detected.

Comparing Anthocyanin and Phenolic Acid Profiles

Similar pigment profiles were obtained for most of the red potato samples, however the relative proportions of different peaks varied (Table 5.2). The major anthocyanin in all *Solanum tuberosum* samples was Pg-3-rut-5-glu acylated with *p*-coumaric acid (pigment 5), which represented between 56.7 and 83.2 % of the total area (on average 67.4%). Differences were found in the proportions of different pigments, especially for the minor peaks. Pg-3-rut-5-glu acylated with ferulic acid (Peak 6) ranged from 3.9 to 19.5% total area and Pg-3-rut-5-glu (Peak 1) ranged from 0.3 to 14.4% total area. Those pigments were very minor in breeding clones such as NDC4069-4, while in clone NDOP5538-3 they represented more than 30% of the total pigment, becoming substantial peaks in some of the clones analyzed. The anthocyanin profile of cultivars from *Solanum stenotomum* showed more qualitative variability. Cultivar Pokko lomocho accumulated Pg-3-rut (67.4%) and had a minor proportion of Pg-3-rut-5-glu acylated with *p*-coumaric acid (12.2%) which suggests cultivar differences with respect to anthocyanin biosynthesis and in the enzymes responsible for anthocyanin glycosylation and/or acylation. Cultivar Leque chaqui also showed a high proportion of Pg-3-rut (16.4%) as compared to the other samples.

Breeding clones grown in the greenhouse showed a lower proportion of Pg-3-rut-5-glu acylated with *p*-coumaric (16 ± 8 %) and an increased proportion of Pg-3-rut-5-glu acylated with ferulic (44 ± 16) than breeding clones grown in the field. The changes in anthocyanin proportions differed among cultivars, being more noticeable for Pg-3-rut-5-glu for which no clear pattern was found. Some samples showed increased proportions up to 80% while others showed a decrease.

The proportions of phenolic acids (Fig. 5.5) of field-grown potato samples varied (Table 5.3). This was particularly true for chlorogenic acid isomers. Chlorogenic acid and its isomers were the major phenolic acids in colored potato tubers and represented 70% of the total phenolic area.

Table 5.2: Relative anthocyanin composition of Red/Purple varieties expressed as % of total peak area at 520 nm

Sample	Pigments												
	1		2		3		4		5		6		7
	F	G	F	G	F	G	F	G	F	G	F	G	F
NDC4069-4	1.8		2.3		4.2		1.5		82.0		4.5		1.3
ND5538-2	0.9		0.5		2.3		3.1		78.9		7.7		0.5
ND5849-1	1.6		1.8		2.2		1.7		80.2		11.8		
6236-1	1.9		1.6		2.7		1.5		75.7		13.9		0.7
6236-2	3.0		3.3		4.0		1.9		73.9		11.3		0.6
4239	0.3		0.8		3.4		1.3		78.8		13.6		0.4
NDOP5847-1	2.4	11.1	0.8	0.9	2.7	2.1	2.3	3.6	80.5	65.6	7.2	11.1	1.3
NDOP5847-2	2.4	5.8	0.4	1.7	2.8	2.3	1.9	3.1	81.8	63.4	8.9	16.3	0.5
NDOP5847-3		9.3		0.6		2.2		4.3		60.0		12.4	
NDOP5589-1	8.0	8.8	1.6	1.6	4.2	2.7	2.8	2.4	70.1	63.8	10.9	17.3	1.6
NDOP5589-2	9.2	7.9	2.3		2.4	2.4	3.8	2.8	66.4	62.4	9.5	17.8	1.4
NDOP5538-1	2.5	6.3	1.1	3.7	2.6	1.9	2.4	2.1	79.4	62.8	9.3	19.7	1.1
NDOP5538-2	11.4		1.3		2.0		3.4		66.4		10.9		0.9
NDOP5538-3	8.0	10.7	2.6	2.9	2.7	2.2	3.8	2.2	64.0	58.4	11.4	19.5	1.6
NDOP5538-4	2.5	14.4	0.5	1.4	1.6	1.7	1.2	2.5	83.2	68.1	8.9	9.2	1.4
NDOP5538-5	4.5	10.7	1.0	2.7	2.0	1.6	3.4	2.8	79.6	63.7	6.4	15.6	1.6
NDOP5538-6	8.9	7.7	1.4	1.4	1.9	2.0	4.2	2.7	71.1	68.9	5.7	15.1	1.3
NDOP5538-7	5.1	8.5	2.2	2.4	2.4	2.1	4.6	2.1	72.3	62.9	6.7	18.7	1.7
NDOP5538-8	13.6		2.4		2.1		4.3		57.9		10.8		1.8
NDOP5538-9	2.9		1.6		2.7		3.8		75.3		7.5		1.5
NDOP5538-10	11.3	9.4	2.2	2.5	3.1	2.3	4.2	2.5	63.5	60.2	9.3	18.6	0.8
NDOP5538-11	9.0	10.7	2.7	2.7	2.4	1.5	3.6	3.2	65.4	56.7	10.4	16.7	0.3
NDOP5538-12	3.4	5.2	2.2	4.0	2.9	2.3	4.1	2.2	73.0	61.9	7.6	19.3	2.3
NDOP5538-13	11.4	11.0	1.4	1.7	2.5	2.2	4.1	2.1	66.0	70.3	8.7	11.8	0.6
ND3261-5R	7.0		2.9		4.7		1.1		60.3		15.2		0.5
Huckberry	7.5		2.1		2.5		2.4		71.3		11.3		0.6
All Red	5.1		3.0		2.6		3.5		64.8		15.1		1.0
Blossom	8.6		2.3		2.9		2.9		61.4		16.4		1.3
Levitts Pink	7.9		2.8		2.0		2.7		60.0		17.5		0.6
Cherry Jubilee	7.7		1.3		2.4		3.3		64.1		15.8		0.7
Wayru	2.2		2.4		2.0		3.3		79.7		8.8		1.6
Leque chaki	1.9		16.4		1.7		1.8		66.1		3.9		1.2
Pokko	4.5		67.4						12.2		1.7		

F: potatoes grown in the field; G: grown in the greenhouse. Pigments same as Fig. 5.1.
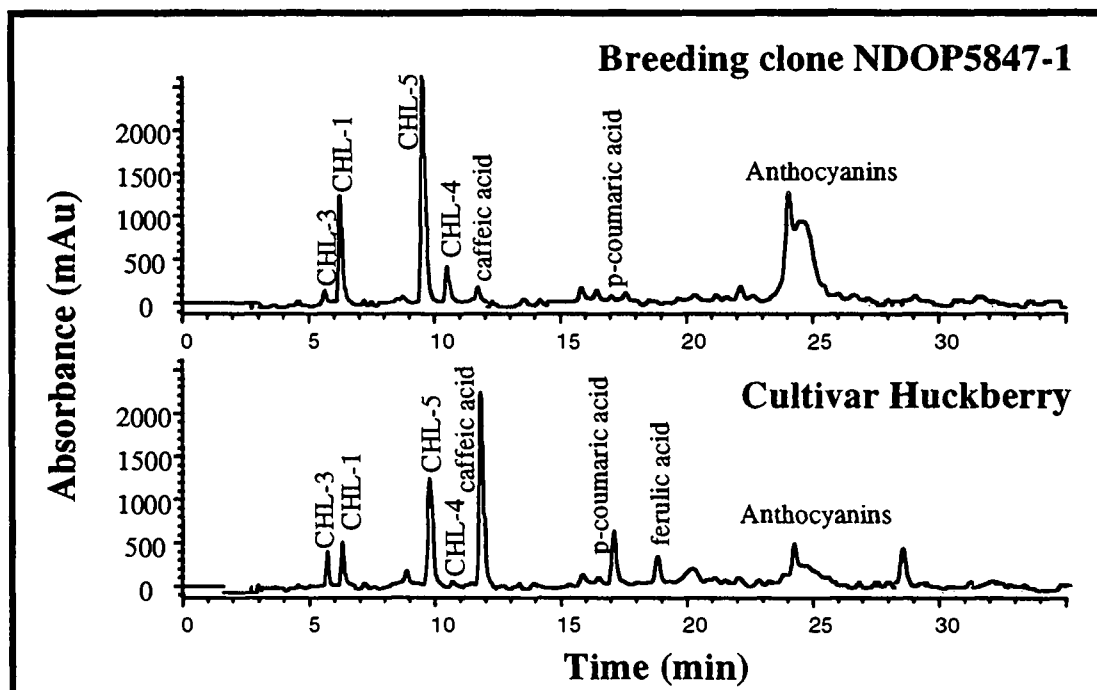
Figure 5.5: HPLC separation (detection at 320 nm) of phenolic acids from red potato cultivars. Supelcosil LC-18, 250 x 4.6 mm i.d. column. Solvent A: methanol, B: 0.07M KH₂PO₄ (pH 2.5). Linear gradient from 20% to 45% A in 30 min, 45% to 70% A in 10 min and isocratic with 70% A for 5 min. Flow rate: 1 mL/min. Injection volume: 50µL.

This was lower, however, than the proportions reported for some chipping types where chlorogenic acid and its isomers represented between 88 and 95% of total phenolics (Ramamurthy et al., 1992; Rodriguez-Saona and Wrolstad, 1997). Cinnamic acids (caffeic, p-coumaric and occasionally ferulic) were present at very low levels, while other phenolics reported in potato tubers such as protocatechuic acid, vanillic acid, gallic acid (Malmberg and Theander, 1984; Onyeneho and Hettiarachchy, 1993; Lewis, 1996) were not found. Cultivar Huckelberry showed a marked difference in phenolic profile, with similar proportions of total chlorogenic and caffeic acids, and high levels of free p-coumaric and ferulic acids.

Changes in Anthocyanin and Phenolic During Cold Storage

The anthocyanin and phenolic profile for NDC4069-4 was evaluated after 1 mo
storage at 4°C. Storage resulted in a decrease in Pg-3-rut-5-glu acylated with *p*-
coumaric (10%) and increases in Pg-3-rut-5-glu acylated with ferulic acid (2.4%) and
total chlorogenic acid (15 %) while no noticeable changes in total monomeric
anthocyanin content were found. There was an important effect of storage on the level
of chlorogenic acid isomers and free *p*-coumaric acid (Table 5.3), with a reduction in
CHL-1 and an increase in CHL-5.

We also monitored the anthocyanin stability of frozen potato tubers. Slices were
frozen, and anthocyanins were extracted and measured at the beginning (time zero) and
after 3 mo storage. A decrease in monomeric anthocyanin content was observed in all
cultivars. Huckberry, All Red, Blossom, and Cherry Jubilee, showed pigment losses of
34, 28, 26 and 28%, respectively, after 3 mo frozen storage at -20°C. We hypothesized
that enzymes are responsible for anthocyanin degradation during frozen storage since we
earlier demonstrated that blanching was critical to preventing anthocyanin destruction
during pigment isolation. These findings also suggest that the monomeric anthocyanin
content of the Peruvian samples may have been low because they were received frozen.

Comparing Anthocyanin and Pigment Profile in Flesh *vs* Skin

We compared the qualitative and quantitative pigment composition of flesh and
skin for selection NDC4069-4 and found concentrations of 28.4 mg/100g flesh and 21.7
mg/100g skin on a fw basis. Expressed in terms of total tuber weight the monomeric
anthocyanin content in the flesh was 25.9 mg/100g while in the skin it was 2 mg/100g,
since the skin represented only 9% of the tuber wt. Lewis (1996) evaluated several
colored potato cultivars with pigmentation in the skin and/or flesh. The red fleshed
cultivar with the highest anthocyanin content had an average of 10.7 mg/100g flesh and
264 mg/100g skin. We found several samples with anthocyanin contents higher than 10
mg/100 g, however, since Lewis did not report anthocyanin content on a tuber wt basis,

comparisons of total pigment content of the different samples cannot be made. We found no qualitative differences in the anthocyanin profile of NDC4069-4 skin and flesh.

Table 5.3: Relative phenolic acid composition of Red/Purple varieties, expressed as % of total peak area at 320 nm.

Sample	CHL-1	CHL-3	CHL-4	CHL-5	Total CHL	Caffeic	p-Coumaric
NDC4069-4							
0 wks	12.4	1.2	5.4	46.2	65.2	1.0	
2 wks	8.2	0.5	10.0	47.6	66.3	1.8	2.5
4 wks	1.1	0.3	2.1	78.0	81.5	1.2	2.6
ND5538-2	5.7	3.4	2.6	62.8	74.5	0.9	2.1
6236-1	22.4		8.3	40.0	70.7	2.3	1.8
6236-2	6.9	1.0	2.0	64.0	73.9	1.7	1.0
4239	25.0		15.9	22.4	63.3		4.6
NDOP5847-1	19.6	1.4	4.7	40.0	65.7	2.4	1.7
NDOP5847-2	2.2	3.4	9.4	30.3	45.3	1.1	3.6
NDOP5589-1	2.3	5.5	16.9	59.5	84.2	1.7	0.4
NDOP5589-2	1.3	5.3	17.5	58.4	82.5	1.5	0.5
NDOP5538-1	2.8	3.4	2.5	64.5	73.2	2.2	2.4
NDOP5538-2	3.5	8.7	17.8	56.9	86.9	2.5	0.3
NDOP5538-3	1.2	23.4	15.6	43.6	83.8	0.3	0.9
NDOP5538-4	3.2	2.7	9.3	47.9	63.1	1.2	0.9
NDOP5538-5	4.5	3.4	11.5	47.5	66.9	1.0	1.0
NDOP5538-7	0.6	5.3	16.7	51.4	74.0	1.2	0.6
NDOP5538-8	9.7	4.5	17.7	44.1	76.0	1.4	0.7
NDOP5538-9	5.2	4.6	12.2	41.7	63.7	0.8	1.2
NDOP5538-11	3.3	8.3	17.1	53.2	81.9	0.5	0.7
NDOP5538-12	1.9	3.1	13.2	38.3	56.5	1.6	1.7
NDOP5538-13	3.3	5.9	11.7	64.0	84.9	2.0	2.0
ND3261-5R	0.6	1.6	8.3	36.9	47.4	1.6	0.8
Huckberry	5.3	3.7	0.8	19.8	29.6	29.0	9.6
All Red	1.2	2.1	8.3	36.6	48.2	1.2	1.5
Blossom	6.7	3.0	18.7	51.6	80.0	2.3	1.0
Levitts Pink	4.4	3.7	17.2	52.8	78.1	4.2	1.0
Cherry Jubilee	3.9	2.1	16.3	50.5	72.0	3.7	1.1
Wayru	0.8	0.5	5.1	54.6	61.0	3.7	1.8
Leque chaki	2.7	1.3	6.4	46.7	57.1	2.7	2.4

The differences in phenolic acids in the skin vs flesh were also evaluated. Tuber skins showed a higher proportion of free phenolic acids, especially chlorogenic and p-coumaric acids, as compared to the anthocyanin content. Phenolic acids are usually accumulated in the peels and they might be important in the defense mechanism to infection for many plants (Friend, 1985; Ramamurthy et al., 1992).

Color Attributes of Potato Anthocyanin Solutions

The color of solutions (pH 3.5) obtained with potato anthocyanins was compared (Table 5.4) to both the artificial dye FD&C Red # 40 and radish anthocyanin extract which gives a color very close to that of the dye (Giusti and Wrolstad, 1996b). We expected potato anthocyanins to provide color characteristics similar to radish anthocyanins since they had similar anthocyanin structures, a pelargonidin triglycoside with sugar substitions at the 3 and 5 positions acylated with cinnamic acids (Giusti and Wrolstad, 1996a).

Table 5.4: Color characteristics (CIELch) of potato (NDO4069-4) and radish (cv Fuego) in model juices at pH 3.5.

Sample	Concentration (mg/100 mL)	L*	Chroma	Hue (Degrees)	Haze
Red Potato	15	79.22	26.74	17.27	1.14
	30	64.35	48.11	21.42	1.05
	45	53.84	63.07	29.51	1.14
Red Radish	15	76.92	40.61	16.23	1.26
	30	64.82	62.11	26.22	1.28
	45	56.45	78.02	37.39	1.37
FD&C Red #40	1.5	67.29	65.45	37.73	1.2

At the highest anthocyanin concentration (45 mg/100mL) potato juice produced a color intensity close to that obtained with 1.5 mg/100 mL FD&C Red # 40 and a more reddish hue (lower hue angle). Potato anthocyanin solutions showed lower chroma (color intensity) values than radish juice at similar concentrations of anthocyanins, and a level comparable to that obtained with FDC Red # 40 only with the higher concentration (45 mg/100mL). The hue angles of potato and radish juices were very close at levels of 15 mg/100mL. Increasing the anthocyanin concentration slightly increased the hue angle (color) of the potato juice towards the more red-orange color obtained with FD&C Red # 40. Greater changes in hue angle with increasing anthocyanin concentration were observed for radish colored solutions. The levels of lightness (L value) obtained with potato and radish anthocyanins were similar at all concentrations, and comparable to that of FD&C Red # 40. The presence of the different sugar substituents (rutinose in potato vs sophorose in radish anthocyanins) and/or the presence of an additional acylating group in radish anthocyanins (malonic acid) might be responsible for color differences in the two anthocyanin extracts. The presence of the purple pigment (Peak 3) may also be important in the final hue of the potato anthocyanin extract.

Natural red food colorant are rare because few natural materials have a bright red color unmixed with other tones (LaBell, 1993). Anthocyanins can impart various shades of red to foods, however, there have been limitations in their application due to low concentration of pigments, flavors and odors associated with the raw material, difficulty in matching desired hues, limited stability to pH, light and oxygen, and cost (Henry, 1996; Murai and Wilkins, 1990). Acylation of the anthocyanin molecule improves pigment stability (Giusti and Wrolstad, 1996a,b; Francis, 1992; Brouillard, 1983). Potential commercial sources of anthocyanin colorants are those obtained from byproduct manufacture of other value-added products such as grapes and cranberries, or from highly pigmented low value crops such as red cabbage and bilberries (Jackman and Smith, 1996). Natural colorant from red cabbage is used by the food industry, although considered more costly than grape color, because of its high stability (Henry, 1996).

The pigment content of some red-fleshed potato breeding clones (NDC4069-4 and NDOP5847-1) is comparable to that of red cabbage. Red-fleshed potato extract provide color characteristics similar to that of FD&C Red # 40 in contrast to the more purplish hue of red cabbage extract at pH 3.5 (Kucza, 1995). Potatoes are a common agronomic crop with the advantage of high production yields (US average yield of 36 MT/Ha) and good long-term storage (10-12 mo). Furthermore, since potatoes are relatively bland, it should be possible to prepare extracts with acceptable flavors.

CONCLUSIONS

The major anthocyanin in red-fleshed potatoes was identified as pelargonidin-3-rutinoside-5-glucoside acylated with p-coumaric acid. Other acylated and non-acylated pelargonidin-3-rutinoside derivatives were present as well as traces of peonidin derivatives. Two breeding clones, NDOP5847-1 and NDC4069-4, showed high anthocyanin content (>35 mg/100g). Potato anthocyanin extract in solution showed color characteristics similar to FD&C Red # 40. In addition, the presence of acylating groups should enhance stability. The presence of alkaloids (α-solanine and α-chaconine) was detected in the pigment extracts.

REFERENCES

Andersen, M., Opheim, S., Aksnes, D.W., and Froystein, N.A. 1991. Structure of petanin, an acylated anthocyanin isolated from Solanum tuberosum, using homo- and hetero-nuclear two-dimensional nuclear magnetic resonance techniques. Phytochem. Anal. 2: 230-236.

Bassa, I.A. and Francis, F.J. 1987. Stability of anthocyanins from sweet potatoes in model beverages. J. Food Sci. 52(6): 1753-1754.

Brouillard, R. 1983. The in vivo expression of anthocyanin colour in plants. Phytochem. 22: 1311-1323.

Broulliard, R. 1981. Origin of the exceptional colour stability of Zebrina anthocyanin. Phytochem. 20: 143-145.

Dangles, O., Saito, N. and Brouillard, R. 1993. Anthocyanin intramolecular copigment effect. Phytochem. 34:119-124.

Francis, F.J. 1987. Lesser-known food colorants. Food Technol 41:62-68.

Francis, F.J. 1989. Food colorants: anthocyanins. Crit. Rev. Food Sci. and Nutr. 28(4): 273-314.

Francis, F.J. 1992. A new group of food colorants. Trends in Food Sci. and Technol. 3: 27-31.

Friend, J., Reynolds, S.B., and Aveyard, M.A. 1985. Phenolic metabolism in potato tuber tissue after infection with Phytophthora infestans. Biochem J. 124: 29

Garcia, F. and Cruz-Remes, L. 1993. The natural xanthophyll pigment industry. Presented at the First International Symposium of Natural Colorants for Food, Nutraceuticals, Beverages and Confectionary. Amherst, Massachusetts, Nov 7-10.

Giusti, M.M. and Wrolstad, R.E. 1996a. Characterization of red radish anthocyanins. J. Food Sci. 61(2): 322-326.

Giusti, M.M. and Wrolstad, R.E. 1996b. Radish anthocyanin extract as a natural red colorant for maraschino cherries. J. Food Sci. 61: 688-694.

Harborne, J.B. 1958. The chromatographic identification of anthocyanin pigments. J. Chrom. 1: 473-488.

Harborne, J.B. 1960. Plant polyphenols: 1: anthocyanin production in the cultivated potato. Biochem. J. 74: 262-269.

Harborne, J.B. 1967. Comparative biochemistry of the flavonoids. Academic Press. London.

Henry, B.S. 1996. Natural food colours. Ch. 2 In *Natural Food Colorants*. (Ed.) Hendry, G.A.F. and Houghton, J.D. 2nd Ed. Blackie A&P Great Britain.

Hong, V. and Wrolstad, R.E. 1990. Use of HPLC separation/photodiode array detection for characterization of anthocyanins. J Agric Food Chem. 38: 708-715.

Hosokawa, K., Fukunaga, Y., Fukushi, E. and Kawabata, J. 1995. Acylated anthocyanins from red *Hyacinthus orientalis*. Phytochem. 39:1437-1441.

Hrazdina, G., Iredale, H. and Mattick, L.R. 1977. Anthocyanin composition of *Brassica oleracea* cv. Red Danish. Phytochem. 16: 297-299.

Hung, C., Murray, J.R., Ohmann, S.M. and Tong, C.B.S. 1997. Anthocyanin accumulation during potato tuber development. J. Amer. Soc. Hort. Sci. 122(1): 20-23.

Idaka, E., Suzuki, K., Yamakita, H., Ogawa, T., Kondo, T. and Goto, T. 1987. Structure of monoacylated anthocyanins isolated from red cabbage, *Brassica oleracea*. Chem. Lett. pp 145-148.

Jackman R.L. and Smith J.L. 1996. Anthocyanins and Betalains. Ch. 8 In *Natural Food Colorants*. (Ed.) Hendry, G.A.F. and Houghton, J.D. 2nd Ed. Blackie A&P Great Britain.

Johnson, C.A. 1995. 1995-1996 seed acres reflect more varieties, market shifts. Valley Potato Grower. 61: 13-16.

Kuhnau, J. 1976. The flavonoids. A class of semi-essential food components: Their role in human nutrition. World Rev. Nutr. Diet. 24: 117-191.

Kucza, M. M. and Wrolstad, R.E. 1995. Color and pigment stability of red natural colorants in a model beverage. Memoire de Fin d'Etudes I.S.A.R.A. Eleve-Ingenieur I.S.A.R.A. Lyon, France.

Kucza, M. M. 1996. Analysis of flavor precursors in radish and radish color extracts. M.S. thesis. Department of Food Science and Technology. Oregon St. Univer. Corvallis, OR.

LaBell, F. 1993. Grapes provide brilliant red color. Food Processing. 54(6): 88-89.

Lauro, G.J. 1991. A primer on natural colors. Am. Assoc. Cereal Chemists 36(11): 949-953.

Lewis, C.E. 1996. Biochemistry and regulation of anthocyanin synthesis in potato and other tuber-bearing *Solanum* species. Ph. D. Thesis. Department of Plant and Plant Microbial Science, University of Canterbury, Christchurch, New Zealand.

Malmberg, A. and Theander, O. 1984. Free and conjugated phenolic acids and aldehydes in potato tubers. Swedish J. Agric. Res. 14: 119-125.

Mazza, G. and Miniati, E. 1993. Introduction. Ch. 1 In *Anthocyanins in fruits, vegetables, and grains*. G. Mazza and E. Miniati (Ed.). CRC Press, Boca Raton, FL.

Murai, K. and Wilkins, D. 1990. Natural red color derived from red cabbage. Food Technol. 44:131

Nagels, L., Van Dongen, W., De Brucker, J. and De Pooter, H. 1980. High performance liquid chromatographic separation of naturally occurring esters of phenolic acids. J Chrom 187:181-187.

Onyeneho, S.N. and Hettiarachchy, N.S. 1993. Antioxidant activity, fatty acids and phenolic acid composition of potato peels. J. Sci. Food Agric. 62: 345-350.

Peng, C.Y. and Markakis, P. 1963. Effect of phenolase on anthocyanins. Nature 199:597.

Price, C.L. and Wrolstad, R.E. 1995. Anthocyanin pigments of Royal Okanogan huckelberry juice. J. Food Sci. 60(2): 369-374.

Ramamurthy, M.S., Maiti, B., Thomas, P. and Nair, P.M. 1992. High performance liquid chromatography determination of phenolic acids in potato tubers (*Solanum tuberosum*) during wound healing. J. Agric. Food Chem. 40: 569-572.

Rodriguez-Saona, L.E. and Wrolstad, R.E. 1997. Influence of potato composition on chip color quality. Am. Pot. J. 74: 87-106.

Sakamura, S., Watanabe, S. and Obata, Y. 1965. Anthocyanase and anthocyanin occuring in eggplant: Part III. Oxidative decoloration of the anthocyanin by polyphenol oxidase. Agr. Biol. Chem. 29(3):181-190.

Sasche, J. 1973. Anthocyane in den kartoffelsorten Urgenta and Desiree. Z. Lebensm. Unters. Forsch. 153: 294-300.

Shi, Z., Bassa, I.A., Gabriel, S.L. and Francis, F.J. 1992a. Anthocyanin pigments of sweet potatoes -*Ipomoea batatas*. J. Food Sci. 57: 755-757, 770.

Shi, Z., Lin, M. and Francis, F.J. 1992b. Stability of anthocyanins from *Tradescantia pallida*. J. Food Sci. 57: 758-759.

Shi, Z., Lin, M. and Francis, F.J. 1992c. Anthocyanins of *Tradescantia pallida*. Potential food colorants. J. Food Sci. 57: 761-765.

Sorensen, E.J. 1992. Speciality potatoes. American Vegetable Grower. Jan. 36-39.

Teh, L.S. and Francis, F.J. 1988. Stability of anthocyanins from *Zebrina pendula* and *Ipomoea tricolor* in a model beverage. J. Food Sci 53: 1580-1581.

Timberlake, C.F., 1988. The biological properties of anthocyanin compounds. NATCOL quarterly bulletin.

Verma, S.C., Purohit, L.K., Sharda, R.T., Purohit, A.N. and Upadhya, M.D. 1972. Anthocyanin in dark- and light-grown sprouts of potato. Potato Res. 15: 166-169.

Wrolstad, R.E. 1976. Color and pigment analyses in fruit products. Oregon St. Univ. Agric. Exp. Stn. Bulletin 624.

Wrolstad, R.E., Culbertson, J.D., Cornwell, C., and Mattick, L.R. 1982. Detection of adulteration in blackberry juice concentrates and wines. J. Assoc. Off. Anal. Chem. 65: 1417-1423.

Wrolstad, R.E., Hong, V., Boyles, M.J. and Durst, R.W. 1995. Use of anthocyanin pigment analyses for detecting adulteration in fruit juices. In Vol. I *Methods to detect adulteration of fruit juice and beverages*. S. Nagy and R.L. Wade (Ed.). AgScience Inc., Auburndale, FL.

ACKNOWLEDGEMENTS

CHAPTER 6

GLYCOALKALOID CONTENT OF RED-FLESHED POTATO BREEDING CLONES AND THEIR STABILITY TO ALKALINE TREATMENTS IN ANTHOCYANIN-CONTAINING POTATO EXTRACTS

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ABSTRACT

Twenty four red-fleshed potato (*Solanum tuberosum*) breeding clones were screened for steroidal glycoalkaloid (SGA) content. SGAs and anthocyanins (ACN) were extracted using 70% acetone, partitioned with chloroform and the aqueous portion was characterized by HPLC and Mass Spectroscopy (MS). The total SGA content varied among potato varieties, ranging from 2.0 to 36.3 mg/100g tuber. Cultivars NDC4069-4 and NDOP5847-1, with highest anthocyanin content, showed low levels of SGAs. The effect of alkali treatments on SGA precipitation, anthocyanin content and profile was studied by adjusting the pH of aqueous extracts (pH ranges of 7.6 to 11). Alkaline conditions resulted in precipitation of SGAs, decreased monomeric anthocyanin content and changes in pigment profile. Complete removal of glycoalkaloids from colored extracts was achieved at pH > 9.2, however it caused substantial anthocyanin loss. Best results were obtained at pH 8.0 with monomeric anthocyanin degradation of 30% and SGA precipitation of up to 90%. The critical factor on the extent of SGAs precipitation is the alkaline pH (> 8.0) rather than the initial alkaloid concentration.

INTRODUCTION

Steroidal glycoalkaloids (SGAs) are a class of nitrogen containing compounds which possess the C_{27} skeleton of cholestane and are produced following the steroid biosynthesis pathway (Friedman and McDonald, 1997; Valkonen et al., 1996). Alkaloids are present in many species of the family *Solanaceae*, including cultivated and wild potatoes (*Solanum* spp.) and tomatoes (*Lycopersicon* spp.).

At least 90 structurally different steroidal alkaloids have been isolated and characterized in over 300 *Solanum* species (Friedman and McDonald, 1997). The major SGAs in commercial potatoes (*Solanum tuberosum*) are α-solanine and α-chaconine, which are glycosylated derivatives of the aglycone solanidine (Stanker et al., 1994). Wild *Solanum* species used in potato breeding to introduce desired traits (frost, disease and pest resistance) may result in increased concentrations of SGAs or incorporation of new

alkaloids (Van Gelder et al., 1989). Shih and Kuc (1974) reported that several alkaloids existing in potatoes may have emerged through hybridization of such species as *S. chacoense* and *S. demissum* with the cultivated potato plant. SGAs are generally present in all parts of many Solanaceous plants, however they tend to accumulate in plant organs with high metabolic activities such as flowers, sprouts, unripe berries, and young leaves (Van Gelder, 1991; Valkonen et al., 1996; Jadhav et al., 1981).

SGAs are natural toxins that probably evolved as protective compounds in response to tissue invasion. SGAs have antimicrobial, insecticidal and fungicidal properties which provides resistance against several insect pests and herbivores (Roddick et al., 1990; Tingey, 1984; Schreiber, 1968). However, they also posses pharmacological and toxicological effects against human beings (Van Gelder, 1991; Jadhav et al., 1981). Since potatoes constitute an important nutritional source in human diet, SGAs along with cyanogenic glycosides are responsible for more of human illnesses and death than any other plant toxicants (Hall, 1992). Toxicity of SGAs is due to their significant anticholinesterase activity and disruption of cell membranes (Roddick and Rijnenberg, 1987; Morris and Lee, 1984). A safety level of SGAs in potatoes was established at 200 mg/kg for acute toxicity, but these levels do not account for possible sub-acute or chronic effects. An upper limit of 60 to 70 mg/kg has been proposed for cultivars to be selected for human consumption (Valkonen et al., 1996; Van Gelder, 1991).

There is potential for the use of red-fleshed potato anthocyanin extracts as an alternative to artificial dyes (Rodriguez et al., 1998), however the levels of SGAs in the pigment extracts are of concern since they might be concentrated with the anthocyanins. SGAs are fairly heat-stable compounds, slightly affected by steaming, boiling, baking, frying, cooking and microwaving of potatoes (Friedman and McDonald, 1997; Van Gelder, 1991). However, SGAs will precipitate under basic conditions, this property is used in purification of alkaloids (Friedman and Dao, 1992; Bomer and Mattis, 1924). However, the stability of anthocyanins is affected with basic pH which increases the rate of destruction (Wrolstad, 1997; Francis, 1989; Harib and Brown, 1956).

In order to produce a red anthocyanin colorant from potatoes, we need information on alkaloid content in the potato tuber and the anthocyanin containing extract. Furthermore, we want to be able to separate the alkaloids from the colored extracts with minimum anthocyanin degradation. The objectives of this study were to identify and quantify the SGAs (α-solanine and α-chaconine), and to determine the ACN/SGAs ratio of different red-fleshed potato breeding clones. An additional objective was to evaluate the effects of alkaline treatment on the stability of SGAs and pigments of an anthocyanin containing extract .

MATERIALS AND METHODS

Plant material

Red fleshed potato (*Solanum tuberosum*) tubers were supplied by the USDA Agriculture Research Service at Prosser, WA (18 breeding clones) during fall 1996 and 1997 and the Oregon State University Crop Science Department, OR where 6 breeding clones were evaluated for two consecutive plantings (Fall 1996 and 1997) at 2 locations (Corvallis and Klamath Falls).

The breeding clones supplied by the USDA Agriculture Research Service fall 1996 were grown in greenhouse pots and were smaller in size (average wt of 38g/tuber) as compared with tubers obtained from OSU Crop Science Department that were larger (average wt of 125g/tuber) and grown in the field. Fresh tubers were washed with cold water upon arrival to eliminate extraneous matter and refrigerated at 4°C until analyzed.

Pigment and SGAs extraction

Potato tubers (ca 50 g) were cut in slices (ca 2 cm thick), blanched at 100°C for 10 min, blended with 100 mL acetone and filtered on a Buchner funnel. The filter cake residue was re-extracted with aqueous acetone (30:70 v/v) until a clear solution was obtained. Filtrates were combined, shaken in a separatory funnel with chloroform (2:1 chloroform:acetone) and stored overnight at 1°C. The aqueous portion (top portion)

was collected and placed on a Buchi rotovapor at 40°C until all residual acetone was evaporated. The aqueous extract was made up to a known volume with distilled water and analyzed for SGAs following the HPLC procedure described by Friedman and Dao (1992). Due to the limited sample size of tubers from Prosser (WA) fall 1996, measurements were made in duplicate and average values are reported. For all other tuber samples we were able to replicate the extraction.

Anthocyanin partial purification

The aqueous extract was passed through a C-18 mini-column (high load C-18 tube), 20 mL capacity and 5 g sorbent weight (Alltech Assoc., Inc., IL), previously activated with methanol followed by 0.01% aqueous HCl (Giusti and Wrolstad, 1996). Anthocyanins and phenolic acids were adsorbed onto the mini-column; sugars and acids were eluted with 2 volumes of 0.01% aqueous HCl and anthocyanins were recovered with methanol containing 0.01% HCl (v/v). The methanol was evaporated using a Buchi rotovapor at 40°C and the pigments were dissolved in deionized water with 0.01% HCl.

Effect of alkaline treatments on anthocyanin and SGA content

Three model juices were prepared using purified potato anthocyanin extracts that contained SGAs. The juices were prepared by diluting different potato anthocyanin extracts in distilled water to an initial monomeric anthocyanin content of 37 mg/100mL. The pH of the juices was adjusted to 2.3 with HCl.

Juice A: Potato juice (250 mL) was divided into 3 beakers (60 mL each) and the pH was increased with $Na_3PO_4.12H_2O$ to the desired pH value (from 7.6 to 11). A total of 9 alkaline treatments and a control (pH 2.3) were tested. Five randomly chosen alkaline treatments and the control were evaluated for each beaker. Treatments were arranged in increasing pH order and at the selected pH level an aliquot (10 mL) was taken and placed in a centrifuge tube.

Juice B:. Potato juice (350 mL) was divided into 3 beakers (100 mL each), the first 2 beakers were treated with $Na_3PO_4.12H_2O$ and the third was treated with KOH to evaluate the effect of different bases (weak vs strong). Nine treatments (pH from 7.6 to 11) and a control were evaluated, the juices were increased to the desired pH and an aliquot (10 mL) was taken and placed in a centrifuge tube. All pH treatments were replicated between beakers.

Juice C: Potato juice (160 mL) was divided into 2 beakers (80 mL each), and treated with KOH. Seven treatments and a control were evaluated, the juices were increased to the desired pH value (from 7.6 to 11), an aliquot (10 mL) was taken and placed in a centrifuge tube. All pH treatments were replicated between beakers.

After alkaline treatment the solutions were placed in a water bath (40°C) for 30 min and then centrifuged at 10,000 RPM for 45 min. The supernatant was collected and phosphoric acid (0.5 mL) was added immediately to acidify the solution. The pellet contained the precipitated SGAs.

Statistical Analysis: Each beaker was repeatedly measured as the pH was changed (repeated measures). Because there was evidence of random beaker effects and due to unbalancedness of the data (different pH levels were studied for each beaker), analyses were conducted with the general mixed model procedure in SAS (The SAS system for windows, release 6.12, SAS Institute Inc., 1997). P-values reported are from an unbalanced split plot model with beaker as the whole plot unit and pH as the subplot unit. There was low power for detecting departures from the Huynt-Feldt assumptions for repeated measures, so only p-values less than 0.01 were considered significant.

Alkaloid precipitation

Solutions (250 mL each) containing three different concentrations of SGAs (15, 30 and 60 mg/100mL) at an initial pH of 2.3 were prepared using precipitated SGAs obtained from potato tubers. Solutions were divided into 4 beakers for each alkaloid concentration and adjusted to different pH values (7.0, 7.5, 8.0, and 8.5) with KOH. After alkaline treatment the solutions were treated as mentioned before.

Statistical Analysis: Each beaker was repeatedly measured as the pH was changed (repeated measures). After finding no evidence of violation of the Huynh-Feldt assumptions (sphericity test p>0.5), the data were analyzed using a split plot model. Alkaloid concentration was the whole plot factor applied to beakers arranged in a completely randomized design with pH as the split plot factor. Because there was no evidence of beaker effects (p >0.5), polynomial regressions were conducted without beaker as a factor in the model. Analyses were conducted with the GLM procedure in SAS (The SAS system for windows, release 6.12, SAS Institute Inc., 1997).

Monomeric anthocyanin content

Monomeric anthocyanin content was determined using a pH-differential method (Wrolstad et al., 1982). A Shimadzu 300 UV spectrophotometer and 1 cm pathlength cells were used for spectral measurements at 420, 510 and 700 nm. Pigment content was calculated as Pg-3-glu, using an extinction coefficient of 31,600 L cm^{-1} mol^{-1} and molecular weight (MW) of 433.2 g mol^{-1} (Wrolstad, 1976).

Analytical HPLC System

Apparatus: An analytical High Performance Liquid Chromatograph (HPLC) Perkin-Elmer Series 400, equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with a Hewlett-Packard $HPLC^{2D}$ ChemStation software were used.

Columns and mobile phase: System I: ODS C-18 column (5 micron), 250 x 4.6 mm ID (Poly LC Inc., Columbia, MD), fitted with a 10 x 4.6 mm ID Spherisorb ODS-2 micro guard column (Alltech, Deerfield, IL). Solvent A: 100% HPLC grade acetonitrile, B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water. **System II**: Spherisorb ODS-2 column (5 micron), 250 x 4.6 mm ID, fitted with a 10 x 4.6 mm ID Spherisorb ODS-2 micro guard column (Alltech, Deerfield, IL). Solvent A: 50% acetonitrile with 5 mM sodium lauryl sulfate and 5 mM sodium sulfate decahydrate. The

pH was adjusted to 4.5 with 1% sulfuric acid. All analytical systems were run at a flow rate of 1 mL/min and an injection volumen of 50μL was used. Solvents and samples were filtered through a 0.45 μm filter type HA (Millipore Corp., Bedford, MA).

Analysis conditions: Potato anthocyanins were separated using System I. The program used a linear gradient from 0 to 30% A in 30 min. Simultaneous detection at 520, 320 and 280 nm was used and spectra were collected for all peaks. Potato SGAs were separated icocratically (100% A) using System II. The signal was monitored at 200 nm. SGAs were quantified using a standard curve at 4 concentrations (0.5, 0.25, 0.125 and 0.0625 mg/mL) of α-solanine and α-chaconine (Sigma Chemical Co., St. Louis, MO).

Semi-preparative HPLC system

Apparatus: A High Performance Liquid Chromatograph (HPLC) Perkin-Elmer Series 400 and a Semi-Preparative Dynamax Rainin Model SD-300 Liquid Chromatograph, equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with a Hewlett-Packard HPLC^{2D} ChemStation software were used.

Columns and mobile phase: Microsorb C-18 reversed phase column (5 micron) 250 x 21.4 mm, fitted with a 50 x 21.4 mm ID guard column (Rainin Instrument Co., Inc., Emeryville, CA). Solvents: same as System I. Flow rate: 20 mL/min. Injection volume: 1 mL. Solvents and samples were filtered through a 0.45 μm filter type HA (Millipore Corp., Bedford, MA).

Isolation of purified pigments: Semi-preparative HPLC system was used. The program followed a linear gradient from 10 to 18% A in 10 min. Simultaneous detection was done at 520, 320 and 280 nm. Purified anthocyanins were concentrated with a C-18 Sep-Pak cartridge and re-disolved in distilled water.

Alkaline hydrolysis of anthocyanins

Semi-preparative isolated anthocyanins were saponified using 10 mL of 10% aqueous KOH for 15 min as described by Giusti and Wrolstad (1996).

Mass spectroscopy (MS) of potato anthocyanins

Low-resolution MS was done using electrospray MS. The instrument was a Perkin-Elmer SCIEX API III+ Mass Spectrometer, equipped with an Ion Spray source (ISV=4700, orifice voltage of 80) and loop injection. Partially purified potato anthocyanin extract and semi-preparative HPLC isolated anthocyanins were injected directly into the system.

RESULTS AND DISCUSSION

Steroidal glycoalkaloid content in red-fleshed potato varieties

The isolation of SGAs from potatoes takes advantage of the fact that the major alkaloids are soluble in slightly acidified water (Jadhav et al., 1981). More than 20 different extraction systems that include solvents such as ethanol, methanol, chlorofom/methanol, water/acetic acid, tetrahydrofuran (THF)/ water/ acetonitrile and other combinations of these methods have been described (Friedman and McDonald, 1997; Friedman and McDonald, 1995). Studying the use of red-fleshed potatoes as a potential source of natural colorants (Rodriguez-Saona et al., 1998), we found that an acidified acetone/chloroform system, commonly used in extraction of anthocyanins from plants (Hong and Wrolstad, 1990), successfully extracted the SGAs present in potato tubers. Further isolation of SGAs was done by solid-phase extraction using a Sep-Pak C-18 column. We determined that most of the potato breeding clones showed reduced amounts of SGAs, in some cases more than 50% loss, when compared to direct HPLC injection of the acidified aqueous extract. The lower recoveries with the Sep-Pak C-18 column might be due to the competition for binding sites between the anthocyanin pigments, phenolic acids and SGAs present in potato tubers, resulting in underestimation of the alkaloid content.

The major SGAs in red-fleshed potato tubers, identified by HPLC (Fig 6.1a) and ESMS (Fig 6.1b), were α-solanine and α-chaconine. The presence of solanidine (aglycon), solanidine plus a hexose and solanidine plus hexose and rhamnose were also

detected by ESMS (Fig 6.1b). These alkaloids most likely had been produced from fragmentation of α-solanine and α-chaconine, since the alkaloid aglycones are rarely found in potatoes (Friedman and McDonald, 1997). However, we cannot disregard their presence in trace amounts in potato tubers since ESMS is a more sensitive technique to detect SGAs than HPLC. The presence of β and γ forms of glycoalkaloids (stepwise cleavage of the sugar moiety) had been reported in potato tubers (Friedman and Dao, 1992; Morris and Petermann, 1985) especially during prolonged storage (Friedman and McDonald, 1997) due to enzymatic hydrolysis.

Red-fleshed potato breeding clones (24) were evaluated for steroidal glycoalkaloid (SGA) content (Table 6.1). The total SGA content varied among potato clones, ranging from 2.0 to 36.3 mg/100g tuber fresh weight (fw) for NDO5849-1 and NDOP5538-12 clones, respectively. All red-fleshed potato breeding clones provided by the OSU Crop Science Dept (Corvallis, OR) showed the lowest SGA levels (average 4.7 ± 2.0 mg/100g tuber fw) while those tubers provided by USDA Agriculture Research Service (Prosser, WA) on fall 1996 showed a higher SGA content (average 17.2 ± 8.7 mg/100g tuber fw). Immature tubers tend to accumulate higher levels of SGAs than fully mature tubers, and upon exposure to light immature tubers are more likely to develop excessive SGA levels (Friedman and McDonald, 1997; van Gelder, 1990). The variability in SGAs levels could be attributed to inherited differences of potato varieties or to environmental factors during growth and storage. Besides location, the growing conditions of the potato tubers were different, with tubers supplied by USDA Agriculture Research Service (Prosser, WA) on fall 1996 being grown in a greenhouse.

Breeding clone NDOP5847-1, provided by USDA Agriculture Research Service (Prosser, WA) on fall 1997, was grown on the field and showed significantly lower SGAs levels than those from previous planting (Table 6.1). Some OSU cultivars grown in Corvallis during fall 1997 (NDO4069-4, NDO6236-1 and NDO6236-2) showed increased SGAs levels. Red-fleshed potato tubers showed high susceptibility to the herbicide treatment applied and most potato plants died or were significantly damaged and stressed.

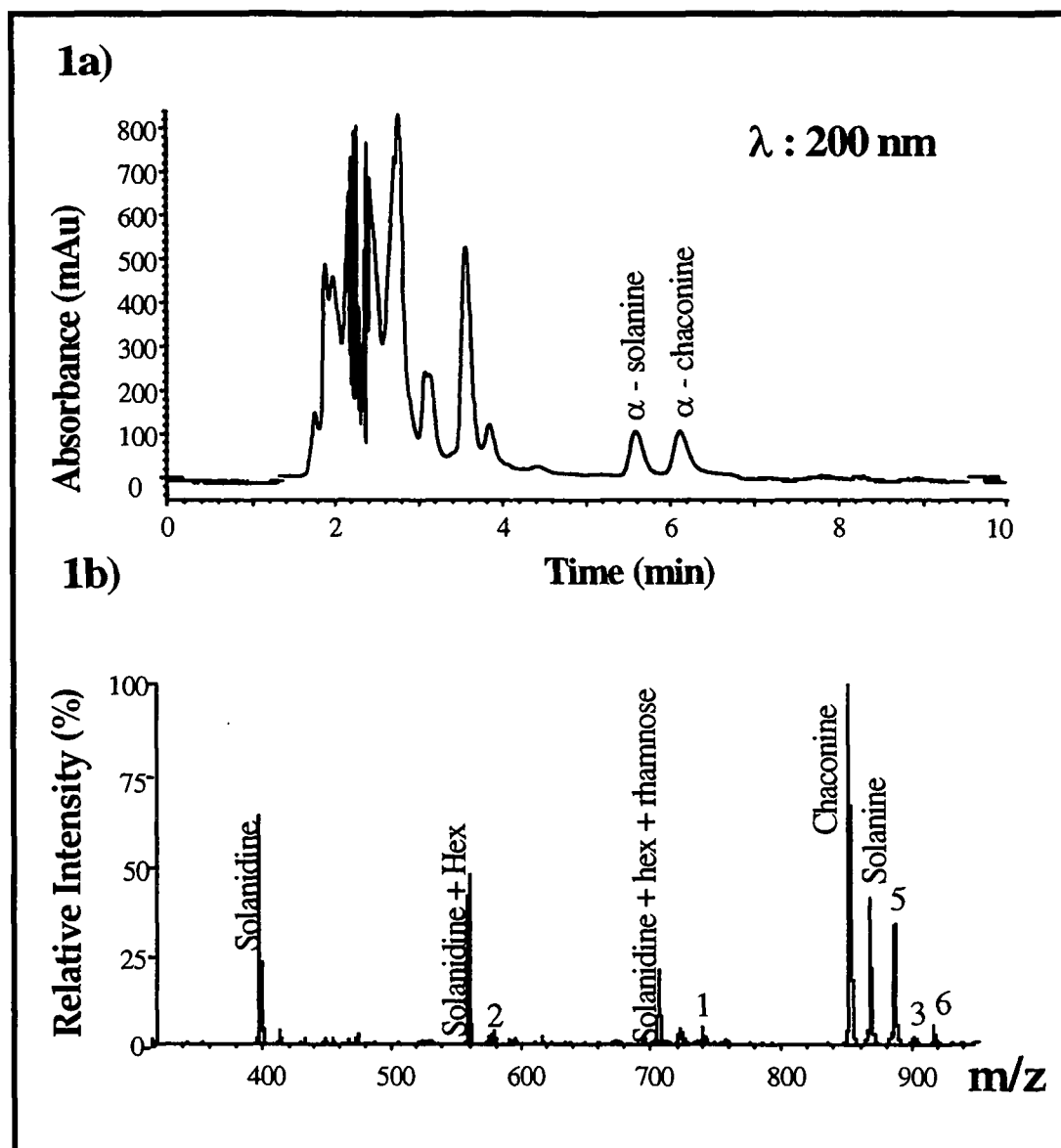Figure 6.1: a) HPLC separation of steroidal glycoalkaloids in red-fleshed potatoes. Spherisorb ODS-2, 250 x 4.6 mm ID column. Solvent: 50% acetonitrile with 5 mM sodium lauryl sulfate and 5 mM sodium sulfate decahydrate, pH 4.5. Isocratic conditions. Flow rate: 1 mL/min and Injection volume: 50FL. b) Mass Spectroscopy of potato pigment extract. The molecular ions correspond to the alkaloids and anthocyanins detected in the extract.

Table 6.1: Alkaloid content (mg/100g tuber) in different varieties of red-fleshed potatoes

Source	Variety	Solanine	Chaconine	ACNS/Alkaloid Ratio
Prosser (WA) 1996	NDOP5847-1	3.4	6.7	3.1
Prosser (WA) 1996	NDOP5847-2	3.5	6.1	0.5
Prosser (WA) 1996	NDOP5847-3	5.5	10.7	0.7
Prosser (WA) 1996	NDOP5538-1	9.1	9.5	0.6
Prosser (WA) 1996	NDOP5538-2	3.7	8.1	0.5
Prosser (WA) 1996	NDOP5538-3	11.4	9.1	0.4
Prosser (WA) 1996	NDOP5538-4	3.4	6.9	0.9
Prosser (WA) 1996	NDOP5538-5	7.4	10.7	0.5
Prosser (WA) 1996	NDOP5538-6	5.8	16.5	0.4
Prosser (WA) 1996	NDOP5538-7	18.8	21.2	0.3
Prosser (WA) 1996	NDOP5538-8	3.7	4.9	1.1
Prosser (WA) 1996	NDOP5538-9	4.2	11.5	0.7
Prosser (WA) 1996	NDOP5538-10	6.6	10.1	0.7
Prosser (WA) 1996	NDOP5538-11	6.7	13.7	0.7
Prosser (WA) 1996	NDOP5538-12	16.1	20.2	0.5
Prosser (WA) 1996	NDOP5538-13	3.9	8.2	1.1
Prosser (WA) 1996	NDOP5589-1	3.0	7.3	0.8
Prosser (WA) 1996	NDOP5589-2	3.7	8.1	0.9
Prosser (WA) 1997	NDOP5847-1	1.4	3.1	8.7
Corvallis (OR) 1996	NDO4069-4	1.6	5.1	5.2
Corvallis (OR) 1997	NDO4069-4	4.6	10.9	2.1
Klamath (OR) 1997	NDO4069-4	2.1	5.4	4.8
Corvallis (OR) 1996	NDO5849-1	0.7	1.3	10.7
Corvallis (OR) 1997	NDO5849-1	1.0	1.3	6.0
Corvallis (OR) 1996	NDO5538-2	1.5	3.5	2.6
Corvallis (OR) 1997	NDO5538-2	1.5	4.6	1.8
Corvallis (OR) 1996	NDO6236-1	1.4	2.4	3.4
Corvallis (OR) 1997	NDO6236-1	4.3	8.2	1.1
Corvallis (OR) 1996	NDO6236-2	1.9	2.6	2.4
Corvallis (OR) 1997	NDO6236-2	3.3	7.2	1.4
Corvallis (OR) 1996	NDO4239	3.4	2.9	1.4
Corvallis (OR) 1997	NDO4239	1.0	1.3	5.4
Corvallis (OR) 1997	All Blue	10.4	16.8	0.7

NDO4069-4 showed the highest susceptibility with few plants producing small tubers (average wt 20g). Imposition of certain environmental conditions such as weather, light exposure, mechanical damage, sprouting, humidity or storage conditions will induce biogenesis of SGAs in potato tuber (Friedman and McDonald, 1997; Salunkhe and Wu, 1979).

The alkaloid ratio (α-chaconine to α-solanine) varied widely among potato breeding clones, ranging from 0.8 to 3.2. Friedman and Dao (1992) reported alkaloid ratios from 1.2 to 2.7 for several potato varieties. Most varieties showed higher content of α-chaconine, with an overall alkaloid ratio of 1.9 ± 0.6. The accumulation α-chaconine in potato tubers could be attributed to variety differences due to germplasm, developmental stages or environmental/stress factors, and is produced by the potato plant because of its severe toxicity (strong cell disruption and anticholinesterase effects). It has been postulated that the production of a mixture of glycoalkaloids (α-chaconine and α-solanine) by the potato tuber results in a synergistic effect with increased toxicity (Friedman and McDonald, 1997; Roddick et al., 1990) and that by producing both SGAs the plant can convert α-chaconine to the non-lytic β_2-chaconine during the plant developmental stages without suppressing protection against pathogens, since β_2-chaconine and α-solanine are acetylcholinesterase inhibitors. As the plant matures, it converts the β_2-chaconine to the α-form in a single energy efficient step (Friedman and McDonald, 1997).

Rodriguez-Saona et al. (1998) reported the monomeric anthocyanin content of these red-fleshed potato breeding clones. The ratio of anthocyanin/SGAs (ACN/SGA) was calculated for the potato clones (Table 6.1). The ratio of ACN/SGA ranged from 0.3 to 10.7 for varieties NDOP5538-7 and ND5849-1, respectively. The potato varieties with highest anthocyanin content (NDC4069-4 and NDOP5847-1) showed relatively low SGAs content and high ACN/SGA ratios which favor their potential use as natural colorants.

Comparison of SGAs content in the potato flesh *vs* skin

The SGAs concentration in tuber peels and flesh was evaluated for cultivar NDOP5847-1. On a mg/100g basis, potato peels were 15 times higher in SGA concentration as compared to tuber flesh. It has been reported that the majority of SGAs in commercial potatoes are found in the peels, concentrated in an 1.5-2.0 mm layer under the skin with the eye-zones containing the highest concentration (Wunsch and Munzert, 1994; Kozukue et al., 1987; Uppal, 1987). The total SGAs levels were 44.2 ± 4.7 mg/100g fw peels and 3.1 ± 0.4 mg/100g fw flesh.

The alkaloid ratio (α-chaconine to α-solanine) was on average 2.4, very close to the ratio found for NDOP5847-1 whole tuber (2.2). When the SGA concentration was expressed in terms of total tuber wt, the alkaloid content in the tuber flesh (2.9 ± 0.5 mg/ 100g) was higher than peels (2.0 ± 0.2 mg/100g) since peels represented only 5% of the total tuber wt.

Effect of alkaline treatment on red-fleshed potato juice extract

Purification of glycoalcaloids by precipitation with ammonium hydroxide (ca pH 10) is a common extraction procedure because all of the SGAs except leptines are only sparingly soluble in water at pH 7 or above (Friedman and McDonald, 1997; Friedman and Dao, 1992). We evaluated the effect of different alkaline treatments, ranging from pH 7.6 to 11, on the extent of alkaloid precipitation and anthocyanin degradation in a red potato juice extract.

The potato juice extracts were standardized according to the monomeric anthocyanin content of the juices, and since we used different red-fleshed potato breeding clones in making the juices the initial SGAs content were different. The SGAs precipitated (Fig. 6.2) as a quadratic function of the pH treatment (p-value < 0.01), and no detectable amounts of SGAs were found at pH values > 9.5. Juice A was significantly different (p-value < 0.01) from the other two juices (Juices B and C) in the total alkaloid response to pH. The alkaloid content in juice A not only started much higher but it

descended more quickly as compared to juices B and C (difference in linear terms, p-value < 0.01). There was no evidence (p-value > 0.1 all effects) of differences between juices B and C, nor between alkali agents (K_3PO_4 vs KOH) within juices B and C.

Figure 6.3 shows the SGAs precipitation in model solutions at different alkaloid concentrations (experiment 2). The pH treatment (p-value < 0.01) and alkaloid concentration (p-value < 0.01) explained 98% of the variability in total alkaloid content ($R^2 = 0.98$).
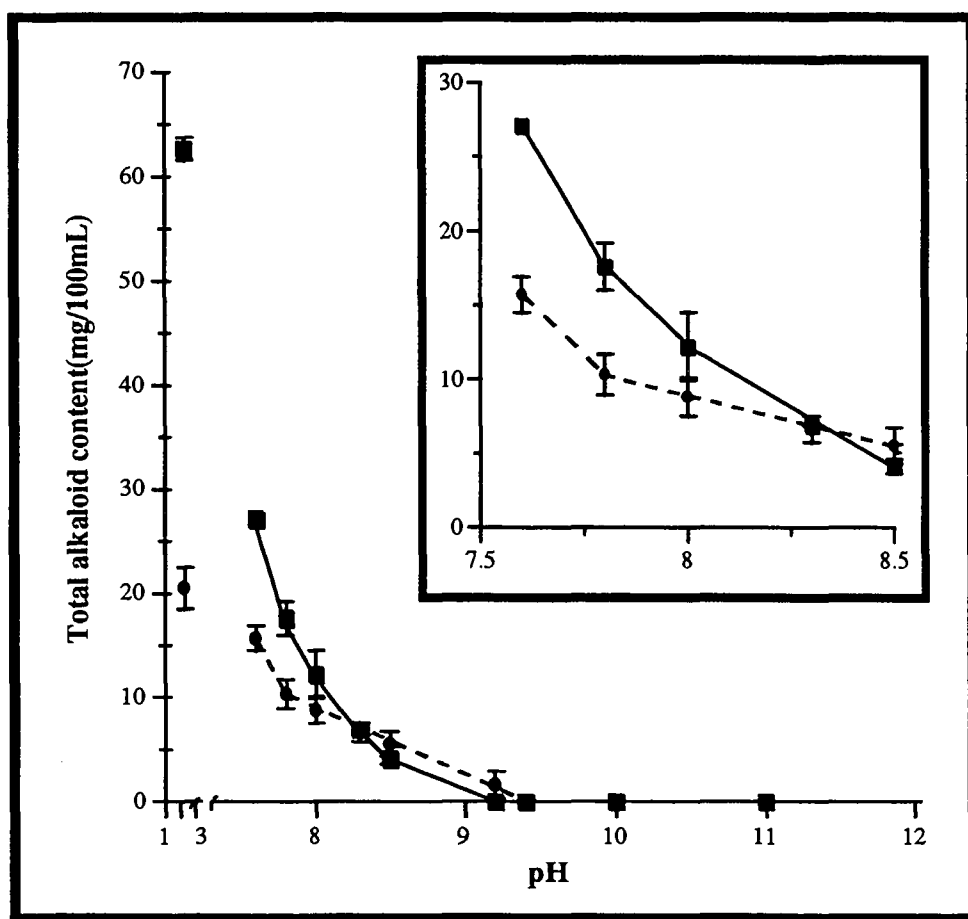

Figure 6.2: Effect of alkaline treatment on the precipitation of steroidal glycoalkaloids in anthocyanin/ SGAs containing extracts. Plot shows connecting points and not fitted regression lines.
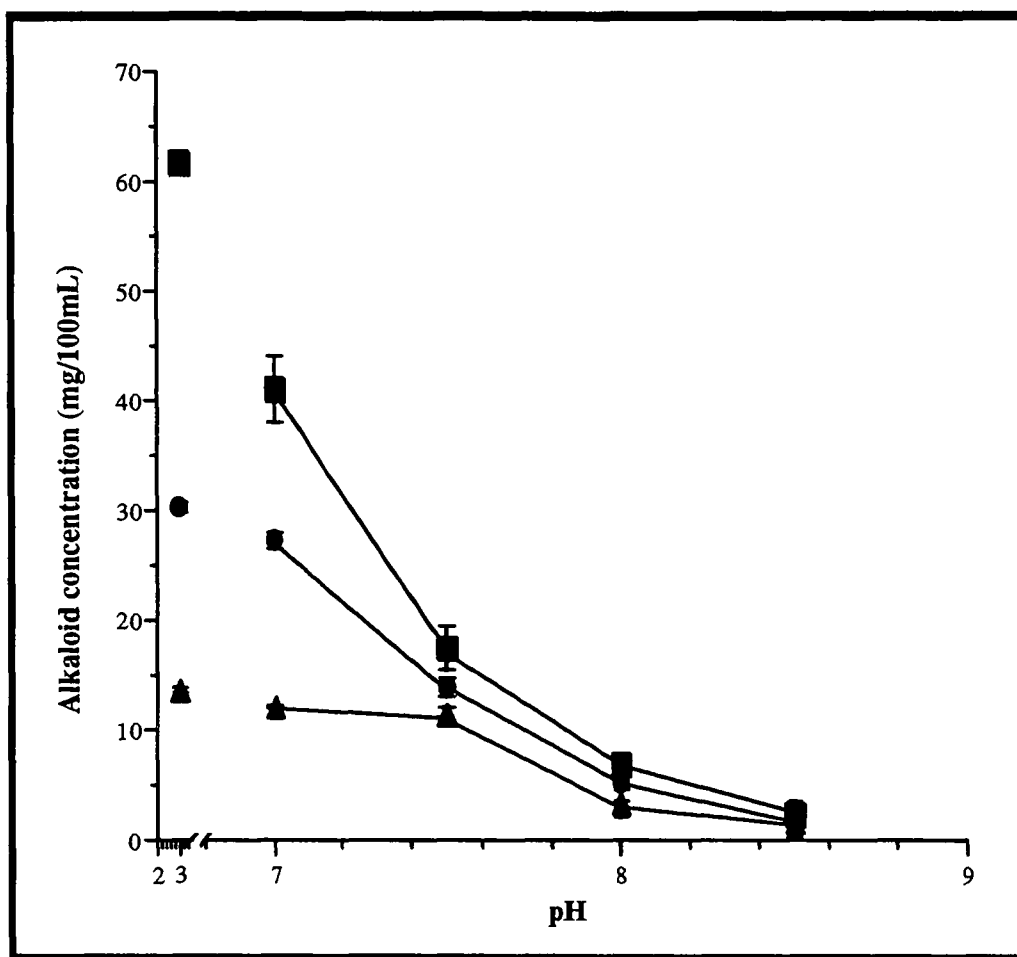
Figure 6.3: Effect of the alkaline treatments and initial alkaloid concentration on the extent of SGAs precipitation using model systems. Plot shows connecting points and not fitted regression lines. Regression equations for the pH ranges between 7.0 and 8.5 are presented.

The higher the initial concentration of alkaloids in the model system the larger the extent of the precipitation. Limited alkaloid precipitation was achieved at pH 7 (34% for C1 and 10% for C2 and C3), increased precipitation was obtained at higher pH (89%, 79% and 75% for C1, C2 and C3 respectively, at pH 8.0), and between 85% (C3) to 95% (C1 and C2) precipitation at pH 8.5. There was a clear evidence of highly significant main effects (alkaloid concentration and pH) and interaction (p-value < 0.0001 in each case). The multivariate model showed different polynomial trends for the

different solutions (Fig. 6.3). Solutions C1 and C2 showed quadratic trends; while solution C3 showed a cubic trend (p-value < 0.001) that was evidenced by the shape of the curve that showed no change in SGAs levels, a pronounced decrease and then it leveled off again. Even though the percent precipitation of glycoalkaloids was different for the treatments, the final SGAs concentration tended to merge at high pH levels (8.0 and 8.5) and no significant difference in SGAs content (p-value 0.72) was observed at pH 8.5. The results suggests that the critical factor on SGAs precipitation is the alkaline pH and not the initial alkaloid concentration. The limited solubility of the alkaloids at basic pH values (> 8.0) might determined the final alkaloid concentration in solution rather than the initial SGAs concentration of the juice extract.

Anthocyanins undergo color changes with pH, displaying their more intense red coloration (flavilium form) and higher stability under acidic conditions (pH < 3). By incresing pH values, the anthocyanin-containing extracts fade to a colorless (carbinol pseudobase and chalcone) form before changing to purple or blue (quinonoidal base) at pH > 6 (Jackman and Smith, 1987; Brouillard, 1983). The basic conditions (pH > 7.5) caused to some extent an irreversible degradation of the anthocyanins in the potato juice extract, resulting in a decrease in monomeric anthocyanin content and an increase in polymeric color of the juices (Fig. 6.4).

The monomeric anthocyanin content of potato juice extracts decreased as a polynomial (p-value < 0.01) function of the alkaline treatment. The extent of pigment degradation also depended on the alkaline agent (p-value < 0.01) used to modify the pH (Fig. 6.5). A mean pigment degradation of 25% and 35% was obtained at pH levels of 7.6 and 8.3, respectively; the pigment loss was significantly increased between pH levels of 8.5 and 10 (42% to 75% loss) and then tended to stabilize at pH > 10. Alkaline treatment at pH higher than 9.0 resulted in substantial degradation (60% to 80%) of anthocyanins.
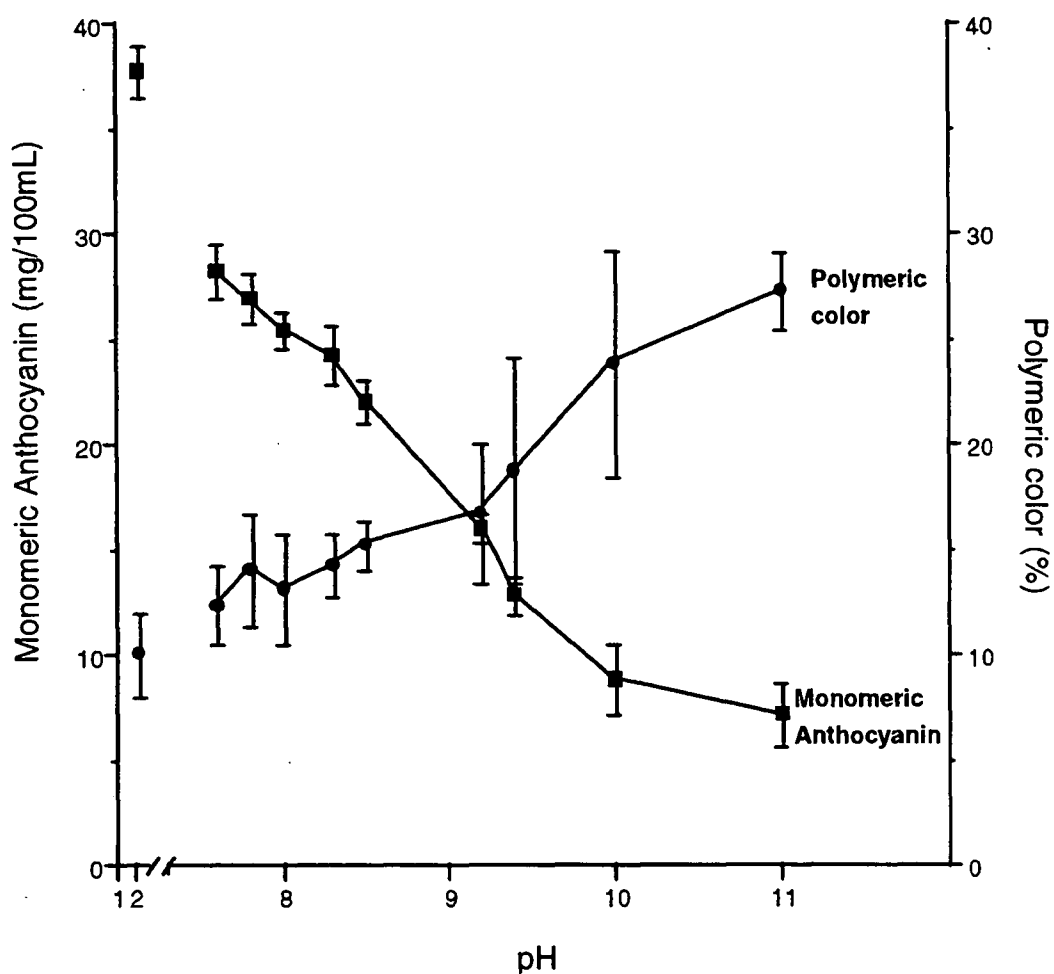
Figure 6.4: Effect of the alkaline treatment on the monomeric anthocyanin and polymeric content of anthocyanin/SGAs containing extracts. Plot shows connecting points and not fitted regression lines. Data shows average values for the different juices evaluated.
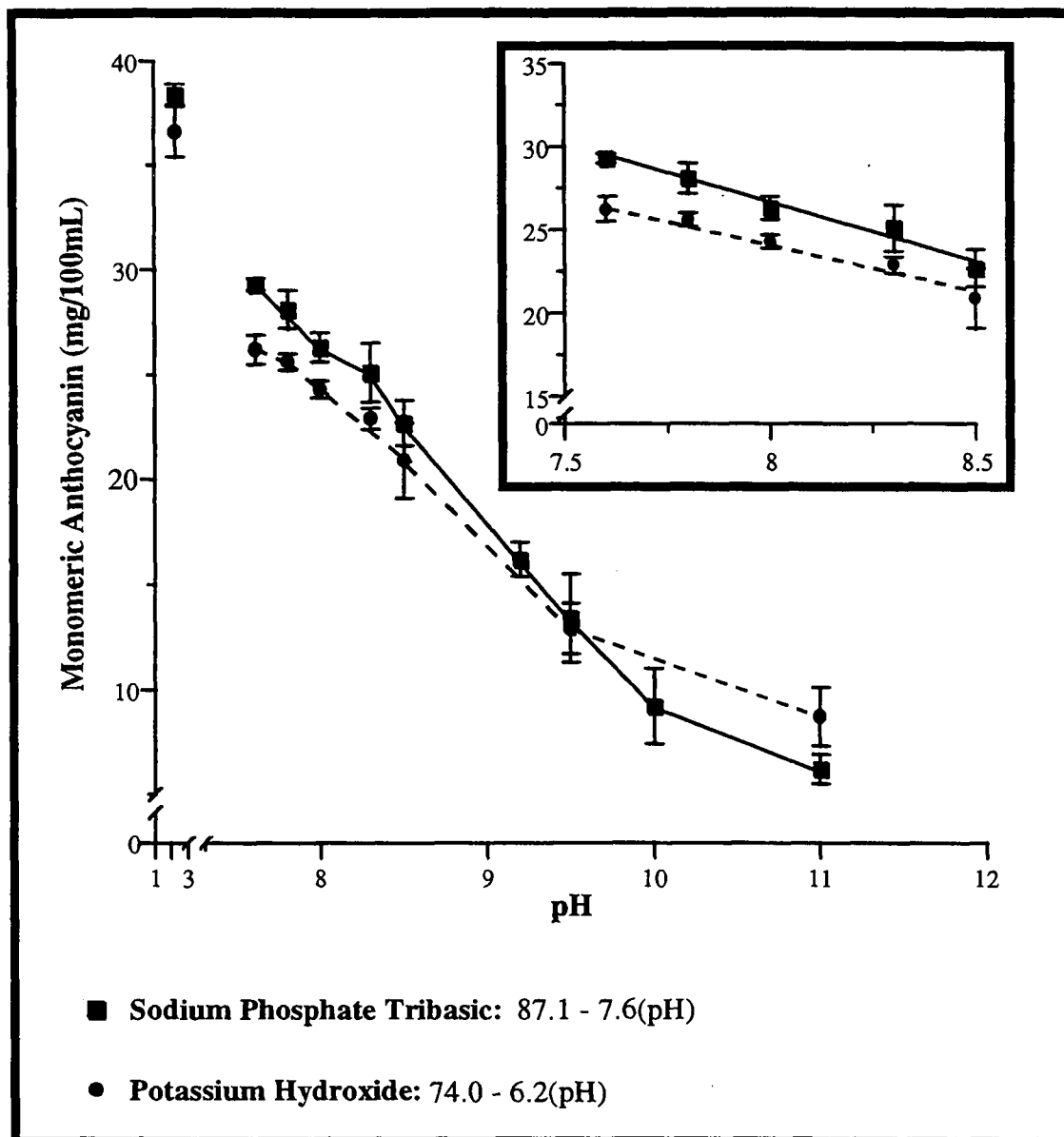
Figure 6.5: Effect of alkaline treatment and agent ($K_3PO_4.12H_2O$ and KOH) on the monomeric anthocyanin content of anthocyanin/SGAs containing extracts. Plot shows connecting points and not fitted regression lines. Regression equations for the pH ranges between 7.6 and 11 are presented.

A data subset using the pH levels ranging from 7.6 to 8.5 was evaluated (Fig. 6.5 inset) in order to obtain a nearly balanced design. There was a strong evidence of an agent effect (p-value < 0.002) and no evidence of a juice effect after adjusting for agents (p-value 0.17). The statistical model showed that the decrease in monomeric anthocyanin is essentially linear in pH over that range. There was evidence that $K_3PO_4.12H_2O$ has a more negative slope (Fig. 6.5), therefore showed an overall higher pigment degradation than KOH treatment. In these pH range, there was no evidence of quadratic or cubic main effects and interactions (p-value 0.28). Other advantages of the use of KOH (strong base) were that pH adjustment of the pigment extracts was faster, required less amounts of the alkali and did not precipitate after further concentration.

The anthocyanin pigments in red-fleshed potatoes have been previously characterized (Rodriguez-Saona et al., 1998; Lewis, 1996; Sachse, 1973; Harborne, 1960) with the major anthocyanin being pelargonidin-3-rutinoside-5-glucoside (Pg-3-rut-5-glu) acylalted with p-coumaric acid, representing more than 70% of the total area (Rodriguez-Saona et al., 1998). The peak assigment has been done as reported by Rodriguez-Saona et al. (1998). The anthocyanin profile changed with the pH treatment (Fig. 6.6). All anthocyanin pigments decreased in area except for peaks 4 and X that increased their areas, especially at pH > 9.0. The peaks 4 & X were isolated by semi-preparative HPLC and analyzed by ESMS and HPLC analyses (alkaline hydrolysis) and were identified as Pg-3-rut-5-glu acylated with p-coumaric. Presumably, the alkaline treatment resulted in geometric isomerization of the major anthocyanin (peak 5), yielding *cis* and *trans* isomers. The presence of *cis* and *trans* isomers of *p-coumaroyl* containing anthocyanins obtained from flowers of *Hyacinthus orientalis* has been reported by Hosokawa et. al. (1995). Other possibility is the esterification of *p-coumaric* at different positions of the anthocyanin molecule as a result of the alkaline treatment. Nagels et al. (1980) synthesized different *caffeoylquinic acid* isomers using heat and a saturated $NaHCO_3$ solution.
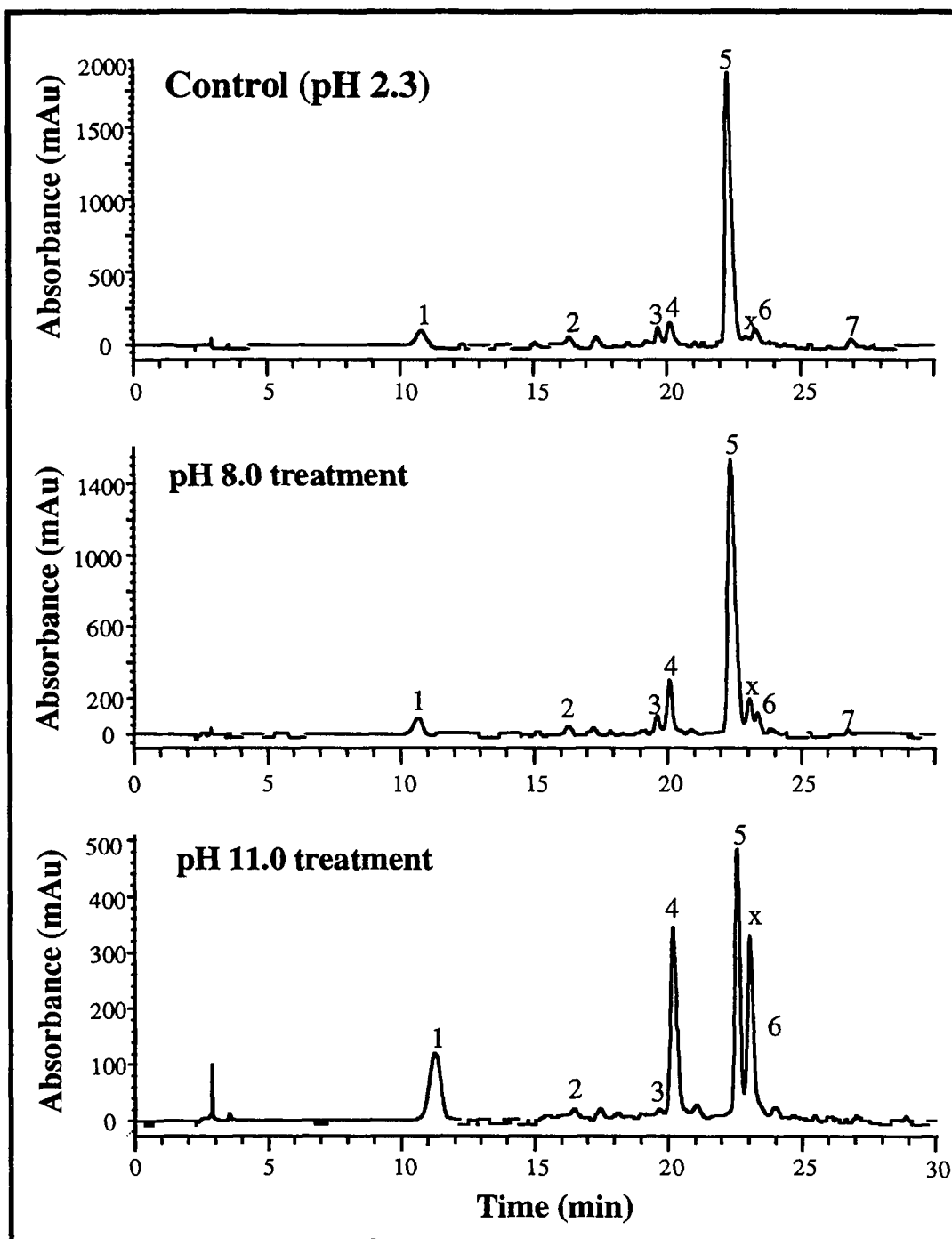
Figure 6.6: Effect of alkaline treatments on the anthocyanin profile of red-fleshed potato extracts. ODS C-18, 250 x 4.6 mm ID column. Solvent A: 100% acetonitrile, B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water. Linear gradient from 0 to 30% A in 30 min. Flow rate: 1 mL/min and Injection volume: 50μL.

Saponification is a common method to determine acylating groups attached to the anthocyanins and uses saturated conditions of KOH for 8-15 min at 25°C to cleave the ester bond (Giusti and Wrolstad, 1996; Hong and Wrolstad, 1990). We expected an increase in the area of Pg-3-rut-5-glu (peak 1) after the alkaline treatment due to saponification of the acylating cinnamic acids, however, its area remained unaltered or slightly decreased after the different treatments. This results suggests that the extent of the alkaline reaction (ca 90 min), temperature (40°C), or the concentration of alkali used to adjust the pH are responsible for the anthocyanin polymerization (Fig. 6.4 and Fig. 6.6). Severe pigment degradation was observed at pH values > 9.5, resulting in up to 90% destruction of pg-3-rut-5-glu acylated with p-coumaric acid.

CONCLUSIONS

The steroidal glycoalkaloid levels determined in different red-fleshed potato breeding clones ranged from 2.0 to 36.3 mg/100g fw tuber. Tubers grown in greenhouse pots showed higher levels of SGAs than field grown tubers. Potato clones with highest anthocyanin content (NDC4069-4 and NDOP5847-1) showed relatively low SGAs content and high ACN/SGA ratios which encourage their use as natural colorants. The major SGAs in potato tubers, α-solanine and α-chaconine, are readily soluble in water and are concentrated along with the anthocyanins. Alkaline treatment was an effective method to precipitate SGAs and separate them from the anthocyanin pigments but resulted in significant pigment degradation at pH levels > 9.0. The agent used to modify the pH had an effect on the monomeric anthocyanin content. We recommend treatment of the anthocyanin-containing juices with KOH to a final pH of 8.0 which precipitated up to 90% of the alkaloids without severe anthocyanin degradation (ca 30%) or changes in pigment profile.

REFERENCES

Bomer, A. And Mattis, H. 1924. Der Solaningehalt der Kartoffein. Z. Unters. Nahr. Genussmittel, 47: 97-127.

Brouillard, R. 1983. The *in vivo* expression of anthocyanin colour in plants. Phytochem. 22(6): 1311-1323.

Bushway, R.J.; Savage, S.A. and Ferguson, B.S. 1987. Inhibition of acetyl cholinesterase by solanaceous glycoalkaloids and alkaloids. Am. Potato J. 64: 409-413.

Fitzpatrick, T.J.; Herb, S.F.; Osman, S.F. and McDermott, J.A. 1977. Potato glycoalkaloids: increases and variations of ratios in aged slices over prolonged storage. Am. Potato J. 54: 539-544.

Francis, F.J. 1989. Food colorants: anthocyanins. Crit. Rev. Food Sci. and Nutr. 28(4): 273-314.

Friedman, M. and Dao, L. 1992. Distribution of glycoalkaloids in potato plants and commercial potato products. J. Agric Food Chem. 40: 419-423.

Friedman, M. and McDonald, G.M. 1995. Extraction efficiency of various solvents for glycoalkaloid determination in potatoes and potato products. Am. Potato J. 72: 66A.

Friedman, M. and McDonald, G.M. 1997. Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. Crit. Rev. Plant Sci. 16(1): 55-132.

Giusti, M.M. and Wrolstad, R.E. 1996. Characterization of red radish anthocyanins. J Food Sci 61: 322-326.

Hall, R.L. 1992. Toxicological burdens and the shifting burden of toxicology. Food Technol. 46: 109-112.

Harborne, J.B. 1960. Plant polyphenols: 1. anthocyanin production in the cultivated potato. Biochem. J. 74: 262-269.

Harib, A.T. and Brown, H.D. 1956. The effect of oxygen and hydrogen ion concentration on color changes in processed beets, strawberries and raspberries. Proc. Am. Soc. Hort. Sci. 68: 482

Hong, V. and Wrolstad, R.E. 1990. Use of HPLC separation/photodiode array detection for characterization of anthocyanins. J Agric Food Chem. 38: 708-715.

Jackman, R.; Yada, R. and Tung, M. 1987. A review: separation and chemical properties of anthocyanins used for their qualitative and quantitative analysis. J. Food Biochem. 11: 279-308.

Jadhav, S.J.; Sharma, R.D. and Salunkhe, D.K. 1981. Naturally occuring toxic alkaloids in food. Crit. Rev. Toxicol. 9:21-104.

Kozukue, N.; Kozukue, E. and Mizuno, S. 1987. Glycoalkaloids in potato plants and tubers. HortScience 22(2): 294-296.

Lewis, C.E. 1996. Biochemistry and regulation of anthocyanin synthesis in potato and other tuber-bearing *Solanum* species. Ph. D. Thesis. Department of Plant and Plant Microbial Science, University of Canterbury, Christchurch, New Zealand.

Linnemann, A.R.; van Es, A. And Hartmans, K.J. 1985. Changes in the content of L-ascorbic acid, glucose, fructose, sucrose and total glycoalkaloids in potatoes (cv Bintje) stored at 7, 16 and 28°C. Potato Res. 28: 271-278.

Morris, S.C. and Lee, T.H. 1984. The toxicity and teratogenicity of Solanaceae glycoalkaloids, particularly those of the potato (*Solanum tuberosum*). Food Technol. Australia 36:118-124.

Morris, S.C. and Petermann, J.B. 1985. Genetic and environmental effects on levels of glycoalkaloids in cultivars of potato (*Solanum tuberosum*). Food Chem. 18: 271-282.

Nagels, L.; Van Dongen, W.; De Brucker, J. and De Pooter, H. 1980. High-Performance liquid chromatographic separation of naturally occuring esters of phenolic acids. J. Chrom. 187:181-187.

Olsson, K. And Roslund, C. 1994. Changes in glycoalkaloid content of potato during long-term storage. In *Abstracts from the Joint Meeting of the Agronomy and Utilization Sections of the European Association of Potato Research*. Villa Real, Portugal, June 24-30 pp 41.

Roddick, J.G. and Rijnenberg, A.L. 1987. Synergistic interaction between the potato glycoalkaloids "-solanine and "-chaconine in relation to lysis of phospholipid/sterol liposomes. Phytochemistry. 26: 1325-1328.

Roddick, J.G.; Rijnenberg, A.L. and Weissenberg, M. 1990. Membrane disrupting properties of the steroidal glycoalkaloids solasonine and solamargine. Phytochemistry 29: 1513-1518.

Rodriguez-Saona, L.E.; Giusti, M.M. and Wrolstad, R.E. 1998. Anthocyanin pigment composition of red-fleshed potatoes (*Solanum tuberosum* and *Solanum stenotomum*). Accepted J. Food Sci.

Salunkhe, D.K. and Wu, M.T. 1979. Control of postharvest glycoalkaloids formation in potato tubers. J. Food Prot. 42(6): 519-525.

Sasche, J. 1973. Anthocyane in den kartoffelsorten Urgenta and Desiree. Z. Lebensm. Unters. Forsch. 153: 294-300.

Schreiber, K. 1968. Steroid alkaloids: the *Solanum* group. In *The alkaloids X - chemistry and physiology*. Eds Manske, R.H.F. Academic Press, NY pp 1-192

Shih, M.J. and Kuc, J. 1974. α and β-solamarine in Kennebec *Solanum tuberosum* leaves and aged tuber slices. Phytochemistry 13: 997-1000.

Stanker, L.H.; Kamps-Holtzapple, C. And Friedman, M. 1994. Development and characterization of monoclonal antibodies that differentiate between potato and tomato glycoalkaloids and aglycons. J. Agric. Food Chem. 42: 2360-2366.

Tingey, W.M. 1984. Glycoalkaloids as pest resistance factors. Am. Potato J. 61: 157-167.

Uppal, D.S. 1987. Varietal and environmental effect on the glycoalkaloid content of potato (*Solanum tuberosum* L.). Plant Foods Hum. Nutr. 37: 333-340.

Valkonen, J.; Keskitalo, M.; Vasara, T. and Pietila, L. 1996. Potato glycoalkaloids: a burden or a blessing? Crit. Rev. Plant Sci. 15(1): 1-20.

Van Gelder, W.M.J.; Tuinstra, L.G.M.; Van der Greef, J. and Scheffer, J.J.C. 1989. Characterization of novel steroidal alkaloids from of solanum species by combined gas chromatography-mass spectrometry. Implications for potato breeding. J. Chrom. 482: 13-22.

Van Gelder, W.M.J. 1991. Chemistry, toxicology, and occurrence of steroidal glycoalkaloids: potential contaminants of the potato (*Solanum tuberosum* L.). In *Poisonous plant contamination of edible plants*. Eds Abbel-Fattah M. Rizk. CRC Press, Boca Raton, FL pp 117-156.

Wrolstad, R.E. 1976. Color and pigment analyses in fruit products. Oregon St. Univ. Agric. Exp. Stn. Bulletin 624.

Wrolstad, R.E. 1997. Colorants. In Food chemistry: principles and applications. Ed. G.L. Christen. AFNC Press, Cutten, CA. In press.

Wu, M.T. and Salunkhe, D.K. 1976. Changes in glycoalkaloid content following mechanical injuries to potato tubers. J. Am. Soc. Hort. Sci. 101: 329-331.

Wunsch, A. And Munsert, M. 1994. Effect of storage and cultivar on the distribution of glycoalkaloids in potato tuber. Potato Res. 37: 3-10.

ACKNOWLEDGEMENTS

CHAPTER 7

COLOR AND PIGMENT STABILITY OF RED RADISH AND RED-FLESHED POTATO ANTHOCYANINS IN JUICE MODEL SYSTEMS

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ABSTRACT

The color and pigment stability of model juices (pH 3.5) colored with 15 mg anthocyanin / 100 mL juice were evaluated during 65 wks of storage at 2°C and 25°C in the dark. Two sources of acylated pelargonidin-based anthocyanins, red-fleshed potatoes (*Solanum tuberosum* L., POT) and red radishes (*Raphanus sativus*, RAE), and 2 different extraction methods, organic solvents/C-18 resin (chemical) and juice processing (physical), were used and compared to juice colored with FD&C Red # 40. Model systems showed color characteristics similar to those of FD&C Red # 40. Color (CIELch) and pigment content showed excellent stability for one year in refrigeration; storage at 25°C increased pigment degradation. Monomeric anthocyanin degraded as a quadratic function of time in juices stored at 25°C, while degradation at 2°C followed zero-order kinetics. At 25°C, higher stability was obtained for juices colored with chemically purified RAE (22 wks half-life) and lowest with potato juice concentrate (10 wks half-life). Anthocyanin composition and extraction method affected pigment stability, with chemically extracted RAE showing the best overall stability.

INTRODUCTION

Along with the demand for natural ingredients in food products there is an increased interest in the development of food colorants from natural sources (Fabre et al., 1993; LaBell, 1990). Food processors applying natural colors need to consider the entire food formulation, processing, packaging and storage conditions for maximum color functionality (Henry, 1996; LaBell, 1990).

Natural red colorants that can serve as alternatives to FD&C Red # 40 or the banned FD&C Red # 2, are of greatest demand by the industry (Francis, 1989; LaBell, 1993). Finding a natural red colorant has been difficult because few natural materials have a bright red color unmixed with other tones (LaBell, 1993). Natural colorants such as carotenoids (paprika), cochineal, anthocyanins, and beet color extract can impart various shades of red to food color, however, there have been limitations in their

application due to solubility, difficulty on matching the desired hue, incompatibility with food matrix, and stability of color shade to pH, light and oxygen (Henry, 1996).

Anthocyanins are a group of natural pigments, present in almost all higher plants, that provide a wide range of colors from orange to blue (Francis, 1989; Newsome, 1986). Concord grapes, grape skin extract (enocyanin), red cabbage, and fruit or vegetable juices (e.g. elderberries, black currents, chokeberries, bilberries) represent commercial sources of natural colorants (Jackman and Smith, 1996; LaBell, 1993; Skrede et al., 1992; Murai and Wilkins, 1990; Francis, 1989). Other proposed anthocyanins being considered as potential food colorants include sweet potatoes (Shi et al., 1992a; Odake et al., 1992; Bassa and Francis, 1987), *Tradescantia pallida* (Shi et al., 1992b), purple-hulled sunflower (Wiesenborn et al., 1991), radishes (Giusti and Wrolstad, 1996a and 1996b), potatoes (Rodriguez-Saona et al., 1998; Sachse, 1973; Harborne, 1960), *Hibiscus sabdariffa* L. (Pouget et al., 1990; Clydesdale et al., 1979), *Zebrina pendula* (Teh and Francis, 1988; Brouillard, 1981) and *Ipoema tricolor* cultivar Heavenly Blue (Goto, 1987; Teh and Francis, 1988; Asen, 1977).

Many factors affect the color and stability of anthocyanins: structure and concentration of the pigment, pH, temperature, light, presence of copigments, self-association, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products, proteins, and sulfur dioxide (Henry, 1996; Mazza and Miniati, 1993; Francis, 1989). Acylation improves the stability of anthocyanins through intramolecular copigmentation (Brouillard, 1983). The presence of aromatic residues of the acyl groups stack hydrophobically with the pyrylium ring of the flavylium cation and greatly decrease the susceptibility of nucleophillic attack of water (reduced hydration reaction), therefore increasing the stability of the chromophore by preventing the formation of a pseudobase or chalcone (Brouillard, 1983). Polyacylated anthocyanins such as red cabbage, *Tradescantia pallida, Ipoema tricolor* cv Heavenly Blue and *Zebrina pendula* have been reported to have unusual stability during processing, storage and to pH changes (Dangles et al., 1993; Shi et al., 1992; Murai and Wilkins, 1990; Teh and Francis, 1988; Brouillard, 1981).

Red radishes (*Raphanus sativus* L.) and red-fleshed potatoes (*Solanum tuberosum* L.) can provide color characteristics similar to FD&C Red #40 (Rodriguez-Saona et al., 1998; Giusti and Wrolstad, 1996b). The major pigments of red radish and red-fleshed potatoes have been identified as pelargonidin-3-sophoroside-5-glucoside acylated with malonic acid, and either *p*-coumaric and/or ferulic acids (Giusti and Wrolstad, 1996a) and pelargonidin-3-rutinoside-5-glucoside acylated with *p*-coumaric acid (Rodriguez-Saona et al., 1998), respectively. Acylation shifts the orange-red hue of pelargonidin to an intense red color and imparts improved stability.

In this study we evaluated color and pigment stability of chemically related anthocyanin obtained from red radish (RAE) and red-fleshed potato (POT) in a juice model system over 65 wks of storage. The effect of pigment purity, determined by extraction method (chemical purification vs physical juice processing), and temperature (2°C vs 25°C) were also evaluated.

MATERIALS AND METHODS

Anthocyanin Extracts

Red radish epidermal tissue (cv Fuego) and red-fleshed potato tuber (cv NDC4069-4) anthocyanins were extracted using 2 different procedures, chemical purification and juice processing operations. Chemical purification of anthocyanins was done using the acetone/chloroform procedure described by Giusti and Wrolstad (1996a). Pigments were partially purified using a C-18 mini-column (high load C-18 tube), 20 mL capacity and 5 g sorbent weight (Alltech Assoc., Inc., IL). The purified fractions were labeled radish anthocyanin extract (RAE) and potato anthocyanin extract (POT). The radish juice concentrate was prepared by blending radish epidermal tissue with distilled water and filtering the slurry through a Whatman filter # 4. The filter cake was re-extracted with water until a clear solution was obtained. The aqueous solution was concentrated using a Buchi rotavapor at 40°C. Potato juice concentrate was produced by blending tubers with acidified water (0.5M citric acid), the juice obtained was

blanched at 100°C for 5 min, and concentrated as described. The concentrated juice was treated with $K_3PO_4.12H_2O$ to precipitate alkaloids as described by Rodriguez-Saona et al. (1998). The concentrates obtained were labeled radish juice (RJ) and potato juice (PJ).

Preparation and Storage of Model Juice Solutions

A 10° Brix solution containing high fructose corn syrup (HFCS, IsoSweet 100, Staley Manufacturing Co. IL.) 0.1% potassium sorbate (w/v), 0.1% sodium benzoate (w/v) and 0.1M citric acid (Sigma Chemical Co., St. Louis, MO) was prepared. Solutions were separated into 8 containers and colored each with 15 mg monomeric anthocyanin of RAE, RJ, POT and PJ /100mL syrup by duplicate. Potassium sorbate and sodium benzoate were also added to restore the 0.1% (w/v) level. The pH of the syrup was adjusted to 3.5 with sodium bicarbonate, except for potato juice where KOH was used.

Colored solutions (15 mL) were placed in 20 mL glass vials, flushed with N_2, capped and pasteurized in water at 85°C for 25 min. The vials were stored in the dark, at 2°C and 25°C for 65 wks.

Determination of pH and °Brix

For pH measurements a Brinkmann 605 pH-meter (Methrohm Herisan, Switzerland) was used. The instrument was calibrated with buffers pH 4.0 and 7.0 and the pH was directly measured in all samples. °Brix was measured using an Auto Abbe refractometer 10500 (Reichert-Jung, Leica Inc., NY, USA). The instrument was set up to measure % soluble solids with temperature compensated mode.

Monomeric Anthocyanin Content, Polymeric Color

Monomeric anthocyanin content and polymeric color were determined using pH differential method and bisulfite bleaching methods, respectively (Wrolstad, 1976). A

Shimadzu 300 UV spectrophotometer and 1 cm pathlength disposable cells were used

for spectral measurements at 420, 510 and 700 nm. Pigment content was calculated as

pelargonidin-3-glucoside, using an extinction coefficient of 31,600 L cm^{-1} mol^{-1} and

molecular weight of 433.2 g mol^{-1} (Wrolstad, 1976).

Color Measurements

Color characteristics (Hunter CIE LCh) were measured using a ColorQuest

Hunter colorimeter (HunterLab, Hunter Associates Laboratories Inc., Reston, VA). The

equipment was set up for transmittance, with specular included, Illuminant C, and 10°

observer angle. Samples of colored syrup were placed in a 1 cm pathlength optical glass

cell (Hellma, Germany) and C.I.E. L*, a*, b*, chroma, hue angle and haze were

measured by duplicate.

HPLC Separation of Anthocyanins

Anthocyanins were separated using an analytical High Performance Liquid

Chromatograph (HPLC) Perkin-Elmer Series 400, equipped with a Hewlett-Packard

1040A photodiode array detector and Gateway 2000 P5-90 computer with a Hewlett-

Packard HPLC^{2D} ChemStation software. Column: ODS C-18 column (5 micron), 250 x

4.6 mm ID (Poly LC Inc., Columbia, MD), fitted with a 10 x 4.6 mm ID Spherisorb

ODS-2 micro guard column (Alltech, Deerfield, IL). Solvent A: 100% HPLC grade

acetonitrile, B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water. The

HPLC system was run at a flow rate of 1 mL/min and an injection volumen of 50μL was

used. Solvents and samples were filtered through a 0.45 μm Millipore filter type HA

(Millipore Corp., Bedford, MA). The program followed a linear gradient from 0 to 30%

A in 30 min. Simultaneous detection at 520, 320 and 280 nm was used and the spectra

were collected for all peaks.

Statistical Analysis

Data from the two temperatures were analyzed separately because they exhibited clearly different patterns and were sampled at different times. The results for pigment content and color measurements were analyzed using linear models with 3 factors (pigment, extract and time) and replicate batch effects within each pigment by extract combination. Analyses were done with the GLM procedure in SAS (The SAS system for windows, release 6.12, SAS Institute Inc., 1997). Focus was on shape of each response curve through time, so that linear and quadratic in time and their interactions with both pigment and extract were tested for significance. All equations are based on linear and quadratic polynomial regression without batch in the model. In the case of the response for monomeric anthocyanin content stored at 2°C, linear polynomial equations are presented even though quadratic term on time were significant. In this case the significance of quadratic and higher order polynomial terms was interpreted as being due to random effects on the instrument that caused the response for all treatments to increase or decrease from day to day.

RESULTS AND DISCUSSION

Pigment Stability

The main effects of pigment structure and extraction method, averaged over time, showed a significant effect ($p < 0.01$) on the degradation of monomeric anthocyanins at 25°C (Fig. 7.1a). The effect of time was dependant on the pigment chemical structure ($p < 0.01$) and showed a quadratic response. The anthocyanin composition of red radish cv Fuego and red-fleshed potato cv NDO4069-4 have been previously reported, being the major pigments pelargonidin 3-sophoroside-5-glucoside acylated with malonic acids and either p-coumaric and ferulic acids and pelargonidin 3-rutinoside-5-glucoside acylated with p-coumaric acid (Giusti and Wrolstad, 1996a; Rodriguez-Saona et al., 1998).
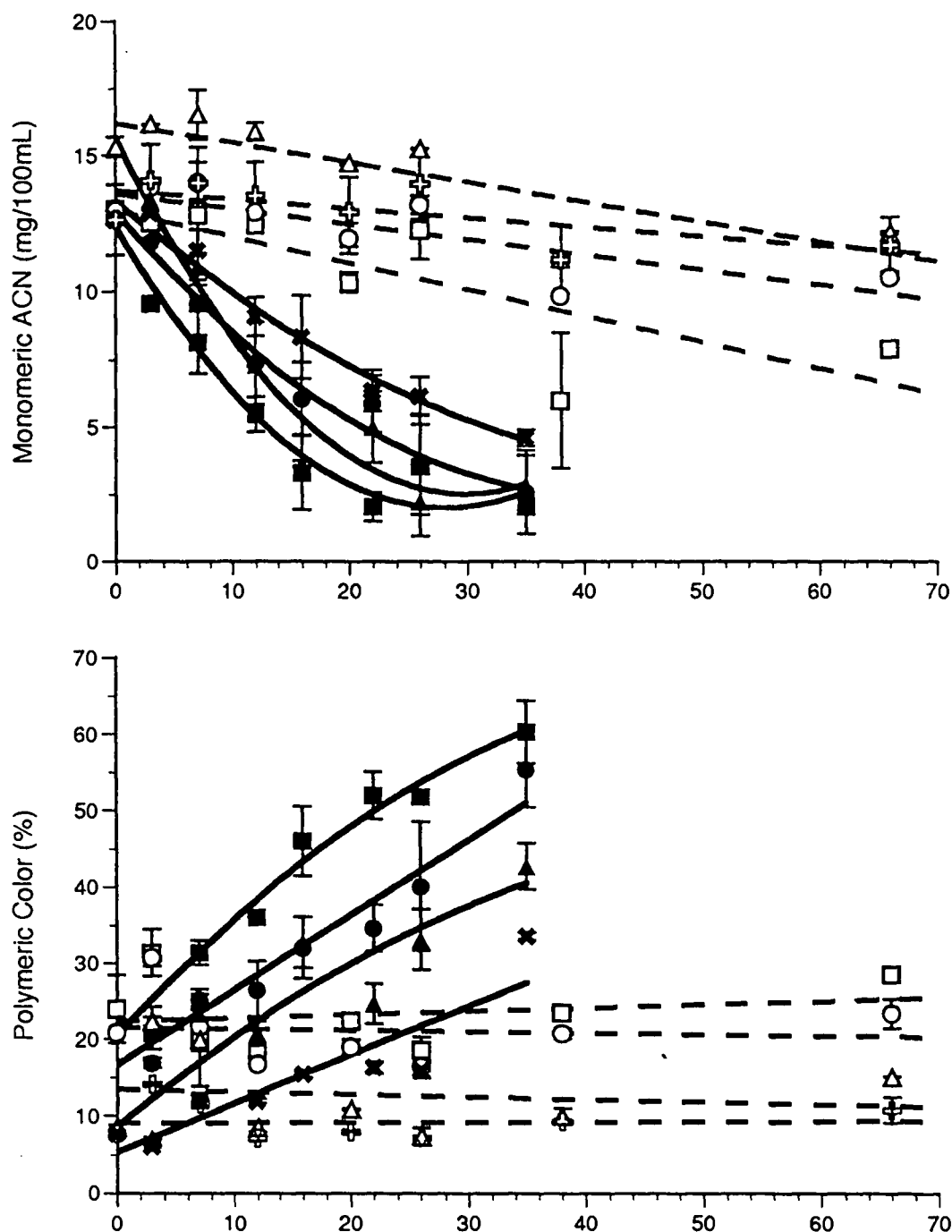
Figure 7.1. Monomeric anthocyanin degradation (a) and polymeric color formation (b) in model juices colored with radish and potato extracts. ■ Potato Juice Concentrate, ▲ Purified Potato Extract, ● Radish Juice Concentrate, ✘ Purified Radish Extract. Fill symbols represent room temperature (25°C) treatments, open symbols represent refrigerated (2°C) treatments. Bars represent the standard deviations, and lines represent the linear or quadratic lines fitted by least squares.

Radish extracts showed higher stability during storage than potato extracts, regardless of extraction procedures. The presence of diacylation in the red radish anthocyanin as compared to monoacylated anthocyanins in red-fleshed potato might be responsible for its enhanced stability. According to Goto (1987), diacylated anthocyanins are stabilized by a sandwich type stacking caused by hydrophobic interactions between the planar aromatic residues of the acyl groups and the positively charged pyrylium nucleus, and thus preventing the addition of nuclophiles, especially water, to the C-2 and C-4 positions of the anthocyanin, diminishing the formation of the pseudobase (Goto and Kondo, 1991; Brouillard, 1981). In the case of monoacylated anthocyanins, only one side of the pyrylium ring can be protected against the nucleophillic attack of water and therefore only a weak intermolecular effect might occur (Bouillard, 1983).

The position of attachment of the acyl group to the sugar and the structure of the sugar are important factors involved in the stacking process (Brouillard, 1983). Red radish anthocyanins has sophorose at the C-3 position of the aglycon and the acyl group is attached to the C-6 of the glucose moiety closest to the anthocyanin (Giusti et al., 1998), allowing free rotation of the acyl group and folding of the planar ring(s) of the aromatic acids over the pyrylium ring. Red-fleshed potato has rutinose at the C-3 position of the aglycon, and although there has not been a conformational study on these potato clones, Andersen et al. (1991) reported that in petanin (an acylated anthocyanin from *Solanum tuberosum*) the acyl group (*p*-coumaric) was attached to the position C-4 of the rhamnosyl moiety of rutinose. These structural differences might account for the improved stability showed by red radish anthocyanins.

Extraction of anthocyanins by C-18 cartridges improved the stability of pigments. The use of the C-18 mini-column allows for the separation of polar compounds such as sugars and acids from the anthocyanins and phenolic acids due to their hydrophobic affinity for the column. Intermolecular co-pigmentation between the anthocyanins and other flavonoid molecules (i.e. rutin, catechin, phenolic acids) could improve stability of anthocyanins by reducing the production of the carbinol pseudobase

and stabilizing the quinonoidal base (Mazza and Brouillard, 1990; Brouillard, 1982; Williams and Hrazdina, 1979; Asen et al., 1972). The different anthocyanin extracts contained phenolic acids (Fig. 7.2), being chlorogenic acid the major phenolic in potatoes. Radish extracts showed a complex phenolic pattern and their spectra suggested the presence of cinnamic esters. Gentistic acid has been reported as the major phenolic acid (Ishikura and Hayashi, 1965) in red radishes. However, stabilization in dilute anthocyanin solutions has been mainly attributed to intramolecular effects (Baublis et al., 1994; Shi et al., 1992) and little or no intermolecular copigmentation takes place (Brouillard, 1983).

Figure 7.1a shows the accelerated degradation of monomeric anthocyanins in model juices colored with vegetable juice concentrates as compared to the chemically extracted pigments, regardless of the storage temperature. Vegetable juice concentrates were crude extracts that contained several compounds likely to react with anthocyanins. Sugar degradation products derived from Maillard reactions, ascorbic acid , presence of enzymes (glycosidases, polyphenoloxidases and peroxidases), and metals, can accelerate the anthocyanin degradation (Jackman and Smith, 1996; Francis, 1989; Markakis, 1982). The complex composition of these crude extracts may also account for the high initial % polymeric color obtained for these treatments (Figure 7.1b), with PJ and RJ showing levels of initial polymerization (ca 20%) as compared to only 8% of POT and RAD.

Storage temperature had a clear effect on the degradation kinetics of monomeric anthocyanin content of colored model juices (Fig. 7.1a). Room temperature storage (25°C) resulted in higher degradation rates (Table 7.1) as compared to refrigerated temperatures (2°C). The estimated monomeric anthocyanin half-lives ($t_{1/2}$) are presented in Table 7.1. At 25°C, the highest stability was obtained with juices colored with RAD (22 wks $t_{1/2}$) and lowest with PJ (10 wks $t_{1/2}$).

Radish cv Fuego Phenolics

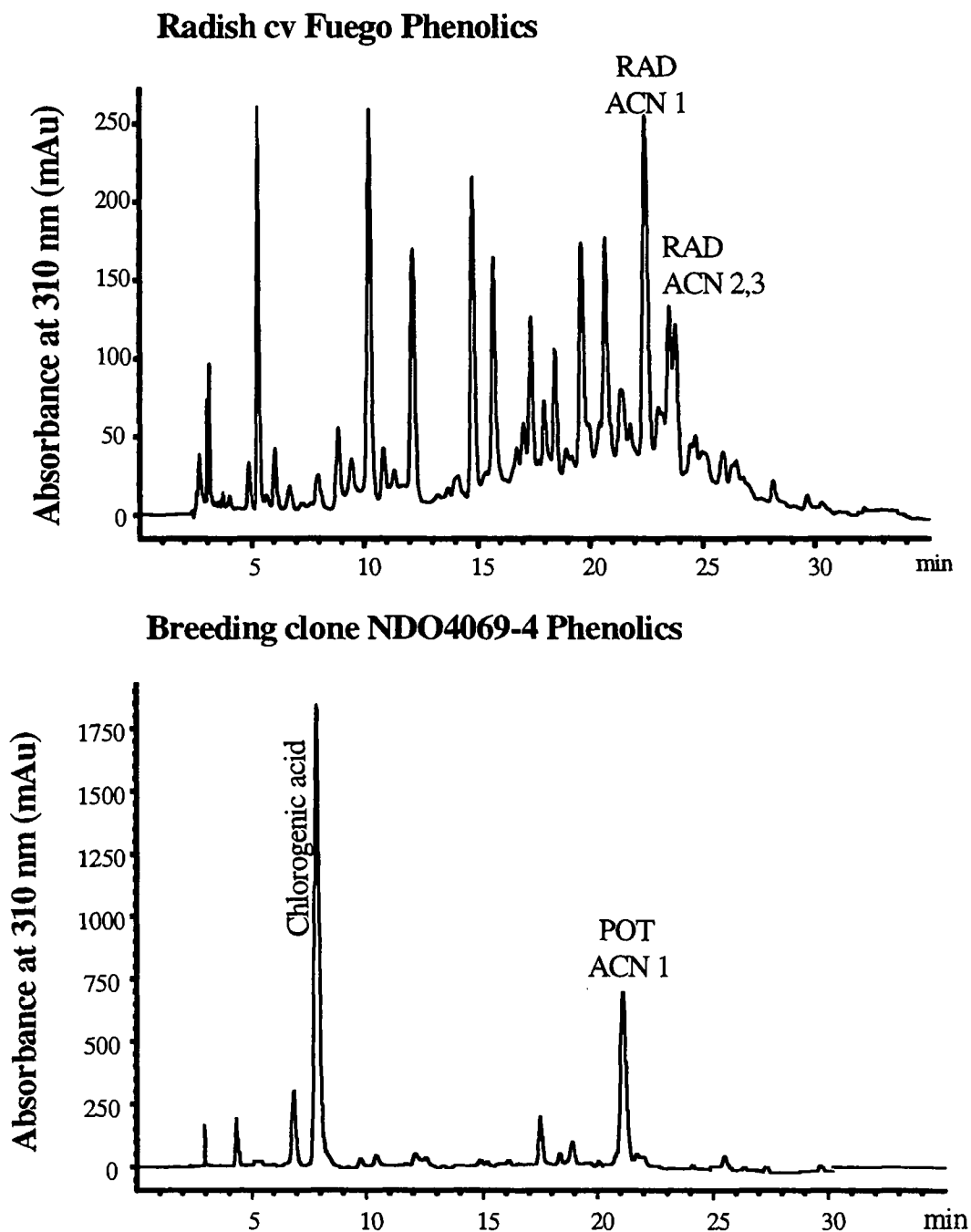## Breeding clone NDO4069-4 Phenolics

Figure 7.2. Phenolic acid content of potato and radish extracts. Column, Poly-LC, 250 x 4.6 mm id. Solvents: A: 100% HPLC grade acetonitrile and B: 1% phosphoric acid, 10% acetic acid, 5% acetonitrile and water. Linear gradient from 0 to 30% A in 30 min. Flow rate: 1 mL/min.

Refrigerated temperatures drastically decreased the rate of anthocyanin degradation with estimated $t_{1/2}$ (Table 7.1) of over a year (60 wks for PJ and 120 wks for RAD). Giusti and Wrolstad (1996b) reported $t_{1/2}$ of 29 wks for syrups (40°Brix) colored with similar concentration of radish anthocyanin extract stored at room temperature. The higher $t_{1/2}$ reported Giusti and Wrolstad (1996a) could be due to the protective effect of sugar addition (syrup) on total monomeric anthocyanin, presumably by lowering the water activity (Jackman and Smith, 1996; Wrolstad et al., 1990).

Table 7.1: Degradation equations for pigment and color of model juices (pH 3.5) colored with potato and radish anthocyanins

Source	RSQ	Room Temperature Storage			
		Radish		Potato	
		C-18 column	Juice Concentrate	C-18 column	Juice Concentrate
Monomeric	0.94	$13.3-0.37t+0.003t^2$	$12.9-0.50t+0.006t^2$	$15.7-0.88t+0.015t^2$	$12.4-0.74t+0.013t^2$
Half life (wks)		24	16	11	10
Source	RSQ	Refrigerated storage			
		Radish		Potato	
		C-18 column	Juice Concentrate	C-18 column	Juice Concentrate
Monomeric	0.72	$14.1-0.044t$	$13.8-0.061t$	$16.5-0.081t$	$13.2-0.111t$
Half life (wks)		>65	>65	>65	60
		(177)*	(120)*	(110)*	

*: predicted half-lives assuming the degradation of anthocyanins follows the same behavior as obtained with the regression model after 65 wks of storage.

Anthocyanin degradation often fits first order kinetics (Cemeroglu et al., 1994; Dravingas and Cain, 1968; Attoe and Von Elbe, 1981). However, it has also been reported that acylated anthocyanin degradation may follow linear (Baublis et al., 1994)

or nonlinear degradation rates, probably due to the folding of the acyl molecules protecting the aglycon (Baublis et al., 1994; Shi et al., 1992d). In this study, an analysis showed that anthocyanin degradation during storage at 25°C fitted a quadratic model for the different treatments. Juices stored at refrigerated temperature showed little degradation and the regression analysis suggested simple linear models.

The degree of polymerization in the juices depended on the storage temperature, pigment, and extraction method (Fig. 7.1b). The effect of time at 25°C was dependant on the extraction method and pigment chemical structure ($p < 0.01$). A quadratic rate of polymeric color development was observed in juices stored at room temperature (25°C), with RAD having the lowest polymeric color levels and PJ the highest. Similar rates of polymer formation were obtained with RJ and POT. Juices stored at refrigerated temperatures showed a very low rate of polymeric color formation, with just detectable levels after 65 wks of storage.

Changes in Anthocyanin Profile (HPLC) During Storage

The anthocyanin composition of the colored juices was monitored by HPLC (Fig. 7.3 & 7.4). We are presenting the changes in anthocyanin profiles for RJ (Fig. 7.3) and PJ (Fig. 7.4) during storage at 25°C since those model juices colored showed accelerated anthocyanin losses. Similar degradation patterns were observed for juices colored with chemically purified radish and potato anthocyanins.

The anthocyanin pigments of radish cv Fuego have been previously characterized by Giusti and Wrolstad (1996a) and peak assignments were done based on the data reported by these authors. The total anthocyanin content decreased with storage time, as showed by a reduction in total peak area at 520 nm (Fig. 7.3). The diacylated peaks 2 and 3 (ca 70% of the total peak area at wk 0) showed 75% and 95% of their area lost in 12 wks and 26 wks, respectively. However, the mono-acylated anthocyanins (peak 1) showed minor changes in total area and after 26 wks of storage at 25°C it had decreased only 24% becoming the major anthocyanin in the radish-colored juices.
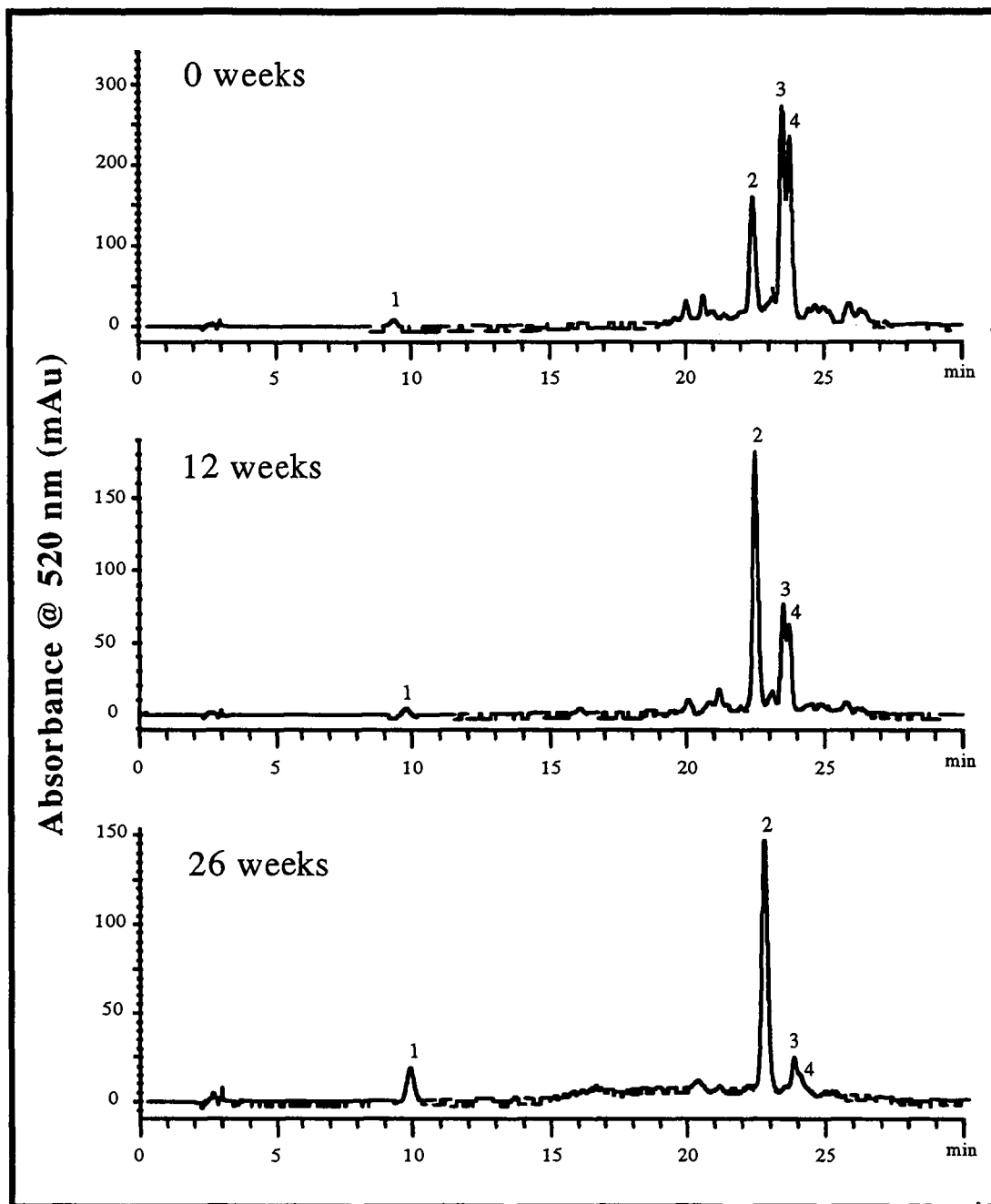
Figure 7.3. Changes in HPLC profile of radish anthocyanins during storage. Peak 1: pg-3-soph-5-glu. Peak 2: pg 3-soph-5-glu acylated with either p-coumaric and ferulic acids. Under the conditions used there was not a separation of these peaks. Peak 3: pg 3-soph-5-glu acylated with malonic and p-coumaric acid, and peak 4: pg 3-soph-5-glu acylated with malonic and ferulic acid. HPLC conditions: same as for Fig. 7.2.
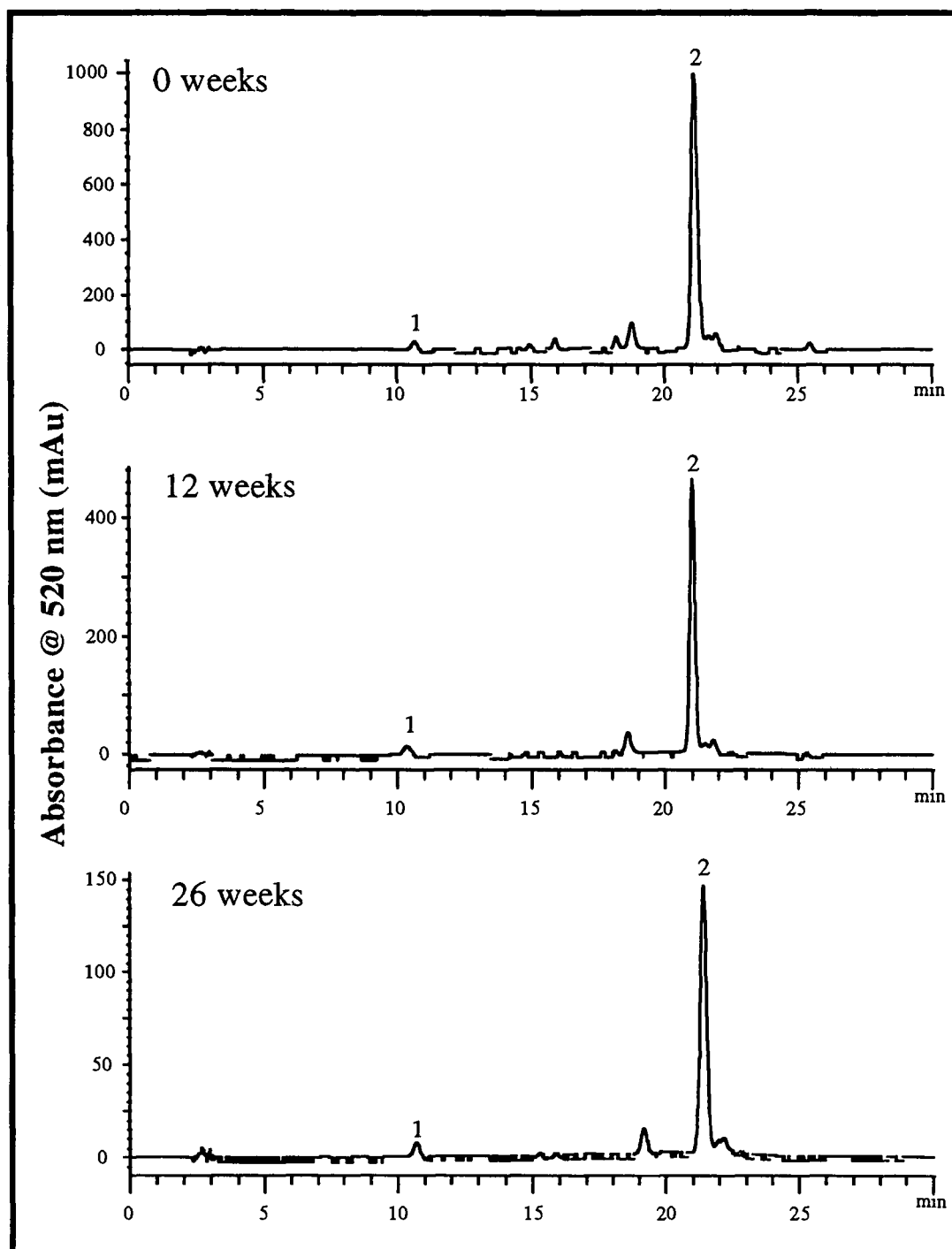
Figure 7.4. Changes in HPLC profile of red-fleshed potato anthocyanins during storage. Peak 1: pg 3-rut-5-glu; peak 2: pg-3-rut-5-glu acylated with *p*-coumaric acid. HPLC conditions: same as for Fig. 7.2.

The results agree with previous reports (Giusti and Wrolstad, 1996a) that the malonic acid acylation is more labile to hydrolysis than the cinnamic acids and its degradation yield pigment 1 as intermediates.

Peak assignments for red-fleshed potato was based on Rodriguez-Saona et al. (1998). The major anthocyanin in potato-colored juices is a mono-acylated pelargonidin derivative (peak 1) which represented 80% of the total peak area at 520 nm (Fig. 7.4) at time 0 wks. After 26 wks of storage (25°C), peak 1 decreased 85-90% of its original area. There was no accumulation of saponified anthocyanin (peak S) for any treatment during storage.

Thermal degradation leads to the formation of the chalcone and its subsequent cleavage yields several degradation products which condense to form complex brown polymeric compounds known as melanoidin pigments (Piffaut et al., 1994). The exact mechanism has not been fully elucidated. Markakis (1982) postulated that the thermal degradation of anthocyanins involves the opening of the heterocycle molecule and the formation of the chalcone glycoside as first step, without the need for the hydrolysis of the glycosidic moiety and formation of the aglycon (Adams, 1973). According to our results, it is possible that mono-acylated anthocyanins could undergo degradation through chalcone glycoside since we did not find partial degradation products from anthocyanin hydrolysis. Di-acylated molecules might be protected against hydration due to sandwich stacking of both acyl molecules. The release of an acyl group (malonic acid) will expose part of the anthocyanin molecule to hydration and further formation of the chalcone form.

Color Changes During Storage

There is a need for a natural colorant that can effectively match the color characteristics of FD&C Red # 40 in food applications. The color characteristics of FD&C Red #40 have been described as bright orange-red (Dziezak, 1987).

The color data (Fig. 7.5) showed the marked effect of storage temperature on color stability of the different treatments. The color (CIEL*ch) of the model juices
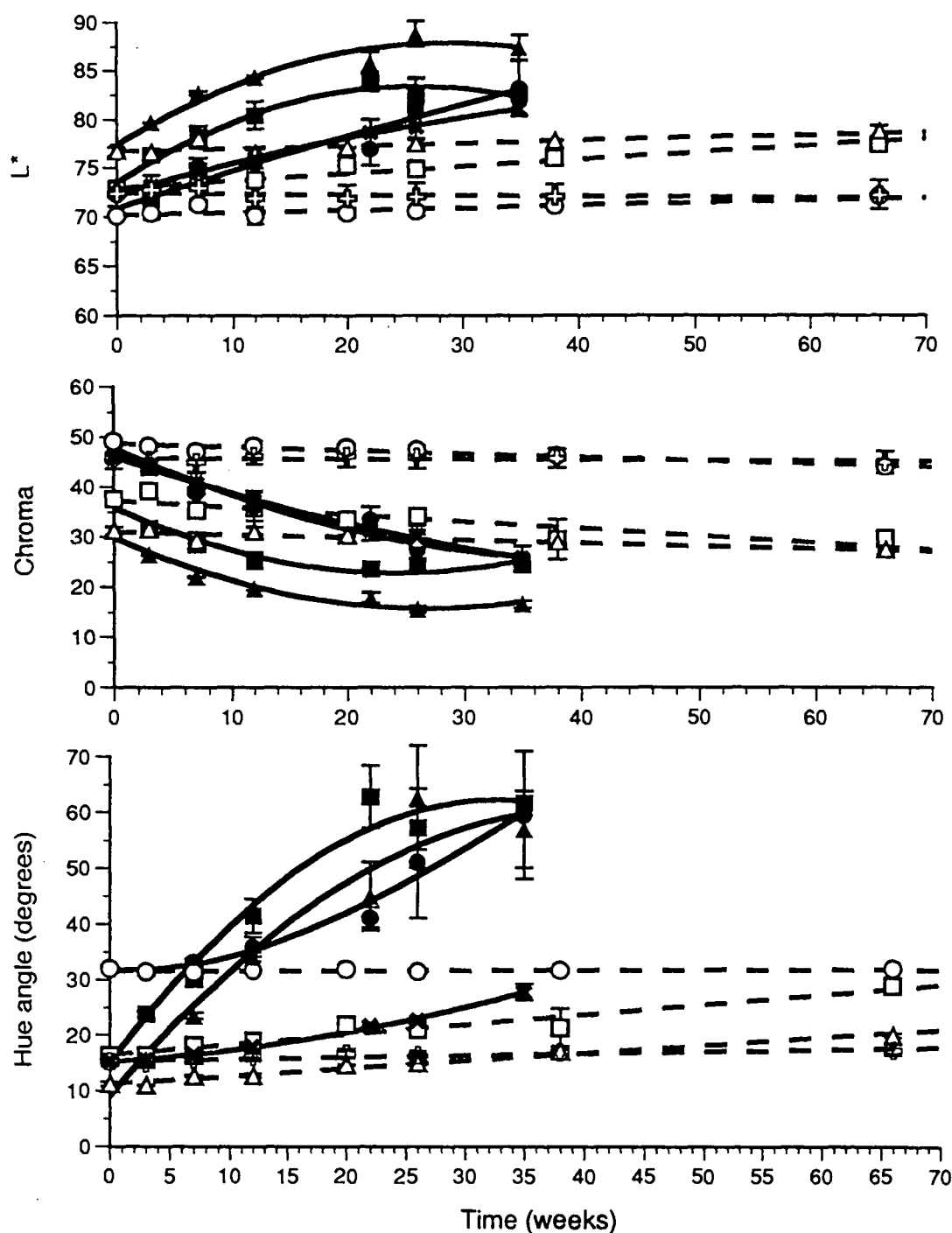
Figure 7.5. Changes in L* (a), chroma (b), and hue angle (c) of model juices colored with potato and radish anthocyanins during storage. ■ Potato Juice Concentrate, ▲ Purified Potato Extract, ● Radish Juice Concentrate, ✕ Purified Radish Extract. Fill symbols represent room temperature (25°C) treatments, open symbols represent refrigerated (2°C) treatments. Bars represent the standard deviations, and lines represent the linear or quadratic lines fitted by least squares.

changed as a quadratic function of storage time (25°C), while during refrigerated storage followed a linear trend. The results for lightness, chroma and hueangle showed significant linear and quadratic time effects (time, time*pigment, time*time and time*time*pigment; p < 0.01). The main effects pigment, extraction method and their interaction were also significant (p <0.01).

The initial lightness (L*) was ≈ 72, except for POT which showed a higher L* (76.7) as compared to the other treatments. All juice treatments showed an increase in lightness (L*) during storage at 25°C (Fig. 7.5a). Juices colored with radish anthocyanins showed lower rate of L* changes; the regression model showed that an increase in L* of 10 units would occur in 42 wks for RAD, 27 wks for RJ and 20 wks for POT and PJ. Little changes in L* were observed in juices stored at 2°C, except for PJ that showed a marked increase in L* as compared to the other treatments.

The initial chroma (color intensity) of the model juices (Fig. 7.5b) depended on the type of pigment used. Radish-colored juices showed higher chroma (chroma ≈ 47.5) than potato-colored juices (chroma ≈ 34.4). RAD-colored juices showed the lowest reduction in chroma during storage at both temperatures evaluated. A reduction of 10 units in chroma would be reached after 16 and 10 wks of storage at 25°C for RAD and RJ, respectively; while potato colored juices (POT and PJ) showed similar decrease in color intensity, ≈ 12 wks for a decrease of 10 units.

Juices colored with vegetable juice concentrates (hue angles of 32° and 16° for RJ and PJ, respectively) showed higher hue angles than juices colored with chemically purified extracts (hue angles of 15° and 11° for RAD and POT, respectively). These differences in hue angle could be attributed to interaction of anthocyanins with other compounds present in the juice concentrates (intermolecular co-pigmentation) since no purification was performed; also other colored compounds besides anthocyanins could be present and contribute to the final color of the colorant extract.

Hue angle gives a numerical estimate of the color of the juices. The hue sequence on a CIELAB diagram is defined with red-purple (0°), yellow (90°), bluish-green (180°) and blue (270°) (McGuire, 1992). Red radish and red-fleshed potato have the same

aglycon, pelargonidin. The hue of pelargonidin derivatives have been reported as orange-red (Mazza and Miniati, 1993) and the presence of acylating groups in the anthocyanin molecule produces a bathochromic shift, changing the hue towards red or red-purple hue, depending on the number of acylations (Dangles et al., 1993). Radish colorant gave higher hue angle than potato colorant even though the major radish anthocyanin have 2 acylating groups and potato anthocyanin only one. These results suggested that malonic acid would not be causing the same bathochromic shift on anthocyanins typical of cinnamic acid acylation.

Increments in hue angle values were observed during storage, with the juices becoming more orange/yellow (Fig. 7.5). These changes were dependent on the storage temperature, pigment structure and extraction method. The vegetable juice composition affected the color stability. The juices colored with red-fleshed potato extracts showed higher rates of color degradation, with fairly linear increases in hue angle observed during 22 wks (PJ) and 26 wks (POT) of storage at 25°C when a plateau was reached at hue angles of ca. 60°, times which also corresponded to a monomeric anthocyanin degradation of ca 80%, with very little monomeric anthocyanin remaining to contribute to color. For the juices colored with both radish extracts, we found a curvilinear increase in hue angle for 35 wks of storage at 25°C. However, RAD colored juices showed little changes in hue angle as compared to the other treatments.

Refrigerated temperatures greatly improved the color stability of the juices (Fig. 7.5). Juices colored with PJ were more susceptible to color changes, especially for L* and hue angle.

The visual appearance of all model juices were very close in color to that of FD&C Red # 40 (150 ppm solution at pH 3.5). The color attributes imparted by RJ to juices (Fig. 7.5) were the closest to the color obtained with FD&C Red # 40 (L*= 67.3; c = 65.5 and h = 37.7°). It has been reported that pigment concentrations have an impact on the color characteristics (Rodriguez-Saona et al., 1998) of the solutions, and the pigment stability (Giusti and Wrolstad, 1996b; Mazza and Miniati, 1983).

Increasing the pigment concentrations of POT, PJ, and RAD could result in juices with color characteristics even closer to those of FD&C Red # 40.

The addition of the colorants to model juices resulted in the formation of haze (Fig. 7.6). Haze was determined in juices after homogenization to account for the material that precipitates during storage. PJ showed the highest initial levels of haze at both temperatures. The model juices colored with the vegetable juice concentrates showed higher haze formation during storage regardless of the temperature, however, lower rates of haze formation were obtained in refrigeration. Cemeroglu et al. (1994) reported that increased soluble solids content promote haze formation. An increase in haze formation in refrigerated juices was observed during the first wks and then it leveled off. Haze formation results from anthocyanin degradation and reactions involving phenolic and proteins (Cemeroglu, 1994). We suspect that the presence of suspended particles (proteins and/or cell wall polysaccharides) in the colorants, especially the vegetable juice concentrates, participated in the haziness of the solutions. Refrigeration appeared to enhance the precipitation of these medium sized compounds.
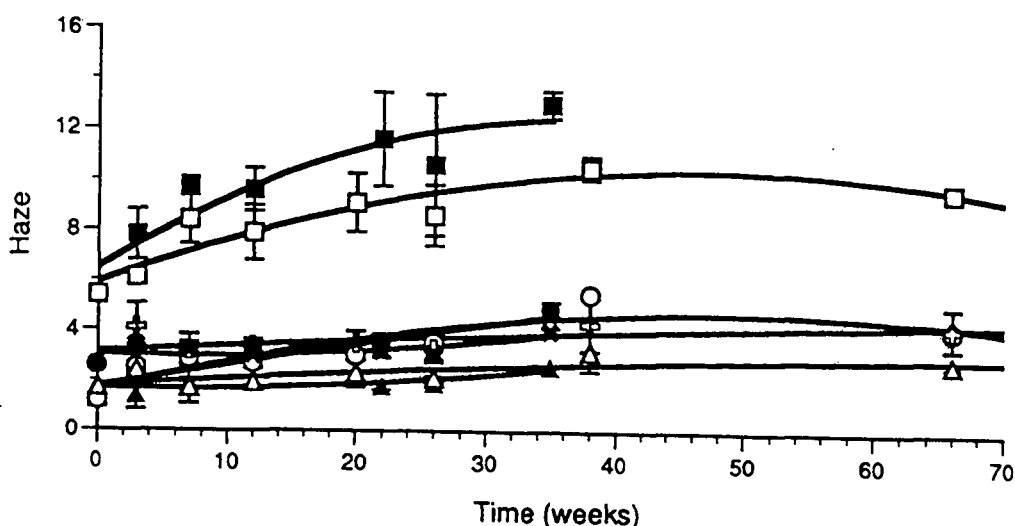
Figure 6. Changes in haze of model juices colored with potato and radish anthocyanins during storage. ■ Potato Juice Concentrate, ▲ Purified Potato Extract, ● Radish Juice Concentrate, ✳ Purified Radish Extract. Fill symbols represent room temperature (25°C) treatments, open symbols represent refrigerated (2°C) treatments. Bars represent the standard deviations, and lines represent the linear or quadratic lines fitted by least squares.

CONCLUSIONS

Color characteristics obtained with radish and potato anthocyanin extracts suggested they could become potential natural alternatives to the use of the artificial dye FD&C Red # 40. Color and pigment degradation greatly depended on storage temperature, with the degradation kinetics following a polynomial model at 25°C and a linear model at 2°C. Anthocyanin degradation was also affected by pigment structure (type of glycoside substituents and acylating groups) and extraction method. At room temperature higher stability was obtained on juices colored with C-18 purified radish anthocyanins (22 wks half-life) and lowest with potato juice concentrate (10 wks half-life). Refrigerated temperatures greatly increases the half-life of the pigment to over a year. Radish anthocyanin extracts showed higher pigment and color stability than red-fleshed potato extracts, with C-18 purified radish anthocyanins having the highest stability. Red radish and red-fleshed potato can impart the desirable orange-red color and adequate stability for different food applications. Model juices colored with radish vegetable juice produced the closest color characteristics to FD&C Red #40. Improved juice processing operations and the use of special concentration techniques (membrane concentration or ultrafiltration) to remove compounds that might accelerate anthocyanin degradation could improve the quality of the pigment extracts, and its stability in food systems.

REFERENCES

Adams, J.B. 1973. Thermal degradation of anthocyanins with particular reference to the 3-glycosides of cyanidin. I. In acidified aqueous solution at 100°C. J. Sci. Food Chem. 24: 747-762.

Andersen, M., Opheim, S., Aksnes, D.W., and Froystein, N.A. 1991. Structure of petanin, an acylated anthocyanin isolated from *Solanum tuberosum*, using homo- and hetero-nuclear two-dimensional nuclear magnetic resonance techniques. Phytochem. Anal. 2: 230-236.

Asen, S.; Stewart, R.N. and Norris, K.H. 1977. Anthocyanin and pH involved in the color of Heavenly Blue morning glory. Phytochem. 16:1118-1119.

Attoe, E.L. and Von Elbe, J.H. 1981. Photochemical degradation of betanine and selected anthocyanins. J. Food Sci. 46:1934-1937.

Bassa, I.A. and Francis, F.J. 1987. Stability of anthocyanins from sweet potatoes in model beverages. J. Food Sci. 52(6): 1753-1754.

Baublis, A.; Spomer, A. and Berber-Jimenez, M.D. 1994. Anthocyanin pigments: Comparison of extract stability. J. Food Sci. 59:1219-1223.

Broulliard, R. 1981. Origin of the exceptional colour stability of *Zebrina* anthocyanin. Phytochem. 20: 143-145.

Brouillard, R. 1983. The *in vivo* expression of anthocyanin colour in plants. Phytochem. 22: 1311-1323.

Cemeroglu, B.; Velioglu, S. and Isik, S. 1994. Degradation kinetics of anthocyanin in sour cherry juice and concentrate. J. Food Sci. 59:1216-1218.

Clydesdale, F.M.; Main, J.H. and Francis, J. 1979. Roselle (*Hibiscus sabdariffa* L.) anthocyanins as colorants for beverages and gelatin desserts. J. Food Prot. 42:204-207.

Dangles, O., Saito, N. and Brouillard, R. 1993. Anthocyanin intramolecular copigment effect. Phytochem. 34:119-124.

Dravingas, G. and Cain, R.F. 1968. Thermal degradation of black raspberry anthocyanin pigments in model systems. J. Food Sci. 33:138-142.

Dziezak, J.D. 1987. Applications of food colorants. Food Technol. 41(4):78-88.

Fabre, C.E.; Santerre, A.L.; Loret, M.O.; Baberian, R.; Pareilleux, A.; Goma, G. and Blanc, P.J. 1993. Production and food applications of *Monascus* ruber. J. Food Sci. 58:1099-1111.

Francis, F.J. 1989. Food colorants: anthocyanins. Crit. Rev. Food Sci. and Nutr. 28(4): 273-314.

Francis, F.J. 1992. A new group of food colorants. Trends in Food Sci. and Technol. 3: 27-31.

Giusti, M.M. and Wrolstad, R.E. 1996a. Characterization of red radish anthocyanins. J. Food Sci. 61: 322-326.

Giusti, M.M. and Wrolstad, R.E. 1996b. Radish anthocyanin extract as a natural red colorant for maraschino cherries. J. Food Sci. 61: 688-694.

Giusti, M.M.; Ghanadan, H. and Wrolstad, R.E. 1998. Elucidation of the structure and conformation of red radish (*Raphanus sativus*) anthocyanins, using one and two dimensional Nuclear Magnetic Resonance techniques. Submitted to J. Agric Food Chem.

Goto, T. 1987. Structure, stability and color variation of natural anthocyanins. Prog. Chem. Org. Nat. Prod. 52:113-158.

Goto, T. and Kondo, T. 1991. Structure and molecular stacking of anthocyanins - Flower color variation. Angew. Chem. Int. Ed. Engl. 30:17-33.

Harborne, J.B. 1960. Plant polyphenols: 1. anthocyanin production in the cultivated potato. Biochem. J. 74: 262-269.

Henry, B.S. 1996. Natural food colours. Ch. 2 In *Natural Food Colorants*. (Ed.) Hendry, G.A.F. and Houghton, J.D. 2nd Ed. Blackie A&P Great Britain.

Jackman R.L. and Smith J.L. 1996. Anthocyanins and Betalains. Ch. 8 In *Natural Food Colorants*. 2nd Ed. G.A.F.Hendry and J.D. Eds. Houghton Blackie A&P Great Britain.

LaBell, F. 1990. Technology diversifies use of natural colors. New cabbage coloring extends stability. Food Processing. 51(4):69-70.

LaBell, F. 1993. Grapes provide brilliant red color. Food Processing. 54(6): 88-89.

Markakis, P. 1992. Stability of anthocyanins in foods. Chp. 6. In *Anthocyanins as food colors*. P. Markakis Ed. Academic Press. NY.

Mazza, G. and Miniati, E. 1993. Introduction. Chp. 1. In *Anthocyanins in fruits, vegetables, and grains*. G. Mazza and E. Miniati Eds. CRC Press, Boca Raton, FL.

McGuire, R.G. 1992. Reporting of objective color measurements. HortScience 27(12):1254-1255.

Murai, K. and Wilkins, D. 1990. Natural red color derived from red cabbage. Food Technol. 44:131

Newsome, R.L. 1986. Food Colors. Food Technol. 40(7):49-56.

Odake, K.; Terahara, N.; Saito, N.; Toki, K. and Honda, T. 1992. Chemical structures of two anthocyanins from purple sweet potato, *Ipomoea batatas*. Phytochem. 31:2127-2130.

Piffaut, B.; Kader, M.; Girardin, M. and Metche, M. 1994. Comparative degradation pathways of malvidin 3,5-diglucoside after enzymatic and thermal treatments. Food Chem. 50:115-120.

Pouget, M.P.; Lejeune, B.; Vennat, B. and Pourrat, A. 1990. Extraction, analysis and study of the stability of Hibiscus anthocyanins. Lebensm. -Wiss. U. -Technol. 23:103-105.

Rodriguez-Saona, L.E.; Giusti, M.M. and Wrolstad, R.E. 1998. Anthocyanin pigment composition of red-fleshed potatoes. Accepted by J. Food Sci.

Sasche, J. 1973. Anthocyane in den kartoffelsorten Urgenta and Desiree. Z. Lebensm. Unters. Forsch. 153: 294-300.

Shi, Z., Bassa, I.A., Gabriel, S.L. and Francis, F.J. 1992a. Anthocyanin pigments of sweet potatoes -*Ipomoea batatas*. J. Food Sci. 57: 755-757, 770.

Shi, Z., Lin, M. and Francis, F.J. 1992b. Anthocyanins of *Tradescantia pallida*. Potential food colorants. J. Food Sci. 57: 761-765.

Shi, Z., Lin, M. and Francis, F.J. 1992c. Stability of anthocyanins from *Tradescantia pallida*. J. Food Sci. 57: 758-759.

Shi, Z.; Francis, F.J. and Daun, H. 1992d. Quantitative comparison of the stability of anthocyanins from *Brassica oleracea* and *Tradescantia pallida* in non-sugar drink model and protein model systems. J. Food Sci. 57:768-770.

Skrede, G.; Wrolstad, R.E.; Lea, P. and Enersen, G. 1992. Color stability of strawberry and black currant syrups. J. Food Sci. 57:172-177.

Teh, L.S. and Francis, F.J. 1988. Stability of anthocyanins from *Zebrina pendula* and *Ipomoea tricolor* in a model beverage. J. Food Sci 53: 1580-1581.

Wiessenborn, D.; Golz, J.; Hanzel, J.; Helgeson, D.; Hettiarachchy, N.; Holm, E. and Lindley, J. 1991. Red food colorant extract derived from purple-hulled sunflower. North Dakota Farm Res. 49:19-21.

Wrolstad, R.E. 1976. Color and pigment analyses in fruit products. Oregon St. Univ. Agric. Exp. Stn. Bulletin 624.

Wrolstad, R.E.; Shrede, G.; Lea, P. and Enersen, G. 1990. Influence of sugar on anthocyanin pigment stability in frozen strawberries. J. Food Sci. 55:1064-1065.

ACKNOWLEDGEMENTS

CHAPTER 8. SUMMARY

The potato tuber composition determines the final chip color characteristics. The role of different potato constituents on chip color was established using potato varieties and model systems. Potato varieties showed that chip color highly correlated with levels of reducing sugars, ascorbic acid, phenolic acids and glutamine. Although we confirmed the major contribution of reducing sugar concentration to the development of chip color, potato varieties and model systems showed that at low reducing sugar content (< 60 mg/100g) other reactants such as glutamine, ascorbic acid and sucrose play an important role. Chlorogenic acid, the major phenolic acid in potato tubers, had no effect on color development and although sucrose affected color quality in model systems, it was not a reliable estimator of chip color quality in potato varieties. Potato breeders rely on reducing sugar levels for selection of new chipping varieties. We have shown that low reducing sugars levels would not ensure low browning if other reactants such as ascorbic acid or glutamine were accumulated.

The use of red-fleshed potato extracts as potential red natural colorants was evaluated using 33 breeding clones. The major anthocyanin (ca 70%) present in red-fleshed potatoes was pelargonidin-3-rutinoside-5-glucoside acylated with p-coumaric acid and two breeding clones, NDOP5847-1 and NDC4069-4, showed high anthocyanin content (>35 mg/100g). The use of potato anthocyanin extracts to color model juices showed that red-fleshed potato can impart the desirable orange-red color, similar to FD&C Red # 40, and adequate stability for different food applications. A comparison between red-fleshed potato anthocyanins and a chemically related pelargonidin-based anthocyanin from radish showed that color and pigment degradation depended on storage temperature, anthocyanin structure and method of pigment extraction. At room temperature higher stability was obtained on juices colored with C-18 purified radish anthocyanins (22 wks half-life) and lowest with potato juice concentrate (10 wks half-life). Refrigeration ($2°C$) greatly increases the half-life of the pigment to over a year. The presence of alkaloids (α-solanine and α-chaconine) was detected in potato pigment

extracts. The levels of steroidal glycoalkaloid in different red-fleshed potato breeding clones ranged from 2 to 36 mg/100g fw tuber, with potato clones that contained the highest anthocyanin content (NDC4069-4 and NDOP5847-1) showing relatively low glycoalkaloid content and high ACN/SGA ratios which encourage their use as natural colorants. The major SGAs in potato tubers, α-solanine and α-chaconine, are readily soluble in water and are concentrated along with the anthocyanins. Alkaline treatment was an effective method to precipitate SGAs, and treatment of the anthocyanin-containing juices with KOH to a final pH of 8.0 precipitates up to 90% of the alkaloids without severe anthocyanin degradation (ca 30%) or changes in pigment profile. These results showed that red-fleshed potatoes could be used as a potential source of natural red colorant providing the right hue, acceptable stability, no undesirable odors and very low alkaloid levels, which make potato anthocyanin extract a suitable alternative to FD&C Red # 40 for food applications.

BIBLIOGRAPHY

Adams, J.B. 1973. Thermal degradation of anthocyanins with particular reference to the 3-glycosides of cyanidin. I. In acidified aqueous solution at 100°C. J. Sci. Food Chem. 24: 747-762.

Aeschbacher, H.U. 1986. Genetic toxicology of browning and caramelization products. In *Genetic toxicology of the diet* - proceedings of a satellite symposium of the 4th international conference of environmental mutagens. Ib Knudsen Ed. Copenhagen, Denmark.

Aeschbacher, H.U. 1990. Anticarcinogenic effect of browning reaction products. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Akhmadieva, A.; Zaichkina, S.I.; Ruzieva, S.I. and Ganassi, E.E. 1993. The protective action of a natural preparation of anthocyan (pelargonidin-3,5-diglucoside). Radiobiologia 33(3):433-435.

Andersen, M., Opheim, S., Aksnes, D.W., and Froystein, N.A. 1991. Structure of petanin, an acylated anthocyanin isolated from *Solanum tuberosum*, using homo- and hetero-nuclear two-dimensional nuclear magnetic resonance techniques. Phytochem. Anal. 2: 230-236.

Anonymous. 1993. Precise color communication. Minolta Camera Co.

Ap Rees, T. and Morrell, S. 1990. Carbohydrate metabolism in developing potatoes. Am. Potato J. 67:835-847.

Artz, W.E.; Pettibone, C.A.; Augustin, J. and Swanson, B.G. 1983. Vitamin C retention of potato fries blanched in water. J. Food Sci. 48:272-273.

Ashoor, S.H. and Zent, J.B. 1984. Maillard browning of common amino acids and sugars. J. Food Sci. 49:1206-1207.

Asen, S.; Stewart, R.N. and Norris, K.H. 1972. Co-pigmentation of anthocyanins in plant tissues and its effect on color. Phytochemistry. 11:1139-1144.

Asen, S.; Stewart, R.N. and Norris, K.H. 1977. Anthocyanin and pH involved in the color of "Heavenly Blue" morning glory. Phytochemistry. 16:1118-1119.

Attoe, E.L. and Von Elbe, J.H. 1981. Photochemical degradation of betanine and selected anthocyanins. J. Food Sci. 46:1934-1937.

Augustin, J.; Johnson, S.R.; Teitzel, C.; Toma, R.B.; Shaw, R.L.; True, R.H.; Hogan, J.M. and Deutsch, R.M. 1978. Vitamin composition of freshly harvested and stored potatoes. J. Food Sci. 40:1295-1299.

Baltes, W. 1990. Roest aroma formation - The role of amino acids during the Maillard reaction. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Bassa, I.A. and Francis, F.J. 1987. Stability of anthocyanins from sweet potatoes in model beverages. J. Food Sci. 52(6): 1753-1754.

Baublis, A.; Spomer, A. and Berber-Jimenez, M.D. 1994. Anthocyanin pigments: Comparison of extract stability. J. Food Sci. 59:1219-1223.

Baxter, J. 1995. Free amino acid stability in reducing sugar systems. J. Food Sci. 60(2):405-408.

Benzig-Purdie, L.M. and Ratcliff, C.I. 1986. A study of the Maillard reaction by ^{13}C and ^{15}N CP-MAS NMR: Influence of time, temperature, and reactants on major products. Dev. Food Sci. 13:193-205.

Bhalerao, S.D. 1993. A factorial approach to identify reactive constituents and their interactions in multicomponent highly acidic model systems simulating lime juice concentrate (32°B). J. Sci. Food Agric. 63(1):91-94.

Bjeldanes, L.F. and Chew, H. 1979. Mutagenicity of 1,2-dicarbonyl compounds: maltol, kojic acid, diacetyl and related substances. Mutat. Res. 67, 367-371.

Bobbio, P.A.; Bobbio, F.O. and Trevisan, L.M.V. 1973. Estudos sobre a reacao de Maillard. I. Efeitos da temperatura e do pH. An. Acad. Brasil. Cienc. 45:419.

Bohart, G.S. and Carson, J.F. 1955. Effect of trace metals, oxygen and light on the glucose-glycine browning reaction. Nature 175:470.

Bomer, A. And Mattis, H. 1924. Der Solaningehalt der Kartoffein. Z. Unters. Nahr. Genussmittel, 47: 97-127.

Bronnun-Hansen, K. and Flink, J.M. 1986. Anthocyanin colorants from Elderberry *(Sambucus nigra* L.) IV. Further studies on production of liquid extracts, concentrates and freeze dried powders. J. Food Technol. 21:605-614.

Brouillard, R. 1981. Origin of the exceptional colour stability of *Zebrina* anthocyanin. Phytochemistry. 20: 143-145.

Brouillard, R. 1982. Chemical structure of anthocyanins. In *Anthocyanins as food colors*. P. Markakis Ed. Academic Press. NY.

Brouillard, R. 1983. The *in vivo* expression of anthocyanin colour in plants. Phytochemistry. 22: 1311-1323.

Brouillard, R.; Mazza, G.; Saad, Z.; Albrecht-Gary, M. and Cheminat, A. 1989. The copigment reaction of anthocyanins: A microprobe for the structural study of aqueous solutions. J. Am. Chem. Soc. 111:2604-2610.

Brown, C.R. 1993. Origin and history of the potato. Am. Potato J. 70:363.

Burton, W.G. 1966. *The Potato*. 2nd edition. Veenman, Wageningen.

Burton, W.G. 1989. *The Potato*. 3rd Edition. Longman Scientific & Technical. England

Burton, W.G. Wilson, A.R. 1978. The sugar content and sprout growth of tubers of potato cultivar Record, grown in different localities, when stored at 10, 2 and 20°C. Potato Res. 21(3):145-161.

Bushway, R.J.; Savage, S.A. and Ferguson, B.S. 1987. Inhibition of acetyl cholinesterase by solanaceous glycoalkaloids and alkaloids. Am. Potato J. 64: 409-413.

Cemeroglu, B.; Velioglu, S. and Isik, S. 1994. Degradation kinetics of anthocyanin in sour cherry juice and concentrate. J. Food Sci. 59:1216-1218.

Chan, R.I. Stich, H.F. Rosin, M.P. and Powrie, W.D. 1982. Antimutagenic activity of browning reaction products. Can. lett. 15, 27-33.

Chiriboga, C.D. and Francis, F.J. 1970. An anthocyanin recovery system from cranberry pomace. J. Am. Soc. Hort. 95:233-236.

Cilliers, J.J.L. Singleton, V.L. 1989. Nonenzymic autoxidative phenolic browning reactions in a caffeic acid model system. J. Agric. Food Chem. 37(4):890-896.

Cilliers, J.J.L. Singleton, V.L. 1990. Nonenzymic autoxidative reactions of caffeic acid in wine. Am. J. Enol. Vitic. 41(1):84-86.

Cilliers, J.J.L. Singleton, V.L.1991.Characterization of the products of nonenzymic autoxidative phenolic reactions in a caffeic acid model system. J. Agric. Food Chem. 39:(7):98-1303.

CIP. 1998. Potato production. In *Post-harvest and marketing*. http://www.cipotato.org/ph&mkt

Claassen, P.A.M.; Budde, M.A.W. and Van Calker, M.H. 1993. Increase in phosphorylase activity during cold-induced sugar accumulation in potato tubers. Potato Res. 36:205-217.

Clydesdale, F.M.; Main, J.H. and Francis, J. 1979. Roselle (*Hibiscus sabdariffa* L.) anthocyanins as colorants for beverages and gelatin desserts. J. Food Prot. 42:204-207.

Clydesdale, F.M.; Main, J.H. and Francis, J. 1979. Cranberry pigments as colorants for beverages and gelatin desserts. J. Food Prot. 42:196-201.

Cooke, J.R. 1974. The chemical estimation of vitamin C. In *Vitamin C*. G.G. Birch and K.J. Parker Eds. Applied Science Publishers, Ltd. London.

Cottrell, J.E.; Duffus, C.M.; Paterson,.L.; Mackay, G.R.; Allison, M.J. and Bain, H. 1993. The effect of storage temperature on reducing sugar accumulation and the activities of three amylolytic enzymes in tubers of the cultivated potato, *Solanum tuberosum* L. Potato Res. 36:107-117.

Damon, G.E. and Janssen, W.F. 1973. Additives for eye appeal. FDA Consumer. July-August.

Danehy, J.P. 1986. Maillard reactions: Nonenzymatic browning in food systems with special reference to the development of flavor. Advances Food Research 30:77-138.

Dangles, O., Saito, N. and Brouillard, R. 1993. Anthocyanin intramolecular copigment effect. Phytochemistry. 34:119-124.

Dao, L. and Friedman, M. 1992. Chlorogenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry. J. Agric. Food Chem. 40:2152-2156.

Dao, L. and Friedman, M. 1994. Chlorophyll, chlorogenic acid, glycoalkaloid and protease inhibitor content of fresh and green potatoes. J. Agric. Food Chem. 42:633-639.

Davies, A.M.C. 1977. The free amino acids of tubers of potato varieties grown in England and Ireland. Potato Res. 20:9-21.

Davies, A.J. and Mazza, G. 1993. Copigmentation of simple and acylated anthocyanins with colorless phenolic compounds. J. Agric. Food Chem. 41:716-720.

Davies, H.V. and Viola, R. 1994. Control of sugar balance in potato tubers. In *The molecular and cellular biology of potato*. 2nd Edition. W.R. Belknap, M.E. Vayda and W.D. Park Eds. CAB International, Wallingford, UK

Dillls, W.L. 1993. Protein fructosylation: fructose and the Maillard reaction. Am. J. Clin. Nutr. 779S-787S.

Dravingas, G. and Cain, R.F. 1968. Thermal degradation of black raspberry anthocyanin pigments in model systems. J. Food Sci. 33:138-142.

Duplessis, P.M.; Marangoni, A.G. and Yada, R.Y. 1996. A mechanism for low temperature induced sugar accumulation in stored potato tubers: the potential role of the alternative pathway and invertase. Am. Potato J. 73:483-494.

Dziezak, J.D. 1987. Applications of food colorants. Food Technol. 41(4):78-88.

Eichner, K. Wolf, W. 1983. Maillard reaction products as indicator compounds for optimizing drying and storage conditions Carrots, varieties. ACS Symposium series - American Chemical Society. 215:317-333.

Einarsson, H. 1987. Lebensm. Wiss. Und Technol. 20:51-55.

Eksari, I. Bonestroo, M.H. Jongen, W.M.F. and Pilnik, W. 1989. Mutagenicity and possible occurrence of flavonol aglycones in heated orange juices. Food Chem. 31(4), 289-294.

Eppendorfer, W.H. and Billie, S.W. 1996. Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower and potatoes as influenced by nitrogen fertilization and phosphorous and potassium deficiency. J. Sci. Food Agric. 71:449-458.

Es, A. van. Hartmans, K.J. 1981. Sugars and starch during tuberization, storage and sprouting. In *Storage of potatoes: post-harvest behaviour, store design, storage practice, handling*. Wageningen, Centre for Agricultural Publ. and Documentation.

Escribano-Bailón, T.; Dangles, O. and Brouillard, R. 1996. Coupling reactions between flavylium ions and catechim. Phytochemistry. 41:1583-1592.

Eskin, N.M. 1990. Biochemistry of food processing: Browning reactions in foods. In *Biochemistry of foods*. Second edition. Academic Press Inc. USA.

Fabre, C.E.; Santerre, A.L.; Loret, M.O.; Baberian, R.; Pareilleux, A.; Goma, G. and Blanc, P.J. 1993. Production and food applications of *Monascus* ruber. J. Food Sci. 58:1099-1111.

Francis, F.J. 1989. Food colorants: anthocyanins. Crit. Rev. Food Sci. and Nutr. 28(4): 273-314.

FDA. 1993. Food Color Facts. Food and Drug Administration (FDA) - International Food Information Council Foundation (IFICF) Brochure. Washington, D.C.

Feather, M.S. 1994. Dicarbonyl sugar derivatives and their role in the Maillard reaction. In *ACS Symposium series: Thermally generated flavors - Maillard, microwave and extrusion processes*. T.H. Parliament, M.J. Morello and R.J. McGorrin Eds. American Chemical Society. Washington DC, USA.

Feeney, R.E. and Whitaker, J.R. 1982. The Maillard reaction and its prevention. In *Food Protein Deterioration*. Am. Chem. Soc. 201-647.

Figueiredo, P.; Elhabiri, M.; Toki, K.; Saito, N.; Dangles, O. and Brouillard, R. 1996. New aspects of anthocyanin complexation. Intramolecular copigmentation as a means for color loss?. Phytochemistry. 41:301-308.

Fitzpatrick, T.J.; Herb, S.F.; Osman, S.F. and McDermott, J.A. 1977. Potato glycoalkaloids: increases and variations of ratios in aged slices over prolonged storage. Am. Potato J. 54: 539-544.

Francis, F.J. 1985. Blueberries as a colorant ingredient in food products. J. Food Sci. 50:754-756.

Francis, F.J. 1987. Lesser-known food colorants. Food Technol 41:62-68.

Francis, F.J. 1989. Food colorants: anthocyanins. Crit. Rev. Food Sci. and Nutr. 28(4): 273-314.

Francis, F.J. 1992. A new group of food colorants. Trends in Food Sci. and Technol. 3: 27-31.

Friedman, M. 1996. Nutritional value of proteins from different food sources. J. Agric. Food Chem. 44:6-29.

Friedman, M. 1997. Chemistry, biochemistry and dietary role of potato polyphenols. J. Agric. Food Chem. 45:1523-1540.

Friedman, M. and Dao, L. 1992. Distribution of glycoalkaloids in potato plants and commercial potato products. J. Agric. Food Chem. 40: 419-423.

Friedman, M. and McDonald, G.M. 1995. Extraction efficiency of various solvents for glycoalkaloid determination in potatoes and potato products. Am. Potato J. 72: 66A.

Friedman, M. and McDonald, G.M. 1997. Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. Crit. Rev. Plant Sci. 16(1): 55-132.

Friend, J., Reynolds, S.B., and Aveyard, M.A. 1985. Phenolic metabolism in potato tuber tissue after infection with Phytophthora infestans. Biochem. J. 124: 29

Fry, L.K. and Stegink, L.D. 1982. Formation of Maillard reaction products in parenteral alimentation solutions. J. Nutrition 112:1631-1637.

Fuller, T.J. and Hughes, J.C. 1984. Factors influencing the relationships between reducing sugars and fry colour of potato tubers of cv. Record. J. Food Technol. 19:455-467.

Furtado, P.; Figueiredo, P.; Cheves das Neves, H. and Pina, F. 1993. Photochemical and thermal degradation of anthocyanidins. J. Photochem. Photobiol. A: Chem. 775:113-118.

Garcia, F. and Cruz-Remes, L. 1993. The natural xanthophyll pigment industry. Presented at the First International Symposium of Natural Colorants for Food, Nutraceuticals, Beverages and Confectionery. Amherst, Massachusetts, Nov 7-10.

Giusti, M.M. and Wrolstad, R.E. 1996a. Characterization of red radish anthocyanins. J. Food Sci. 61(2): 322-326.

Giusti, M.M. and Wrolstad, R.E. 1996b. Radish anthocyanin extract as a natural red colorant for maraschino cherries. J. Food Sci. 61: 688-694.

Giusti, M.M.; Ghanadan, H. and Wrolstad, R.E. 1998. Elucidation of the structure and conformation of red radish (*Raphanus sativus*) anthocyanins, using one and two dimensional Nuclear Magnetic Resonance techniques. Submitted to J. Agric. Food Chem.

Giusti, M. M.; Rodríguez-Saona, L. E.; Baggett J. R.; Reed, G. L.; Durst, R. W. and Wrolstad R. E. 1998. Anthocyanin Pigment Composition of Red Radish Cultivars as Potential Food Colorants. J. Food Sci. 63: 219-224.

Goto, T. 1987. Structure, stability and color variation of natural anthocyanins. Prog. Chem. Org. Nat. Prod. 52:113-158.

Goto, T.; Hoshino, T. and Takase, S. 1979. A proposed structure of commelinin, a sky-blue anthocyanin complex obtained from the flower petals of *commelina*. Tetrahedron Lett. 31:2905-2908.

Goto, T.; Kondo, T.; Imagawa, H. and Miura, I. 1981. Heavenly Blue anthocyanin II. Trans-4-O-(6-O-(trans-3-O-(β-D-glucopyranosyl)caffeyl)-β-D-glucopyranosyl)-caffeic acid, a novel component in Heavenly Blue anthocyanin. Tetrahedron Lett. 22:3213-3216.

Goto, T.; Kondo, T.; Tamura, H. and Imagawa, H. 1982. Structure of gentiodelphin, an acylated anthocyanin isolated from *Gentiana makinoi*, that is stable in dilute aqueous solution. Tetrahedron Lett. 23:3695-3698.

Goto, T.; Kondo, T.; Kawai, T. and Tamura, H. 1984. Structure of Cinerarin, a tetraacylated anthocyanin isolated from the blue garden cineraria, *Senecio cruentus*. Tetrahedron Lett. 25:6021-6024.

Goto, T. and Kondo, T. 1991. Structure and molecular stacking of anthocyanins - Flower color variation. Angew. Chem. Int. Ed. Engl. 30:17-33.

Goto, T.; Tamura, H.; Kawai, T.; Hoshino, N.; Harada, N. and Kondo, T. 1986. Chemistry of metalloanthocyanins. Annals New York Acad. Sci. 471:155-173.

Griffiths, D.W.; Bain, H. and Dale, M.F.B. 1995. Photo-induced changes in the total chlorogenic acid content of potato (Solanum tuberosum) tubers. J. Sci. Food Agric. 68:105-110.

Habib, A.T. and Brown, H.D. 1956. Factors influencing the color of potato chips. Food Technol. 10(7):332-336.

Hagen, S., Augustin, J.,Grings, E. and Tassinari, P. 1993. Pre-column phenylisothiocyanate derivatization and liquid chromatography of free amino acids in biological samples. Food Chem. 46:319-323.

Hall, R.L. 1992. Toxicological burdens and the shifting burden of toxicology. Food Technol. 46: 109-112.

Hallagan, J.B. 1991. The use of certified food color additives in the United States. Cereal Foods World. 36:945-948.

Handwerk, R.L. and Coleman, R.L. 1988. Approaches to the citrus browning problem. J. Agric. Food Chem. 36 (1):231-236.

Harborne, J.B. 1958. The chromatographic identification of anthocyanin pigments. J. Chrom. 1: 473-488.

Harborne, J.B. 1960. Plant polyphenols: 1. anthocyanin production in the cultivated potato. Biochem. J. 74: 262-269.

Harborne, J.B. 1967. Comparative biochemistry of the flavonoids. Academic Press. London.

Harib, A.T. and Brown, H.D. 1956. The effect of oxygen and hydrogen ion concentration on color changes in processed beets, strawberries and raspberries. Proc. Am. Soc. Hort. Sci. 68: 482

Hawkes, J.G. 1990. The Potato - Evolution, Biodiversity and Genetic Resources. Belhaven Press. London, Great Britain.

Hayase, F.; Kim, S.B. and Kato, H. 1986. Analyses of the chemical structures of melanoidins by ^{13}C NMR, ^{13}C and ^{15}N CP-MAS spectrometry. Agric. Biol. Chem. 50:1951-1957.

Hayase, F. and Kato, H. 1986. Low molecular Maillard reaction products and their formation mechanisms. In *Amino-carbonyl reactions in food and biological systems*. Proceedings of the 3rd international symposium on the Maillard reaction. M. Fujimaki, M. Namiki, and H. Kato Eds. Shizuoka. Japan.

Hayase, F. 1996. Scavenging of active oxygen by melanoidins. In *The Maillard Reaction - Consequences for the chemical and life sciences*. Chp. 5. R. Ikan Ed. John Wiley & Sons. West Sussex. Great Britain.

Hayase, F.; Hirashima, S.; Okamoto, G. and Kato, H. 1990. Scavenging of active oxygens by melanoidin. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Hayashi, T.; Hoshi, Y. and Namiki, M. 1984. Purple pigment produced by the reaction of dehydro-L-ascorbic acid with phenylalanine. Agric. Biol. Chem. 48:2377-2378.

Hayashi, T.; Mase, S. and Namiki, M. 1985. Formation of the N,N□-dialkylpyrazine cation radical from glyoxal dialkylimine produced on reaction of a sugar with an amine or amino acid. Agric. Biol. Chem. 49:3131-3137.

Hayashi, T. and Namiki, M. 1980. Formation of two carbon sugar fragment at an early stage of the browning reaction with amine. Agric. Biol. Chem. 44:2575-2580.

Hayashi, T. and Namiki, M. 1981. On the mechanism of free radical formation during browning reaction of sugar with amino compounds. Agric. Biol. Chem. 45:933-939.

Hayashi, T.; Namiki, M. and Tsuji, K. 1983. Formation mechanism of the free radical product and its precursor by the reaction of dehydro-L-ascorbic acid with amino acid. Agric. Biol. Chem. 47:1955-1960.

Hayashi, T. and Namiki, M. 1986. Role of sugar fragmentation in the Maillard reaction. In *Amino-carbonyl reactions in food and biological systems*. Proceedings of the 3rd international symposium on the Maillard reaction. M. Fujimaki, M. Namiki, and H. Kato Eds. Shizuoka. Japan.

Hayashi, T.; Ohta, Y.; Namiki, M. 1977. Electron spin resonance spectral study on the structure of novel free radical products formed by the reactions of sugars with amino acids. J. Agric. Food Chem. 25:1282-1287.

Hayashi, K. and Takeda, K. 1970. Further purification and component analysis of commelinin showing the presence of magnesium in this blue complex molecule. Studies on anthocyanins LXII. Proc. Japan Acad. 46:535-540.

Henry, B.S. 1996. Natural food colours. Ch. 2 In *Natural Food Colorants*. (Ed.) Hendry, G.A.F. and Houghton, J.D. 2nd Ed. Blackie A&P Great Britain.

Herrman, T.J.; Love, S.L.; Shafii, B. and Dwelle, R.B. 1996. Chipping performance of three processing potato cultivars during long-term storage at two temperature regimes. Am. Potato J. 73(9):411-425.

Hettiarachchy, N. 1992. Natural red colorant from sunflower seeds could replace red dye No 40. Industrial Bioprocessing. 14(9):2.

Hill, L.M.; Reimholz, Rroder, R.; Nielsen, T.H. and Stitt, M. 1996. The onset of sucrose accumulation in cold stored potato tubers is caused by an increase rate of sucrose synthesis and coincides with low levels of hexose-phosphates, an activation of sucrose phosphate synthase and the appearance of an new form of amylase. Plant Cell Env. 19:1223-1237.

Ho, C. 1996. Thermal generation of Maillard aromas. In *The Maillard Reaction - Consequences for the chemical and life sciences*. Chp. 5. R. Ikan Ed. John Wiley & Sons. West Sussex. Great Britain.

Hodge, J.E. 1953. Dehydrated foods: chemistry of browning reactions in model systems. Agric. Food Chem. 1(15):929-943.

Hodge, J.E. and Rist, C.E. 1953. The Amadori rearrangement under new conditions and its significance for non-enzymatic browning reactions. J. Am. Chem. Soc. 75:316-322.

Hodge, J.E.; Fisher, B.E. and Nelson, E.C. 1963. Dicarbonyls, reductones and heterocyclics produced by the reactions of reducing sugars with secondary amine salts. Proc. Am. Soc. Brew. Chem. 84-92.

Hoff, J.E.; Jones, C.M.; Wicox, C.E. and Castro, M.D. 1971. The effect of nitrogen fertilization on the composition of the free amino acid pool of potato tubers. Am. Potato J. 48:390-394.

Hong, V. and Wrolstad, R.E. 1990. Use of HPLC separation/photodiode array detection for characterization of anthocyanins. J. Agric Food Chem. 38: 708-715.

Hoshino, T. 1992. Self-association of flavylium cations of anthocyanidin 3,5-diglucosides studied by circular dichroism and ^{1}H NMR. Phytochemistry. 31:647-653.

Hoshino, T.; Matsumoto, U. and Goto, T. 1980. The stabilizing effect of the acyl group on the co-pigmentation of acylated anthocyanins with c-glucosylflavones. Phytochemistry. 19:663-667.

Hoshino, T.; Matsumoto, U. and Goto, T. 1981. Self-association of some anthocyanins in neutral aqueous solution. Phytochemistry. 20:1971-1976.

Hoshino, T.; Matsumoto, U.; Goto, T. and Harada, N. 1982.Evidence for the self-association of anthocyanins IV: PMR Spectroscopic evidence for the vertical stacking of anthocyanin molecules. Tetrahedron Lett. 23:433-436.

Hosokawa, K., Fukunaga, Y., Fukushi, E. and Kawabata, J. 1995. Acylated anthocyanins from red Hyacinthus orientalis. Phytochemistry. 39:1437-1441.

Hosokawa, K. Fukushi, E. Kawabata, J. Fujii, C. Ito, T. and Yamamura, S. 1997. Seven acylated anthocyanins in blue flowers of Gentiana. Phytochemistry. 45(1):167-171.

Hrazdina, G.; Borzell, A. J. and Robinson, W. B. 1970. Studies on the stability of the anthocyanin-3,5-diglucosides. [WINE]. Amer. J. Enol. Vitic. 21(4):201-204.

Hrazdina, G., Iredale, H. and Mattick, L.R. 1977. Anthocyanin composition of Brassica oleracea cv. Red Danish. Phytochemistry. 16: 297-299.

Hughes, J.C. and Fuller, T.J. 1984. The effect of nitrogen during growth on sugar content and fry colour of cv. Record during extended storage at 10°C. Abstracts of conference

papers of the 9th Triennial Conference of the European Association of Potato Research. Hung, C., Murray, J.R., Ohmann, S.M. and Tong, C.B.S. 1997. Anthocyanin accumulation during potato tuber development. J. Amer. Soc. Hort. Sci. 122(1): 20-23.

Hurrel, R.F.; Finot, P.A.; and Cuq, J.L. 1982. Protein-polyphenol reactions. 1. Nutritional and metabolic consequences of the reaction between oxidized caffeic acid and the lysine residues of casein. Br. J. Nutr. 47:191-211.

Hurrel, R.F. and Finot, P.A. 1984. Nutritional consequences of the reactions between proteins and oxidized polyphenols. In Nutritional and toxicological aspects of food safety. M. Friedman Ed. Plenum, NY.

Idaka, E..; Ogawa, T. and Kondo, T. 1987a. Isolation of highly acylated anthocyanins from Commelinaceae plants, Zebrina pendula, Rhoeo spathacea and Setcreasea purpurea. Agric. Biol. Chem. 51:2215-2220.

Idaka, E., Suzuki, K., Yamakita, H., Ogawa, T., Kondo, T. and Goto, T. 1987b. Structure of monoacylated anthocyanins isolated from red cabbage, Brassica oleracea. Chem. Lett. pp 145-148.

Isherwood, F A. 1976. Mechanism of starch-sugar interconversion in Solanum tuberosum [potatoes]. Phytochemistry. 15 (1):33-41.

Isla, M.I. Vattuone, M.A. Sampietro, A.R. 1991. Proteinaceous inhibitor from Solanum tuberosum invertase. Phytochemistry. 30(3):739-743.

Jackman R.L. and Smith J.L. 1996. Anthocyanins and Betalains. Ch. 8 In Natural Food Colorants. (Ed.) Hendry, G.A.F. and Houghton, J.D. 2nd Ed. Blackie A&P Great Britain.

Jackman, R.; Yada, R. and Tung, M. 1987. A review: separation and chemical properties of anthocyanins used for their qualitative and quantitative analysis. J. Food Biochem. 11: 279-308.

Jackson, M.T. 1987. Breeding strategies for True Potato Seed. In The production of new potato varieties. G.J. Jellis and D.E. Richardson Eds. Cambridge University Press. Great Britain.

Jadhav, S.J.; Mazza, G. and Desai, U.T. 1991. Post-harvest handling and storage. In Potato: production, processing, and products. D. K. Salunkhe, S. S Kadam, and S. J. Jadhav Eds. CRC Press. Boca Raton, FL.

Jadhav, S.J.; Sharma, R.D. and Salunkhe, D.K. 1981. Naturally occurring toxic alkaloids in Food. Crit. Rev. Toxicol. 9:21-104.

Jagerstad, M. Grivas, S. Olsson, K. Reutersward, A.L. Negishi, C. and Sato, S. 1986. Formation of food mutagens via Maillard reactions. In *Genetic toxicology of the diet* – proceedings of a satellite symposium of the 4th international conference of environmental mutagens. Ib Knudsen Ed. Copenhagen, Denmark.

Johnson, C.A. 1995. 1995-1996 seed acres reflect more varieties, market shifts. Valley Potato Grower. 61: 13-16.

Johnson, P.E.; Lykken, G.; Mahalko, J.; Milne, D.; Inman, L.; Garcia, W.J. and Inglett, G.E. 1983. The effect of browned and unbrowned corn products on absorption of zinc, iron, and copper in humans. ACS Symp. Series 215:3349-3360.

Jurd, L. 1966. The formation of metal and co-pigment complexes of cyanidin 3-glucoside. Phytochemistry. 5:1263-1271.

Jurd, L. 1972. Some advances in the chemistry od anthocyanin-type plant pigments. In *The chemistry of plant pigments*. Chichester, Co. Academic Press. NY

Kadam, S.S.; Dhumal, S.S. and Jambhale, N.D. 1991. Structure, nutritional composition, and quality. In *Potato: processing, production, and products*. D.K. Salunkhe, S.S. Kadam and S.J. Jadhav Eds. CRC Press. USA.

Kalyanaraman, B., Premovic, P.I. and Sealy, R.C. 1987. Semiquinone anion radicals from addition of amino acids, peptides, and proteins to quinone derived from oxidation of catechols and catecholamines. J. Biol. Chem. 262:11080-11087.

Kamei, H.; Kojima, T.; Hasegawa, M.; Koide, T.; Umeda, T.; Yukawa, T. and Terabe, K. 1995. Suppression of tumor cell growth by anthocyanins in vitro. Cancer Invest. 13(6):590-594.

Karaivanova, M.; Drenska, D. and Ovcharov, R. 1990. A modification of the toxic effects of platinum complexes with anthocyans. Eksp. Med. Morfol. 29(2):19-24.

Kato, H. and Tsuchida, H. 1981. Estimation of melanoidin structure by pyrolysis and oxidation. Prog. Food Nutr. Sci. 5:147-156.

Kato, K.; Watanabe, K. and Sato, Y. 1981. Effect of metals on the Maillard reaction of ovalbumin. J. Agric. Food Chem. 29:540.

Khanbari, O.S. and Thompson, A.K. 1993. Effects of amino acids and glucose on the fry colour of potato crisps. Potato Res. 36:359-364.

Kim, S.B.; Hayase, F. and Kato, H. 1985. Decolorization and degradation products of melanoidins on ozonolysis. Agic. Biol. Chem. 49:785-792.

Kim, S.B.; Hayase, F. and Kato, H. 1986. Desmutagenic effects of melanoidins against amino acid and protein pyrolysates. Dev. Food Sci. 13:383-392.

Kincal, N.S. and Giray, C. Kinetics of ascorbic acid degradation in potato blanching. Intl. J. Food Sci. Technol. 22:249-254.

Kondo, T. Yoshida, K. Nakagawa, A. Kawai, T. Tamura, H. Goto, T. 1992. Structural basis of blue colour development in flower petals from Commelina communis. Nature. 358:515-518.

Koppanyi, T.; Vivino, A.E. and Veitch, Jr., F.P. 1945. A reaction of ascorbic acid with amino acids. Science. 101:541-542.

Kozukue, N.; Kozukue, E. and Mizuno, S. 1987. Glycoalkaloids in potato plants and tubers. HortSci. 22(2): 294-296.

Kramer-Schafhalter, A.; Fuchs, H.; Silhar, S.S.; Kovac, M. and Pfannhauser, W. 1996. Process consideration for extraction from black chokeberry. In *The second international symposium on natural colorants INF/COL II*. The Hereld Organization. Acapulco, Mexico.

Kucza, M. M. 1996. Analysis of flavor precursors in radish and radish color extracts. M.S. thesis. Department of Food Science and Technology. Oregon St. Univer. Corvallis, OR.

Kucza, M. M. and Wrolstad, R.E. 1995. Color and pigment stability of red natural colorants in a model beverage. Memoire de Fin dⒺtudes I.S.A.R.A. Eleve-Ingenieur I.S.A.R.A. Lyon, France.

Kuhnau, J. 1976. The flavonoids. A class of semi-essential food components: Their role in human nutrition. World Rev. Nutr. Diet. 24: 117-191.

Kurata, T.; Fujimaki, M. and Sakurai, 1973. Red pigment produced by the oxidation of dehydro-L-ascorbic acid with amino acid. Agric. Biol. Chem. 37:1471-1477.

LaBell, F. 1990. Technology diversifies use of natural colors. New cabbage coloring extends stability. Food Processing. 51(4):69-70.

LaBell, F. 1993. Grapes provide brilliant red color. Food Processing. 54(6): 88-89.

Labuza, T.P. 1994. Interpreting the complexity of the kinetics of the Maillard reaction. In *Maillard reactions in chemistry, food and health*. The proceedings of the 5th international symposium on the Maillard reaction. T.P. Labuza, G.A. Reineccius, Monnier, V., O'Brien, J. and Baynes, J. Eds. The Royal Society of Chemistry. Cambridge, Great Britain.

Larisch, B. Pischetsrieder, M. and Severin, T. 1996. Reactions of dehydroascorbic acid with primary aliphatic amines including N alpha-acetyllysine. J. Agric Food Chem. 44(7):1630-1634.

Lauro, G.J. 1991. A primer on natural colors. Am. Assoc. Cereal Chemists 36(11): 949-953.

Ledl, F. 1990. Chemical pathway of the Maillard reaction. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Ledl, F.; Fritsch, G.; Hiebl, J.; Pachmayr, O. And Severin, T. 1986. Degradation of Maillard products. In *Amino-carbonyl reactions in food and biological systems*. Proceedings of the 3rd international symposium on the Maillard reaction. M. Fujimaki, M. Namiki, and H. Kato, Eds. Shizuoka. Japan.

Ledl, F. and Schleicher, E. 1990. New aspects of the Maillard reaction in foods and in the human body. Angewandte Chemie 29(6):565-706.

Lee, I.E.; Chuyen, N.V.; Hayase, F. and Kato, H. 1994. Desmutagenicity of melanoidins against various kinds of mutagens and activated mutagens. Biosci. Biotechnol. Biochem. 58:18-23.

Lenoble, R.; Richheimer, S.L.; Bank, V.R.; and Bailey, D.T. 1996. Enhanced stability and improved color of anthocyanins by the use of natural extracts. In *The second international symposium on natural colorants INF/COL II*. The Hereld Organization. Acapulco, Mexico.

Leszczynski, W. 1989. Introduction. In *Potato Science and Technology*. Chp. 1 & 2. G. Lisinska and W. Leszczynski Eds. Elsevier Applied Science. London. Great Britain.

Leszczynski, W. 1989. Potato tubers as a raw material for processing and nutrition. In *Potato Science and Technology*. Chp. 2. G. Lisinska and W. Leszczynski Eds. Elsevier Applied Science. London. Great Britain.

Leszkowiat, M.J.; Baricello, V.; Yada, R.Y.; Coffin, R.H.; Lougheed, E.C. and Stanley, D.W. 1990. Contribution of sucrose to nonenzymatic browning in potato chips. J. Food Sci. 55:281-282.

Lewis, C.E. 1996. Biochemistry and regulation of anthocyanin synthesis in potato and other tuber-bearing *Solanum* species. Ph. D. Thesis. Department of Plant and Plant Microbial Science, University of Canterbury, Christchurch, New Zealand.

Lietti, A.; Cristoni, A. and Picci, M. 1976. Studies on Vaccinium myrtillus anthocyanosides. I. Vasoprotective and anti-inflammatory activity. Arzneim-Forsch. 26(5):829-832.

Lingnert, H. 1990. Development of the Maillard reaction during food processing. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Lingnert, H. and Eriksson, C.E. 1980. Antioxidative Maillard reaction products. I. Products from sugars and free amino acids. J. Food Process. Preserv. 4:161-172.

Linnemann, A.R.; Van Es, A. and Hartmans, K.J. 1985. Changes in the content of L-ascorbic acid, glucose, fructose, sucrose and total glycoalkaloids in potatoes (cv. Bintje) stored at 7, 16 and 28°C. Potato Res. 28:271-278.

Lipman, A.L. 1996. Current regulations for certification exempt color additives in the USA. In *The second international symposium on natural colorants INF/COL II*. The Hereld Organization. Acapulco, Mexico.

Lisinska and W. Leszczynski Eds. Elsevier Applied Science. London. Great Britain.

Lu, T.S.; Saito, N.; Yokoi, M.; Shigihara, A. and Honda, T. 1992. Acylated peonidin glycosides in the violet-blue cultivars of *Pharbis nil*. Phytochemistry. 31:659-663.

Maccarone, E.; Ferrigno, V.; Longo,M.L. and Rapisarda, R. 1987. Effects of light on anthocyanins. Kinetics and photodegradation products in acidic aqueous solution. Ann. Chim. (Rome) 77:409-508.

Malmberg, A. and Theander, O. 1984. Free and conjugated phenolic acids and aldehydes in potato tubers. Swedish J. Agric. Res. 14: 119-125.

Markakis, P. 1992. Stability of anthocyanins in foods. Chp. 6. In *Anthocyanins as food colors*. P. Markakis Ed. Academic Press. NY.

Marmion, D.M. 1991. Handbook of U.S. colorants: Foods, drugs, cosmetics and medical devises. 3rd Edition. John Wiley and Sons, Inc. NY.

Marquez, G. and Añon, M.C. 1986. Influence of reducing sugars and amino acids in the color development of fried potatoes. J. Food Sci. 51:157-160.

Mazza, G. 1983. Correlations between quality parameters of potatoes during growth and long-term storage. Amer. Potato J. 60:145-159.

Mazza, G. and Brouillard, R. 1990. The mechanism of co-pigmentation of anthocyanins in aqueous solutions. Phytochemistry. 29:1097-1102.

Mazza, G. and Miniati, E. 1993. Introduction. Chp. 1. In *Anthocyanins in fruits, vegetables, and grains*. G. Mazza and E. Miniati Eds. CRC Press, Boca Raton, FL.

McCay, C.M.; McCay, J.B. and Smith, O. 1987. The nutritive value of potatoes. In *Potato processing*. Fourth edition. W.F. Talburt and O. Smith Eds. The AVI Publishing Co., Inc. Westport, CT.

McGuire, R.G. 1992. Reporting of objective color measurements. HortSci 27(12):1254-1255.

Meggos, H.N. 1994. Effective utilization of food colors. Food Technol. 48(1):112.

Mok, C. and Hettiarachchy, N.S. 1991. Heat stability of sunflower-hull anthocyanin pigment. J. Food Sci. 56:553-555.

Mondy, N.I. and Gosselin, B. 1989. Effect of irradiation on discoloration, phenols and lipids of potatoes. J. Food Sci. 54:982-984.

Mondy, N.I. and Munshi, C.B. 1993. Effect of maturity and storage on ascorbic acid and tyrosine concentrations and enzymatic discoloration of potatoes. J. Agric. Food Chem. 41:849-852.

Monsalve, A., Powers, J.R. and Leung, H.K. 1990. Browning of dehydroascorbic acid and chlorogenic acid as a function of water activity. J. Food Sci. 55:1425-1428.

Morazzoni, P. and Magistretti, M.J. 1986. Effects of Vaccinium myrtillus anthocyanosides on prostacyclin-like activity in rat arterial tissue. Fitoterapia 57 (1):11-14.

Morris, S.C. and Lee, T.H. 1984. The toxicity and teratogenicity of Solanaceae glycoalkaloids, particularly those of the potato (*Solanum tuberosum*). Food Technol. Australia 36:118-124.

Morris, S.C. and Petermann, J.B. 1985. Genetic and environmental effects on levels of glycoalkaloids in cultivars of potato (*Solanum tuberosum*). Food Chem. 18: 271-282.

Mottram, D.S. 1994. Flavor compounds formed during the Maillard reaction. In *ACS Symposium series: Thermally generated flavors - Maillard, microwave and extrusion*

processes. T.H. Parliament, M.J. Morello and R.J. McGorrin Eds. American Chemical Society. Washington DC, USA.

Mulder, E.G. and Bakena, K. 1956. Effect of nitrogen, phosphorus and magnesium nutrition of potato plants on the content of free amino acids and on the amino acid composition of the protein of the tubers. Plant Soil 7:135-166.

Murai, K. and Wilkins, D. 1990. Natural red color derived from red cabbage. Food Technol. 44:131

Nafisi, K. and Markakis, P. 1983. Inhibition of sugar-amine browning by aspartic and glutamic acids. J. Agric. Food Chem. 31:1115-1117.

Nagels, L.; Van Dongen, W.; De Brucker, J. and De Pooter, H. 1980. High-Performance liquid chromatographic separation of naturally occurring esters of phenolic acids. J. Chrom. 187:181-187.

Nakabayashi, T. and Shibata, A. 1967. Formation of red color by dehydrated food in the presence of ascorbic acid. Nippon Shokuhin Gakkaishi. 14:11-14.

Nakatani, N.; Fukuda, H. and Fuwa, H. 1979. Major anthocyanin in Bolivian purple corn (*Zea mays* L.). Agric. Biol. Chem. 43:389.

Namiki, M. 1988. Chemistry of Maillard reactions: Recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. Adv. Food Res. 32:115-184.

Namiki, M. and Hayashi, T. 1975. Development of novel free radicals during the amino-carbonyl reaction of sugars with amino acids. J. Agric. Food Chem. 23:497-491.

Namiki, M. and Hayashi, T. 1981. Formation of novel free radical products in an early stage of Maillard reaction. Prog. Food Nutr. Sci. 5: 81-91.

Namiki, M. and Hayashi, T. 1983. A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. ACS symposium series 215:21-46.

Newsome, R.L. 1986. Food Colors. Food Technol. 40(7):49-56.

Niederhauser, J.S. 1993. International cooperation and the role of the potato in feeding the world. Am. Potato J. 70:385

Noonan, J.E. 1972. Color additives in Food. In *CRC Handbook of Food Additives*. Chp. 14. 2nd Edition. CRC Press. USA.

Noonan, J. E. 1985. An analysis of the factors affecting the future of FD&C colors. Cereal Foods World. 30:265-267.

Odake, K.; Terahara, N.; Saito, N.; Toki, K. and Honda, T. 1992. Chemical structures of two anthocyanins from purple sweet potato, *Ipomoea batatas*. Phytochemistry. 31:2127-2130.

Ogawa, M. and Uritiani, L. 1979. Tissue browning of potato tubers induced by gamma irradiation. Agric. Biol. Chem. 34:870-877.

Ohtsuka, M.; Kurata, T. and Arakawa, N. 1986. Isolation and characterization of a degradation product derived from 2,3-diketo-L-gulonic acid. Dev. Food Sci. 13:77-84.

Olsson, K. And Roslund, C. 1994. Changes in glycoalkaloid content of potato during long-term storage. In *Abstracts from the Joint Meeting of the Agronomy and Utilization Sections of the European Association of Potato Research*. Villa Real, Portugal, June 24-30 pp 41.

Onyeneho, S.N. and Hettiarachchy, N.S. 1993. Antioxidant activity, fatty acids and phenolic acids compositions of potato peels. J. Sci. Food Agric. 62:345-350.

Onyeneho, S.N. and Hettiarachchy, N.S. 1993. Antioxidant activity, fatty acids and phenolic acid composition of potato peels. J. Sci. Food Agric. 62: 345-350.

O'Brien, J. and Morrissey, P.A. 1989. Nutritional and toxicological aspects of the Maillard browning reaction in foods. Crit. Rev. Food Sci. Nutr. 28(3):211-248.

Palamidis, N. and Markakis, P. 1975. Stability of grape anthocyanin in carbonated beverage. J. Food Sci. 40:1047-1049.

Pandell, A.J. 1983. Mechanism of Fe(III)-catalyzed reaction for pyrocatechase. J. Org. Chem. 48:3908-3912.

Pendharkar, M.B. and Nair, P.M. 1987. Alteration of phenylpropanoid metabolism in gamma irradiated potatoes. Potato Res. 30:589-601.

Pendharkar, M.B. and Nair, P.M. 1995. A comparative study of phenylpropanoid metabolism in gamma irradiated and unirradiated potato tubers. Potato Res. 38:187-198.

Peng, C.Y. and Markakis, P. 1963. Effect of phenolase on anthocyanins. Nature 199:597.

Pham, C.B. Cheftel, J.C. 1990. Influence of salts, amino acids and urea on the non-enzymatic browning of the protein-sugar system. Food Chem. 37 (4):251-260.

Piffaut, B.; Kader, M.; Girardin, M. and Metche, M. 1994. Comparative degradation pathways of malvidin 3,5-diglucoside after enzymatic and thermal treatments. Food Chem. 50:115-120.

Poei-Langston, M.S. and Wrolstad, R.E. 1981. Color degradation in an ascorbic acid - anthocyanin - flavonol model system. J. Food Sci. 46:1218-1222.

Pouget, M.P.; Lejeune, B.; Vennat, B. and Pourrat, A. 1990. Extraction, analysis and study of the stability of Hibiscus anthocyanins. Lebensm. -Wiss. U. -Technol. 23:103-105.

Pressey, R. 1969. Role of invertase in the accumulation of sugars in coldstored potatoes. Am. Potato J. 46(8):291-297.

Price, C.L. and Wrolstad, R.E. 1995. Anthocyanin pigments of Royal Okanogan huckelberry juice. J. Food Sci. 60(2): 369-374.

Pritchard, M.K. and Adam, L.R. 1994. Relationships between fry color and sugar concentration in stored Russet Burbank and Shepody potatoes. Am. Potato J. 71:59-68.

Quattrucci, E. 1988. Heat treatments and nutritional significance of Maillard reaction products. In *Nutritional and toxicological aspects of food processing* - proceedings of an international symposium. Rome, Italy.

Ramamurthy, M.S., Maiti, B., Thomas, P. and Nair, P.M. 1992. High performance liquid chromatography determination of phenolic acids in potato tubers (*Solanum tuberosum*) during wound healing. J. Agric. Food Chem. 40: 569-572.

Rao, N.M. and Rao, M.N. 1972. Effect of non-enzymatic browning on the nutritive value of casein-sugar system. J. Food Sci. Technol. 9:66.

Reeve, R.M.; Hautala, E. and Weaver, M.L. 1969. Anatomy and compositional variation within potatoes. Phenolics, enzymes and other minor components. Am. Potato J. 46:374-386.

Reineccius, G.A. 1990. The influence of Maillard reactions on the sensory properties of foods. In *The Maillard reaction in food processing, human nutrition and physiology*. P.A. Finot, H.U. Aeshbacher, R.F. Hurrell, R. Liardon Eds. Birkhauser Verlag, Switzerland.

Renz, A.; Merlo, L. and Stitt, M. 1993. Partial purification from potato tubers of three fructokinases and three hexokinases which show differing organ and development specificity. Planta 190:156-165.

Reyes, F.R.; Poocharden, B. and Wrolstad, R.E. 1982. Maillard browning reaction of sugar-glycine model systems: changes in sugar concentration, color and appearance. J. Food Sci. 47:1376-1377.

Rice-Evans, C.; Miller, N.J. and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol. Med. 20(7):933-956.

Richardson, T. and Hyslop, D.B. 1985. "Enzymes" from Food Chemistry. O. R. Fennema Ed. Marcel Dekker, Inc. New York.

Rizzi, G.P. 1994. The Maillard reaction in foods. In *Maillard reactions in chemistry, food and health.* The proceedings of the 5th international symposium on the Maillard reaction. T.P. Labuza, G.A. Reineccius, Monnier, V., O'Brien, J. And Baynes, J. Eds. The Royal Society of Chemistry. Cambridge, Great Britain.

Roddick, J.G. and Rijnenberg, A.L. 1987. Synergistic interaction between the potato glycoalkaloids α-solanine and α-chaconine in relation to lysis of phospholipid/sterol liposomes. Phytochemistry. 26: 1325-1328.

Roddick, J.G.; Rijnenberg, A.L. and Weissenberg, M. 1990. Membrane disrupting properties of the steroidal glycoalkaloids solasonine and solamargine. Phytochemistry 29: 1513-1518.

Rodriguez-Saona, L.E.; Giusti, M.M. and Wrolstad, R.E. 1998. Anthocyanin pigment composition of red-fleshed potatoes. J. Food Sci. In Press

Rodriguez-Saona, L.E. and Wrolstad, R.E. 1997. Influence of potato composition on chip color quality. Am. Potato J. 72:87-106.

Roe, M.A., Faulks, R.M. and Belsten, J.L. 1990. Role of reducing sugars and amino acids in fry colour of chips from potatoes grown under different nitrogen regimes. J. Sci. Food Agric. 52:207-214.

Roe, M.A. and Faulks, R.M. 1991. Color development in a model system during frying: Role of individual amino acids and sugars. J. Food Sci. 56:1711-1713.

Rosa, J. 1973. Stability of anthocyanin dye concentrates obtained from black current press-cake. I. Production and storage on concentrates as well as possibilities of application. PR Inst. Lab. Badaw. Przem. Spozyw. 23(2):269-300.

Rosa, J. 1973. Stability of anthocyanin pigment concentrates obtained from black current press cake. II. Studies on the rate of pigment destruction during storage. PR Inst. Lab. Badaw. Przem. Spozyw. 23(3):447-462.

Rumore, M.M.; Strauss, S. and Kothari, A.B. 1992. Regulatory aspects of color additives. Pharm. Technol. 16(3):68-82.

Sakamura, S., Watanabe, S. and Obata, Y. 1965. Anthocyanase and anthocyanin occurring in eggplant: Part III. Oxidative decoloration of the anthocyanin by polyphenol oxidase. Agr. Biol. Chem. 29(3):181-190.

Salunkhe, D.K. and Wu, M.T. 1979. Control of postharvest glycoalkaloids formation in potato tubers. J. Food Prot. 42(6): 519-525.

Sapers, G.M. 1993. Browning of foods: Control by sulfites, antioxidants and other means. Food Technol. 47(10): 75-84.

Sapers, G.M.; Douglas, F.W.; Ziolowski, M.A.; R.L. Miller and Hicks, K.B. 1990. Determination of ascorbic acid, dehydroascorbic acid and ascorbic acid-2-phosphate in infiltrated apple and potato tissue by high performance liquid chromatography. J. Chrom. 503:431-436.

Sapers, G.M.; Jones, S.B. and Maher, G.T. 1983. Factors affecting the recovery of juice and anthocyanin from cranberries. J. Am. Soc. Hort. Sci. 108:246.

Sasche, J. 1973. Anthocyane in den kartoffelsorten Urgenta and Desiree. Z. Lebensm. Unters. Forsch. 153: 294-300.

Schreiber, K. 1968. Steroid alkaloids: the *Solanum* group. In *The alkaloids X - chemistry and physiology*. Eds Manske, R.H.F. Academic Press, NY pp 1-192

Shallenberger, R.S. and Birch, G.G. 1975. Sugar Chemistry. AVI Publishing Co. Inc., Westport, CT.

Shallenberger, R.S., Smith, O. and Treadway, R.H. 1959. Role of the sugars in the browning reaction in potato chips. J. Agric. Food Chem. 7(4):274-277.

Shi, Z., Bassa, I.A., Gabriel, S.L. and Francis, F.J. 1992. Anthocyanin pigments of sweet potatoes -*Ipomoea batatas*. J. Food Sci. 57: 755-757, 770.

Shi, Z.; Francis, F.J. and Daun, H. 1992. Quantitative comparison of the stability of anthocyanins from *Brassica oleracea* and *Tradescantia pallida* in non-sugar drink model and protein model systems. J. Food Sci. 57:768-770.

Shi, Z., Lin, M. and Francis, F.J. 1992. Anthocyanins of *Tradescantia pallida*. Potential food colorants. J. Food Sci. 57: 761-765.

Shi, Z., Lin, M. and Francis, F.J. 1992. Stability of anthocyanins from *Tradescantia pallida*. J. Food Sci. 57: 758-759.

Shih, M.J. and Kuc, J.1974. α and β-Solamarine in Kennebec *Solanum tuberosum* leaves and aged tuber slices. Phytochemistry 13: 997-1000.

Sieczka, J.B. and Maatta, C. 1986. The effects of handling on chip color and sugar content of potato tubers. Am. Potato J. 63:363-372.

Singleton, V.L. 1987. Oxygen with phenols and related reactions in musts, wines and model systems: Observations and practical implications. Am J Enol Vitic 38:69-77.

Sinha, N.K., Cash, J.N. and Chase, R.W. 1992. Differences in sugars, chip color, specific gravity and yield of selected potato varieties grown in Michigan. Am. Potato J. 69:385-389.

Skrede, G.; Wrolstad, R.E.; Lea, P. and Enersen, G. 1992. Color stability of strawberry and black currant syrups. J. Food Sci. 57:172-177.

Smith, H.B. 1927. Chromosome counts in the varieties of *Solanum tuberosum* and allied wild species. Genetics 12: 84-92.

Smith, O. 1968. Potatoes: Production, Storing, Processing. AVI publishing Co. Westport. CO.

Smith, O. 1987. Potato chips. In *Potato processing*. Fourth edition. Van Nostrand Reinhold Company Inc. USA.

Sorensen, E.J. 1992. Specialty potatoes. Amer. Veg. Grower. Jan. 36-39.

Sowokinos, J.R. 1994. Post-harvest regulation of sucrose accumulation in transgenic potatoes: role and properties of potato tuber UDP-glucose pyrophosphorylase. In The molecular and cellular biology of the potato. 2nd Edition. W.R. Belknap, M.E. Vayda and W.D. Park Eds. CAB international. Wallingford, UK

Sowokinos, J.R.; Orr, P.H.; Knoper, J.A. and Varns, J.L. 1987. Influence of potato storage and handling stress on sugars, chip quality and integrity of the starch (amyloplast) membrane. Am. Potato J. 64:213-226.

Sowokinos, J.R. and Preston, D.A. 1988. Maintenance of potato processing quality by chemical maturity monitoring (CMM). Station Bulletin 586. Minnesota Agricultural Experiment Station. University of Minnesota.

Spanos, G.A. and Wrolstad, R.E. 1987. Anthocyanin pigment, nonvolatile acids and sugar composition of red raspberry juice. F. Assoc. Off. Anal. Chem. 70:712-722.

Spanos, G.A. and Wrolstad, R.E. 1990. Influence of variety, maturity, processing and storage on the phenolic composition of pear juice. J. Agric. Food Chem. 38:817-824.

Spark, A.A. 1969. Role of amino acids in non-enzymic browning. J. Sci. Food Agric. 20:308.

Spears, K. 1988. Developments in food colourings: the natural alternative. TIBTECH 6:283-288.

Stanker, L.H.; Kamps-Holtzapple, C. And Friedman, M. 1994. Development and characterization of monoclonal antibodies that differentiate between potato and tomato glycoalkaloids and aglycons. J. Agric. Food Chem. 42: 2360-2366.

Stich, H.F. Rosin, M.P. Wu, C.H. and Powrie, W.D. 1981. Clastogenicity of furans found in food. Cancer letters. 113, 89-95.

Takeda, K. Yanagisawa, M. Kifune, T. Kinoshita, T. Timberlake, C.F. 1994. A blue pigment complex in flowers of Salvia patens. Phytochemistry. 35(5):1167-1169.

Takizawa, Y., Munakata, T., Iwasa, Y. Suzuki, T. and Mitsuhashi, T. 1985. Novel oxidative coupling of monophenols in the system of cupric chloride-Oxygen-Alcohol. J. Org. Chem. 50:4383-4386.

Talburt, W.F. 1987. History of potato processing. In Potato Processing. 4th Edition. W.F. Talburt and O. Smith Eds.The AVI Publishing Co., Inc. Westport, CT.

Talburt, W.F.; Schwimmer, S. and Burr, H.K. 1987. Structure and chemical composition of the potato tuber. In Potato processing. Fourth edition. Van Nostrand Reinhold.

Talburt, W.F.; Schwimmer, S. and Burr, H.K. 1987. Structure and chemical composition of the potato tuber. In Potato processing. 4th Edition. W.F. Talburt and O. Smith Eds.The AVI Publishing Co., Inc. Westport, CT.

Talley, E.A. and Porter, W.L. 1970. Chemical composition of potatoes. Relationship of the free amino acid concentrations to specific gravity and storage time. Am. Potato J. 47:214-224.

Tamura, H. and Yamagami, A. 1994. Antioxidative activity of mono-acylated anthocyanins isolated from Muscat Bailey A grape. J. Agric. Food Chem. 42:1612-1615.

Teh, L.S. and Francis, F.J. 1988. Stability of anthocyanins from *Zebrina pendula* and *Ipomoea tricolor* in a model beverage. J. Food Sci. 53: 1580-1581.

Timberlake, C.F., 1988. The biological properties of anthocyanin compounds. NATCOL quarterly bulletin.

Timberalke, C.F. and Bridle, P. 1982. Distribution of anthocyanins in food plants. Chp. 5. In *Anthocyanins as food colors*. P. Markakis Ed. Academic Press. NY.

Timberlake, C.F. and Henry, B.S. 1988. Anthocyanins as natural food colorants. Prog. Clin. Biol. Res. 280:107-121.

Tingey, W.M. 1984. Glycoalkaloids as pest resistance factors. Am. Potato J. 61: 157-167.

Trevanion, S.J. and Kruger, N.J. 1991. Effect of temperature on the kinetic properties of pyrophosphate: fructose 6-phosphate phosphotransferase from potato tuber. J. Plant Physiol. 137:753-759.

Uppal, D.S. 1987. Varietal and environmental effect on the glycoalkaloid content of potato (*Solanum tuberosum* L.). Plant Foods Hum. Nutr. 37: 333-340.

Valkonen, J.; Keskitalo, M.; Vasara, T. and Pietila, L. 1996. Potato glycoalkaloids: a burden or a blessing? Crit. Rev. Plant Sci. 15(1): 1-20.

Van Gelder, W.M.J. 1991. Chemistry, toxicology, and occurrence of steroidal glycoalkaloids: potential contaminants of the potato (*Solanum tuberosum* L.). In *Poisonous plant contamination of edible plants*. Eds Abbel-Fattah M. Rizk. CRC Press, Boca Raton, FL pp 117-156.

Van Gelder, W.M.J. 1991. Chemistry, toxicology, and occurrence of steroidal glycoalkaloids: potential contaminants of the potato (*Solanum tuberosum* L.). In *Poisonous plant contamination of edible plants*. Eds Abbel-Fattah M. Rizk. CRC Press, Boca Raton, FL pp 117-156.

Verma, S.C., Purohit, L.K., Sharda, R.T., Purohit, A.N. and Upadhya, M.D. 1972. Anthocyanin in dark- and light-grown sprouts of potato. Potato Res. 15: 166-169.

Vernin, G. and Parkanyi, C. 1982. Mechanism of formation of heterocyclic compounds in Maillard and pyrolysis reactions. In *Chemistry of heterocyclic compounds in flavours and aromas*. G. Vernin Ed. Ellis Horwood Limited. Chichester, England.

Wang, H.; Cao, G. and Prior, R.L. 1997. Oxygen radical absorbing capacity of anthocyanins. J. Agric. Food Chem. 45:304-309.

Wang, T.S.C., Chen, J. and Hsiang, W. 1985. Catalytic synthesis of humic acids containing various amino acids and dipeptides. Soil Sci. 140:3-10.

Weisburger, J.H. 1994. Specific Maillard reactions yield powerful mutagens and carcinogens. In *Maillard reactions in chemistry, food and health*. The proceedings of the 5th international symposium on the Maillard reaction. T.P. Labuza, G.A. Reineccius, Monnier, V., O'Brien, J. and Baynes, J. Eds. The Royal Society of Chemistry. Cambridge, Great Britain.

Whitfield, F.B. 1992. Volatiles from interactions of Maillard reactions and lipids. Crit. Rev. Food Sci. Nutr. 31(1/2):1-58.

Wiessenborn, D.; Golz, J.; Hanzel, J.; Helgeson, D.; Hettiarachchy, N.; Holm, E. and Lindley, J. 1991. Red food colorant extract derived from purple-hulled sunflower. North Dakota Farm Res. 49:19-21.

Wiessenborn, D.; Golz, J.; Hanzel, J.; Helgeson, D.; Hettiarachchy, N.; Holm, E. and Lindley, J. 1991. Red food colorant extract derived from purple-hulled sunflower. North Dakota Farm Res. 49:19-21.

Wiessenborn, D.; Zbikowski, Z.; Glowacki, A. and Hettiarachchy, N. 1994. Bench-scale processing of red-colored extracts from sunflower hulls. Trans. ASAE. 37:515-520.

Williams, M. and Hrazdina, G. 1979. Anthocyanins as food colorants: Effect of pH on the formation of anthocyanin-rutin complexes. J. Food Sci. 44:66-68.

Wills, R.B.H. 1987. Composition of Australian fresh fruit and vegetables. Food Technol. Aust. 39:523-526.

Wills, R.B.H. and Silalahi, J. 1990. Effect of storage temperature on loss of vitamin C in potato crisps. J. Sci. Food Agric. 53:275-278.

Wolfrom, M.L.; Kashimura, N. and Horton, D. 1974. Factors affecting the Maillard browning reaction between sugars and amino acids. Studies on the nonenzymic browning of dehydrated orange juice. J. Agric. Food Chem. 22:796-800.

Wong, J.W. and Shibamoto, T. 1996. Genotoxicity of Maillard reaction products. In *The Maillard Reaction - Consequences for the chemical and life sciences*. Chp. 7. R. Ikan Ed. John Wiley & Sons. West Sussex. Great Britain.

Wrolstad, R.E. 1976. Color and pigment analyses in fruit products. Oregon St. Univ. Agric. Exp. Stn. Bulletin 624.

Wrolstad, R.E. 1997. Colorants. In Food chemistry: principles and applications. Ed. G.L. Christen. AFNC Press, Cutten, CA. In press.

Wrolstad, R.E., Culbertson, J.D., Cornwell, C., and Mattick, L.R. 1982. Detection of adulteration in blackberry juice concentrates and wines. J. Assoc. Off. Anal. Chem. 65: 1417-1423.

Wrolstad, R.E., Hong, V., Boyles, M.J. and Durst, R.W. 1995. Use of anthocyanin pigment analyses for detecting adulteration in fruit juices. In Vol. I *Methods to detect adulteration of fruit juice and beverages*. S. Nagy and R.L. Wade (Ed.). AgScience Inc., Auburndale, FL.

Wrolstad, R.E.; Shrede, G.; Lea, P. and Enersen, G. 1990. Influence of sugar on anthocyanin pigment stability in frozen strawberries. J. Food Sci. 55:1064-1065.

Wu, M.T. and Salunkhe, D.K. 1976. Changes in glycoalkaloid content following mechanical injuries to potato tubers. J. Am. Soc. Hort. Sci. 101: 329-331.

Wunsch, A. And Munsert, M. 1994. Effect of storage and cultivar on the distribution of glycoalkaloids in potato tuber. Potato Res. 37: 3-10.

Yamaguchi, T. and Nakagawa, K. 1983. Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivatives. Agr. Biol. Chem. 47, 2461-2465.

Yaylayan, V.A. and Forage, N.G. 1992. A kinetic model for the reaction of tryptophan with glucose and mannose - the role of diglycation in the Maillard reaction. Food Chem. 44:201-208.

Yaylayan, V.A. and Hughes-Despointes, A. 1994. Chemistry of Amadori rearrangements products: analysis, synthesis, kinetics, reactions and spectroscopic properties. Crit. Rev. Food Sci. Nutr. 34(4): 321-369.

Yen, G.C. and Hsieh, P.P. 1994. Mechanism of antimutagenic effect of Maillard reaction products prepared from xylose and lysine. In *Maillard reactions in chemistry, food and health*. The proceedings of the 5th international symposium on the Maillard reaction. T.P. Labuza, G.A. Reineccius, Monnier, V., O'Brien, J. And Baynes, J. Eds. The Royal Society of Chemistry. Cambridge, Great Britain.

Yen, G.C. Tsai, L.C. and Lii, G.C. 1992. Antimutagenic effect of Maillard browning products obtained from amino acids and sugars. Food Chem. Toxicol. 30, 127-132.

Yen G.C. and Tsai, L.C. 1993. Antimutagenicity of a partially fractionated Maillard reaction product. Food Chem. 47, 11-15.

Yoshitama, K. 1977. An acylated delphinidin 3-rutinoside-5,3',5'-triglucoside from *Lobelia erinus*. Phytochemistry. 16:1857-1858.

Zhang, L.; Porter, G.A. and Bushway, R.J. 1997. Ascorbic acid and glycoalkaloid content of Atlantic and Superior potato tubers as affected by supplemental irrigation and soil amendments. Am. Potato J. 74:285-304.

Zucker, M. 1963. Influence of light on synthesis of protein and of chlorogenic acid in potato tuber tissues. Plant Physiol. 38:575-580.