

PRODUCTION OF AN ANTHOCYANIN-RICH VEGETABLE JUICE
CONCENTRATE FROM CULL RED RADISHES FOR USE AS A FOOD
COLORANT

THESIS

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By

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Abstract

Anthocyanins are widely distributed compounds in nature that serve a dual purpose: they produce a wide array of vibrant colors and they possess potent antioxidant properties.

The ability to harvest the natural rich colors of anthocyanins as a potential replacement for synthetic dyes such as FD&C Red No. 40 will fulfill the modern consumer's demand for a more natural and healthful product. Anthocyanins present in red radish skins provide color intensity, under acidic conditions, similar to that of FD&C Red No. 40 and could provide a useful tool for the food industry. Additionally, radish anthocyanins exhibit higher stability than most other anthocyanin extracts.

The objective of this work was to harvest an anthocyanin-rich natural colorant from cull radishes, a waste product, in order to produce a high quality product free of undesirable aromas that provides color and added value suitable for food applications.

From cull or premium radish skins, the color was extracted by abrasive action or steam to produce a colored juice. Filtration, evaporation, salt and acid addition and heat were evaluated as means of removing undesirable aromas and flavors or minimize their

formation during processing. Samples were analyzed using UV-vis spectroscopy, HPLC and sensory analyses.

Premium and cull radishes produced very similar products. A chemical extraction of red radish skins yielded 107mg pelargonidin-3-glucoside equivalents (pg-3-glu eq)/100g of skin. Abrasive peeler effectively extracted pigments from cull radishes, salt and acid significantly decreased aroma formation, but created the new challenge of salt removal. Nano-filtration reduced the anthocyanin yield from 83.6mg/100g skin to 72.6mg pg-3-glu eq/100g of skin. Concentration of the juice in a rotary evaporator for 1-10 min helped decrease aroma intensity and produced a juice with 224-206mg pg-3-glu equivalents/L. Steam was effective at safely removing between 25-50% of the anthocyanins from the skins while preventing aroma and flavor formation.

A sensory panel found the quality of color to be comparable to FD&C Red No. 40, and found that samples extracted with salt, acid or steam had lower aroma intensity.

Despite the lower pigment yields, the steam extraction method was preferred because of its simplicity, and quality of the final product. Additionally, the product being created is from a waste material naturally generated by the radish company. This waste can be turned into a high quality food colorant. Using this resource potentially increases the sustainability of a product and the land by making the end product more cost effective for the business, while providing consumers with a healthy alternative to synthetic dyes.

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Chapter 1: Literature Review

1.1 Color and Its Importance:

Color, flavor, and texture are three characteristics that determine food acceptance, but color has a greater impact on our judgment of a particular product (Wrolstad and Smith 2010). Color is a visual sensation/perception that allows one to differentiate between various objects (Wrolstad and Smith, 2010); for instance, the difference between an unripe (green) and ripe (yellow) lemon. This sensation is experienced by an observer when photons from the visible spectrum reflect off an object and enter the retina, as seen in the figure below.

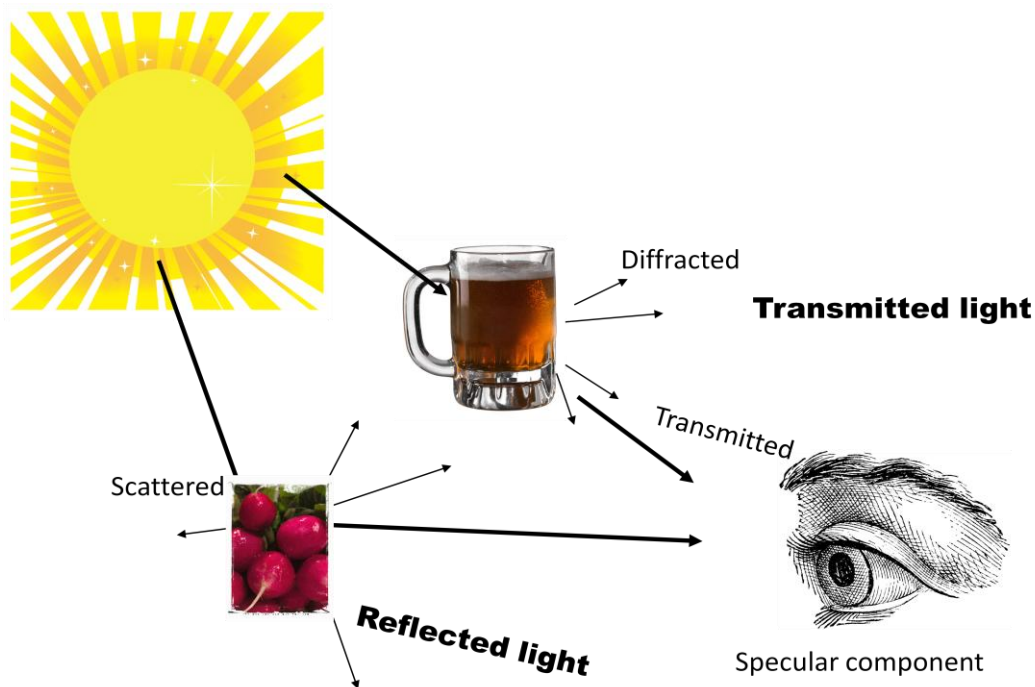


Figure 1.01: A Source, Object, and Observer are Needed to See Color.

Thus, the color of a food may act as a “fingerprint” by displaying the potential quality of the item to a consumer. For instance, whether the food is under or over ripe, potentially flavorful, its potential quality, and possible nutrient content (Mateus and de Freitas, 2009). Moreover, color is the first characteristic of food and beverages to be recognized and is positively correlated to standards of quality by consumers (Mateus and de Freitas, 2009). In other words, it is the physical appearance of food that affects our liking and initial impression of a particular product (Lyman, 1989). Zellner et al. (1991) showed that color has an influence on whether consumers like a flavored beverage or not and how the color and perceived taste is correlated. Consumers generally expect the flavor/ripeness of the food to match the color (Mateus and de Freitas, 2009). For instance, limes with green, cherries with red, carrots with orange, etc. Thus, color helps provides identity to a product. Color is thus added to food products for a multitude of reasons: (1) to replace lost color after processing, (2) enhance the already present color, (3) to minimize batch variations, and (4) to color uncolored materials (Mortensen, 2006).

There are many subclasses of food colorants and these are organized based on their respective source (Mateus and de Freitas, 2009; Mortensen, 2006) and include:

- a) Natural colors: These are derived from natural edible sources such as caramel, betanins and anthocyanins.

- b) Nature identical colors: These are manufactured via chemical synthesis and are identical to their natural counterparts; for instance, riboflavin and β -carotene.
- c) Synthetic colors: These are colors that are not naturally found in nature and are produced via chemical synthesis; both carmoisine and tartrazine are examples.
- d) Inorganic colors: These are manufactured or mined and include titanium dioxide, gold, and silver.

1.2 History of Colorants in Foods and Certification

Color plays such a central role in the appearance and/or liking of food products. Because of this fact man has been using colorants, both natural and artificial, since ancient times (Sharma et al., 2011). Color adulteration was a common practice; for instance, toxic and brightly colored inorganic salts such as mercury sulfide, copper sulfate, and lead chromate were used in jams, butter, and candy to improve a foods appearance (McKone, 1991). It is believed that in the mid 19th century one could not purchase food or drink that had not been adulterated in some fashion; for instance, lead chromate (yellow) was used to correct the blue hue of watered down milk (Sharma et al., 2011). However, detection of inorganic salt colorants by balance and the microscope helped consumers detect what was in their food (Sharma et al., 2011). Organic coal tar dyes soon became popular due the variety of colors they could provide (Garfield, 2000). Yet, there was concern over these colorants and other food additives; which gave way to the Pure Food and Drug Act of 1906 (Sharma et al., 2011). One of the aspects of the act was to thwart

the use of dyes, chemicals, and/or other ingredients that make the product unfit for consumers (Sharma et al., 2011). Initially, seven coal tar dyes were considered safe or certified for food matrixes, as seen in table 1.01.

Common Name	FDA Name
Ponceau 3R	FD&C Red No. 1
Amaranth	FD&C Red No. 2
Erythrosine	FD&C Red No. 3
Indigotine	FD&C Blue No. 2
Light Green SF	FD&C Green No. 2
Naphthol Yellow	FD&C Yellow No. 1
Orange 1	FD&C Orange No. 1

Table 1.01: Food, Drug, and Cosmetic (FD&C) Dyes Recognized as Safe by the U.S. FDA in 1906 (Adapted from Sharma et al., 2011)

According to the FDA, “Color Certification is a congressionally mandated program for FDA enforcement of specifications by batch certification of the certifiable color additives that are added to foods, drugs, cosmetics, and medical devices in the United States. Under this program, the color additive manufacturers provide FDA's Color Certification Branch, CCB, with a representative sample of each color additive batch. CCB analyzes the color additive sample to ensure that it meets the specifications listed in the Code of Federal Regulations (21 CFR Part 74), which identify the certifiable color additives.”

Natural colorants are exempt from this certification process since they have been consumed by people for years without any complications. However, the law does not allow these products known as “juices” to be called “natural colorants.” For instance, either fruit (21 CFR 73.250) or vegetable (21 CFR 73.260) juice color are permitted;

anthocyanins from either of these sources are thus permitted (Rodriguez-Saona et al., 2000). Additionally, dried extracts are under this umbrella, but the extracting solvent must be water (Rodriguez-Saona et al., 2000).

Over time the list of approved dyes has been amended for the safety of consumers; both FD&C Red No. 2 and FD&C Red No. 4 are two examples of colors that have been decertified (Hopkins, 1976). The colors that are now certified and their overall prevalence in the US are below in table 1.02. However, consumers have favored more natural colorants in recent years since they may not produce undesired effects (Mateus and de Freitas, 2009).

Dye	Mass, kg	% of Total
Blue 1	240,654	4.1
Blue 2	208,646	3.6
Green 3	5,739	0.1
Red 3	70,746	1.4
Red 40	2,330,576	40
Yellow 5	1,558,756	26.7
Yellow 6	1,406,060	24.1
Totals	5,830,177	100

Table 1.02: Comparison of Quantities of FD&C Dyes Certified for Use in the U.S. by the FDA in 2007 (Adapted from Sharma et al., 2011)

1.3 Anthocyanins:

1.3.1 Anthocyanin Background

Anthocyanins are a class of water soluble pigments that are responsible for the red, purple, and blue colors dispersed in various flowers, fruits, vegetables, cereals, roots, etc

(Mateus and de Freitas, 2009). The word anthocyanin was derived from the Greek words: anthos and kyanos, anthos meaning flower and kyanos meaning dark blue (Delgado-Vargas et al., 2003). They are found in various flowers, fruits, vegetables, cereals, roots, etc (Mateus and de Freitas 2009). People have consumed anthocyanins for centuries without adverse reactions and anthocyanins can be incorporated into food systems easily due to their water solubility (Giusti and Wrolstad, 2003).

Anthocyanins are believed to be important in the sense that they attract pollinators due to their bright colors and protect the plant from ultraviolet radiation, which can cause damage to the DNA, proteins, and various membranes of the plant (Vanderauwera et al., 2005). There has been more interest in anthocyanins due to their potential as potent antioxidants, antibacterial agents, and natural food colorants (Kang et al., 2003).

Anthocyanins are sensitive to their respective environment. When exposed to heat, light, pH changes, and other factors they have the potential to degrade, which is a limiting factor for the molecule when attempting to incorporate into food systems.

1.3.2 Anthocyanin Chemical Structure

The aglycons of anthocyanins are made up of eight conjugated double bonds forming a C-6 (A ring)-C-3 (C ring)-C-6 (B ring) carbon skeleton as seen in figure 1.02 (He and Giusti, 2009).

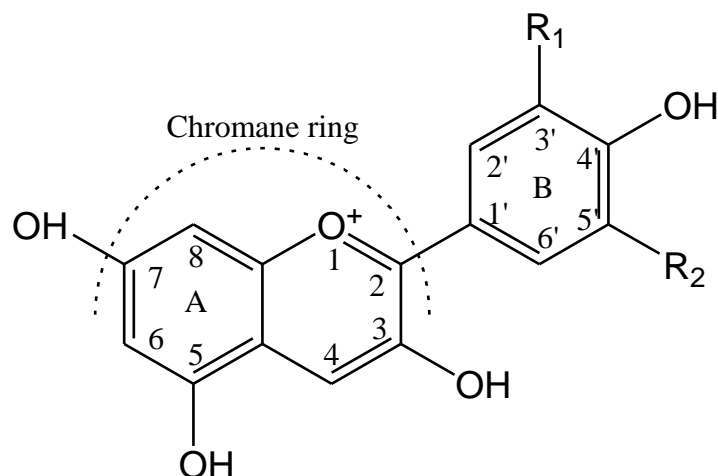


Figure 1.02: Basic Chemical Structure of a Common Anthocyanidin
(Adapted from He and Giusti, 2009).

On the B ring there are two R subunits: R-1 and R-2 respectively. These are substituted with oxymethyl, hydroxyl, and hydryl groups. These different combinations in the R-groups contribute to make the six common anthocyanidin chromophores: cyanidin (Cy), delphinidin (Dp), malvidin (Mv), pelargonidin (Pg), peonidin (Pn), and petunidin(Pt) (He and Giusti 2009). The color of the various alglycones will be dependent on the subunits (table 1.03); the more methoxyl groups will produce a more red color while hydroxyl groups will produce a more blue color (Delgado-Vargas et al., 2003).

Name	R ₁	R ₂	Color	λ_{\max} (nm) in HCl acidified MeOH
Pelargonidin	H	H	Red	520
Cyanidin	OH	H	magenta	535
Peonidin	OCH ₃	H		532
Malvidin	OCH ₃	OCH ₃	Purple	542
Delphinidin	OH	OH		546
Petunidin	OCH ₃	OH		543

Table 1.03: Differences in the Chemical Structure, Color and λ_{\max} of the Six Most Common Anthocyanins (Source: He and Giusti, 2009).

1.3.1 The Effect of Varying pH on Anthocyanin Structure

The structures of anthocyanins allow them to easily reverse confirmation when exposed to varying pH conditions. This contributes to the four major anthocyanin forms that exist in equilibrium: the red flavylum cation, the blue quinonoidal base, the colorless carbinol pseudobase, and the colorless chalcone (Brouillard and Delaporte, 1977). Thus, the pH of the environment has a tremendous effect on the color the anthocyanin displays. For instance, in a highly acidic environment, pH 1.0, a bright red to purple color will appear due to the oxonium form present. An anthocyanin under these ideal stabilizing conditions has the potential to be used in food applications as natural colorants. At pH 4.5, the anthocyanin appears colorless due to the hemiketal state it is in. At pH 7.0, there is a blue to green color based on the anthocyanin being in its quinonoidal base form. These changes are seen in figure 1.03 below.

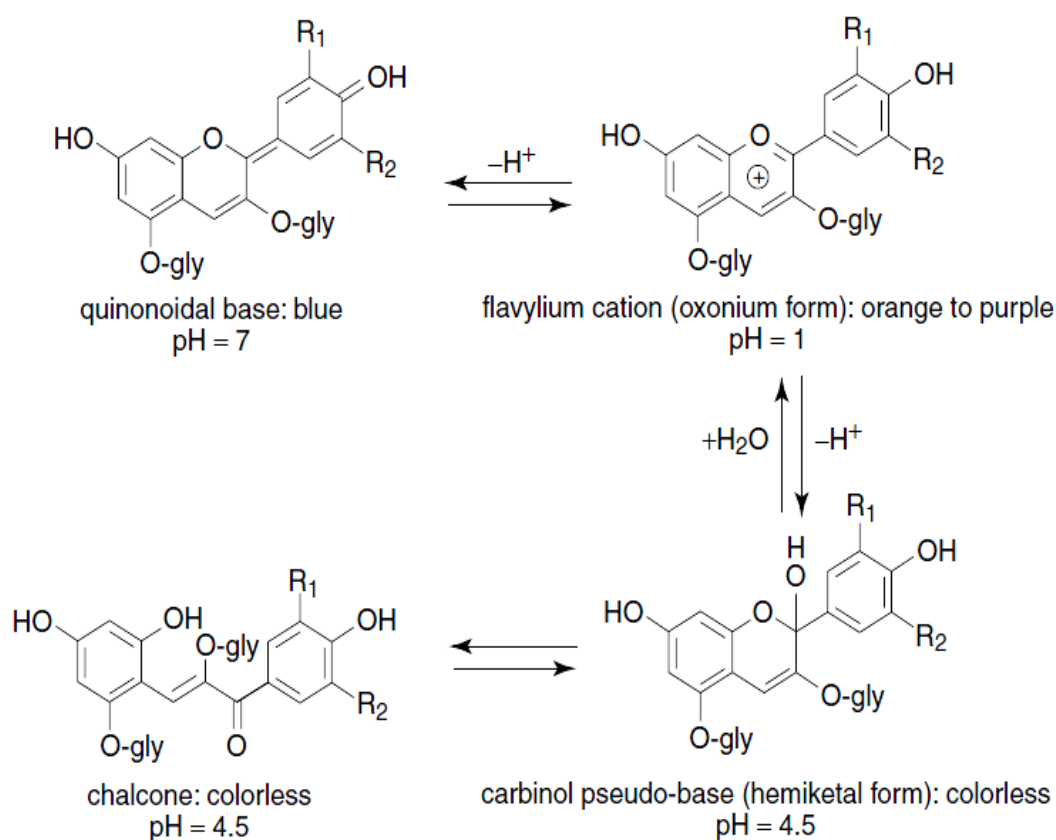


Figure 1.03: Anthocyanin Chemical Structure in Various pH Environments
(Source: Dangles et al., 1993b).

1.3.4 Glycosylation and Acylation

Anthocyanins show increased stability at low pH and when in complex with sugars, glycosylation, and acids, acylation (Giusti and Wrolstad, 2003). The hydroxyl groups present on the anthocyanin aglycone may be bound to one or more sugar moieties via glycosidic bonds; the sugars may then be acylated with various organic acids (aliphatic and cinnamic acids) via ester bonds (Giusti and Wrolstad, 1996a). Due to stability issues the aglycone form is rarely found in nature; instead, anthocyanins are generally found to be bound to acylated sugars. The presence of the glycosylation and acylation,

anthocyanins are more resistant to pH, heat, light, and oxidative stress; thus showing greater resistance to degradation (Manach et al., 2004). The presence of these groups changes both the size of the molecule and the polarity. Increased glycosylation increases the polarity and the affinity for water; while acylation reduces the polarity and affinity for water. The presence of both glycosylation and acylation contribute to the stability of the anthocyanin molecule by forming an intramolecular H-bonding network (Giusti and Wrolstad, 2003). There are many derivatives of anthocyanins because they have the potential to have various types of attached sugars and acyl substituents.

Glycosidic linkage is most common at the 3 and 5 positions, but there may be additional sugar substituents attached to other positions on the aglycon (Wrolstad et al., 2004). The most commonly attached sugars are rhamnose and glucose derivatives, but galactose, arabinose, xylose, and sophorose were found as well (He and Giusti, 2009). Glucosides of cyanidin, delphinidin, and pelargonidin are the most common anthocyanins (Borkowski et al., 2005). Previous work has shown that anthocyanins linked to a sugar moiety at both the 3 and 5 position impact both the spectral characteristics of the anthocyanin as well as shifting to a blue color (Giusti and Wrolstad, 1996a).

Additionally, it has been documented that glycosylation at the 3 position caused an increased spectral absorbance versus glycosylation at the 5 and both the 3 and 5 positions respectively (Giusti and Wrolstad, 1996a).

There are various acylating groups that can attach to their respective glycosylated aglycones; including cinnamic and/or aliphatic acids. Cinnamic acids are composed of

an aromatic ring and include p-coumaric, ferulic, caffeic, gallic, and sinapic acids.

Aliphatic acids are generally straight or branched carbon chains and include malonic, malic, succinic, oxalic, and acetic acids. These acylations are commonly bound to the C-3 sugar or esterified to the 6-OH position on the sugar moiety, as seen in figure 1.04.

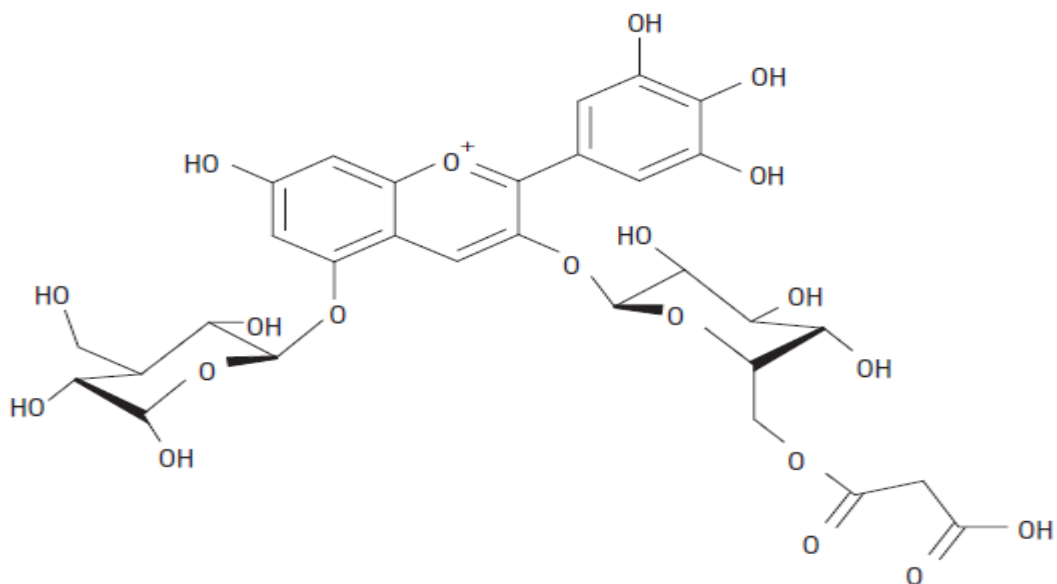


Figure 1.04: Complex Chemical Structure of a Delphinidin Acylated Anthocyanin
(Source: Barczak, 2005)

Acylation plays a central role in the color of a pigment; since cinnamic acids cause both a bathochromic shift and a hyperchromic effect (Giusti et al., 1999). A bathochromic shift indicates an increase in the visible absorption maximum (nm) and while a hyperchromic shift indicates an increase in peak intensity (mAU) (Wallace and Giusti, 2008).

Acylation also improves both color and tinctorial strength. Additionally, they help stabilize anthocyanins in stressful environments such as extreme pH, heat, and light (Anderson et al. 2005). These benefits give acylated anthocyanins the potential to be incorporated into various food systems; especially as natural food colorants. High

amounts of acylated anthocyanins exist in red onions, red cabbage, red potatoes, radishes, purple potatoes, black carrots, and grapes. Of these samples both red radishes and red potatoes stand out in the fact that they are very stable and may be possible alternatives to FD&C Red No. 40 (Giusti and Wrolstad, 2003).

The increased color stability of anthocyanins is due to the many structural variations; including the glycosylation, hydroxylation, and methoxylation of the B-ring and the presence of acylated groups (Giusti et al., 1999). Glycosylation increases molecular stability by allowing intramolecular H-bonding to occur between the anthocyanin and the sugar moiety (Giusti and Wrolstad, 2003). Acylation allows for copigmentation, stability through intermolecular and intramolecular interactions, to occur (Harborne and Williams, 2000). The C-ring, the electrophilic center, can react with nucleophiles during copigmentation (Jing, 2006). This type of intermolecular and intramolecular stacking is pictured in figure 1.05.

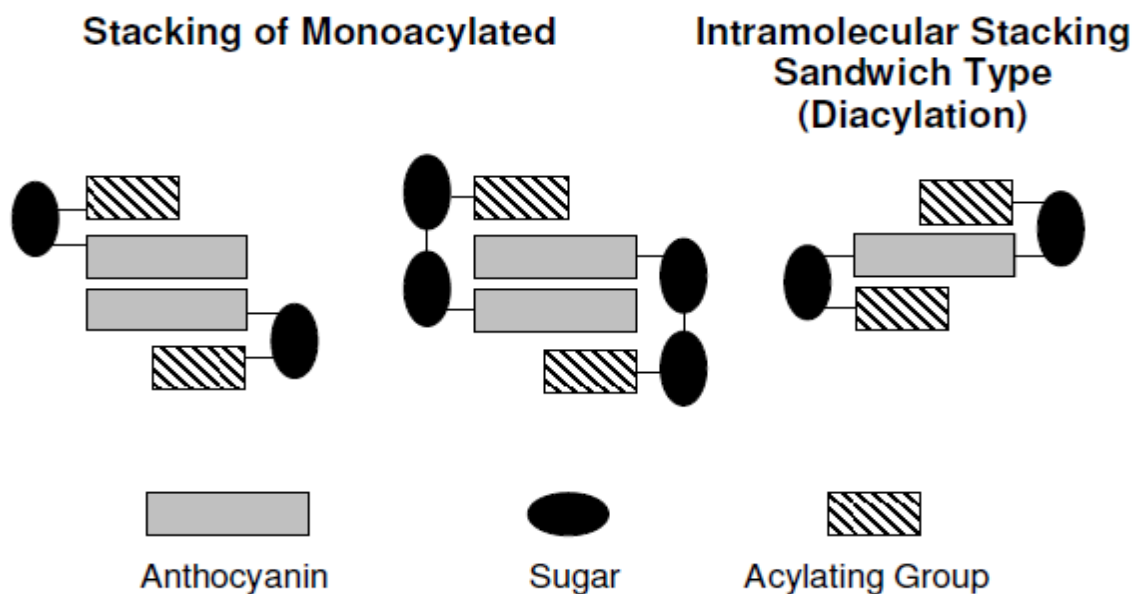


Figure 1.05: Intermolecular and Intramolecular Stacking of Acylated Anthocyanins
(Adapted from: Giusti and Wrolstad, 2003 and Yoshida et al., 1992).

This spatial configuration and stacking of the acylated anthocyanins provides greater stability. Additionally, anthocyanins can be acylated with more than one group which creates variations in stacking (Giusti and Wrolstad, 2003).

1.4 Synthetic and Natural Colorants:

1.4.1 Synthetic and Natural Colorants Background:

Since color is one of the first characteristics perceived by a consumer, it appears that artificial colorants are a way to make food more appealing (Radomski, 1974). Sharma et al. (2011) agree saying that synthetic or artificial colorants are used to correct or enhance the appearance of food after processing. Many colors are produced from aromatic compounds, since they allow electrons to move freely about their conjugated structure. One of the first synthetic dyes using this property is called mauve, a dark purple color. It

was made by accident while British chemist Sir William H. Perkin discovered aniline while experimenting with coal tar derivatives (Ettlinger, 2007). Coal tar was leftover from the burning and processing of converting coal into coke, which was used in the iron industry (Ettlinger, 2007). Now most dyes are from petroleum derivatives instead of coal tar. Regardless, many beautiful colors have been developed over the years, but there have been some issues.

In the early 1950s there were two instances of toxicity of dyes in humans: FD&C Orange No. 1 and FD&C Red No. 32. Black and orange Halloween candy, made with the azo dye FD&C Orange No. 1, figure 1.06, had given children diarrhea (Radomski, 1974). This cathartic effect continued when consumers consumed popcorn colored by FD&C Red No. 32, as well as butter and margarine colored with FD&C Yellow No. 4 (Radomski, 1974). In the case of FD&C Orange No. 1, it was observed that reductive fission of bacterial gut flora easily split the N-N, azo, linkage; this was seen in humans and animals and seems to be a general trend for azo colorants (Radomski, 1974). However, he believes that the more “toxic” structures are possibly the ones that are only sulfonated on one of the aromatic rings and the less “toxic” are sulfonated on at least two of the aromatic rings.

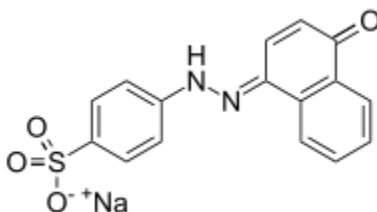


Figure 1.06: FD&C Orange No. 1

FD&C Red No. 1, a cherry red color, was decertified in 1961 due to the fact that it caused liver cancer in rats. FD&C Red No. 2, a red-purple color, was decertified by the FDA in 1976 for being a possible carcinogen (Sharma et al., 2011). Powdered FD&C Red No. 3, a cherry pink color, is considered a safe food colorant in the United States, but the lake form is banned due to causing a carcinogenic response in rats (Sharma et al., 2011). FD&C Red No. 4, a scarlet red, was used in maraschino cherries and produced a “brilliant” hue and was stable to sulfites and light (Wrolstad, 2008); it was banned for use since it damaged both the adrenal gland and the urinary bladder (Wrolstad, 2008). Despite this evidence, processors lobbied for its use as there was not an adequate replacement; the color was reinstated and restricted to maraschino cherries at 150 ppm (Wrolstad, 2008). FD&C Red No. 40 or Allura Red AC, an orange-red color, was certified in 1971 by the FDA and was used as a replacement for FD&C Red No. 2 and FD&C Red No. 4 (Sharma et al., 2011). Some of these red colorants and some alternatives are seen below in figure 1.07. FD&C Red No. 40 has the highest intake among the FD&C certified dyes at 100 mg/day; FD&C Yellow No. 5, which is second, is consumed at a rate of 43 mg/day (Giusti and Wrolstad 1996). Allura Red AC can be found in many products: Gelatins, puddings, dairy products, confections, beverages, condiments. Despite FD&C Red No. 40’s certified status there are concerns on its safety; as there are with many of the synthetic dyes.

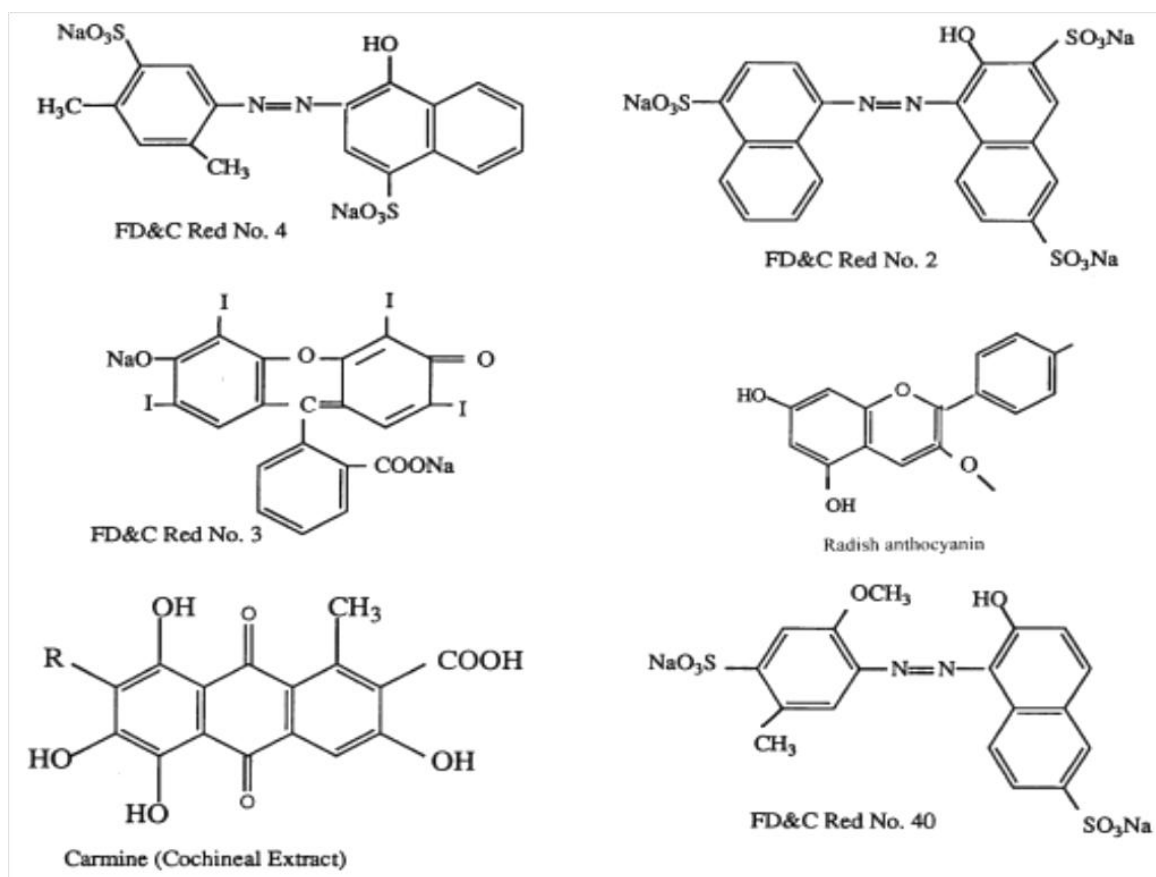


Figure 1.07: Structures of both Synthetic and Natural Red Colorants (Source: Wrolstad, 2009).

1.4.2 Concerns of Synthetic Dyes:

The concerns over the use of synthetic dyes are not new. In the 1950s the issues with FD&C Orange No. 1 and other colorants led to the safety reevaluation of synthetic food colorants (Radomski, 1974). In the 1970s there were several studies that concluded that FD&C Yellow No. 5 and No.6 causes allergic reactions and possibly hyperactivity (Sharma et al., 2011). Additionally, dyes presence in foods has increased five-fold over the last fifty years; figure 1.08 depicts how consumption has increase from 12 mg/day in 1955 to 59mg/day in 2007 (Jacobson, 2007).

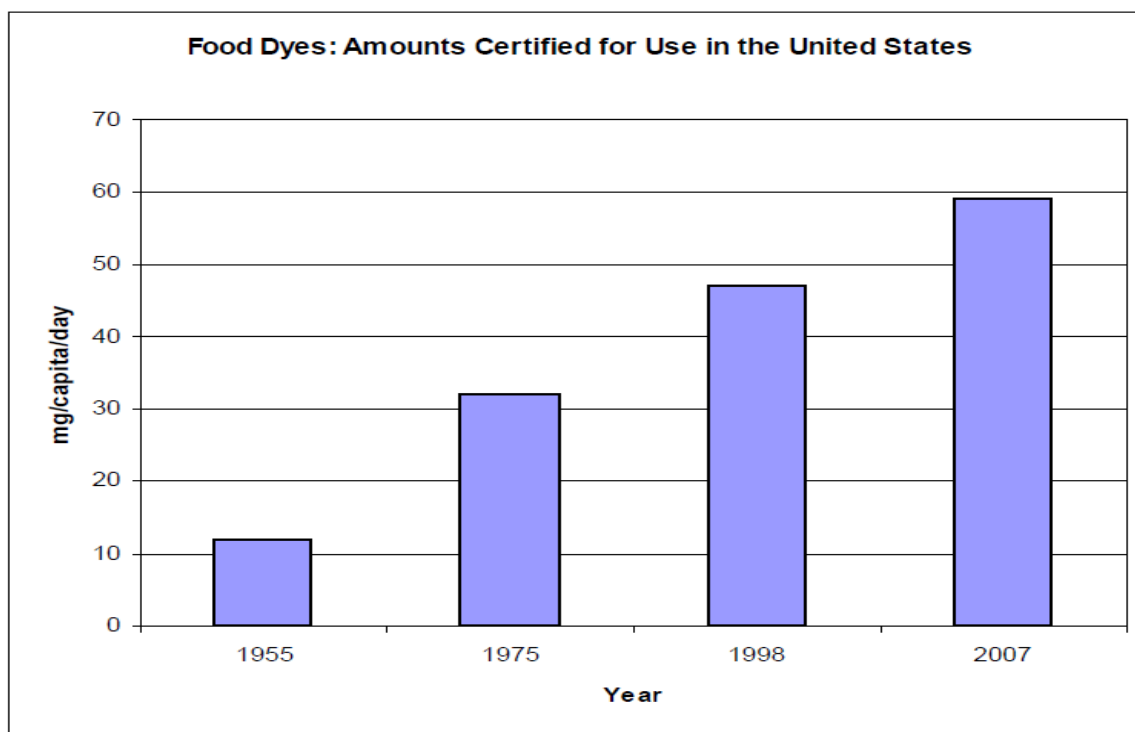


Figure 1.08: Synthetic Food Dye Consumption in the United States (Source: Jacobson, 2008).

Many believe this increase in consumption is correlated with higher rates of behavioral disturbances, such as ADHD (Jacobson, 2007). McCann et al. tested how the intake of artificial food colorants and additives affected the behavior of children, in their randomized, double-blinded, placebo-control, crossover trial. They found that synthetic colorants and/or Na benzoate in the diet of 3-year-old and 8/9-year-old general population children increased the rate of hyperactivity in those exposed (2007). Due to the findings in this study the European Food Safety Authority is evaluating the findings that synthetic colorants cause increased hyperactivity in children (Sharma et al., 2011). Their findings could catalyze the FDA reevaluate the safety of the seven certified FD&C dyes that are currently in use (Sharma, et al. 2011). Many consumer groups would like

food manufactures to voluntarily substitute natural colorants for artificial dyes sooner than later. These consumer groups are hesitant that change will occur since the link to issues and food dyes was hypothesized some thirty years ago and there are still food dyes present in a number of products (Jacobson, 2007). As figure 1.08 demonstrates, food dyes are ever present and increasingly being used in American food products.

1.4.3 Natural Colorants:

There are many sources for natural colorants; they generally come from plant materials (carrots and grapes), but can come from scale insects (cochineal), fungi (*Monascus* spp.), and even cyanobacteria (*Arthrospira* spp.), as table 1.04 details (Mortensen, 2006).

There are some limitations to natural colorants though (Mortensen, 2006; Giusti and Wrolstad, 2003).

- a) Low concentration of pigment in colored food product- Therefore, more / larger quantities of a material would have to be used to get the desired effects.
- b) Unwanted/undesired flavors – vegetable flavors in a beverage.
- c) Insoluble matter – peels and seeds.

However, many of these issues are fixed or overcome by extracting and concentrating the material (Mortensen, 2006).

EU Name	E-number	U.S. Name	U.S. 21 CFR
Curcumin	E 100	Turmeric	73.600, 73.615
Riboflavin	E 101	Riboflavin	73.450
Cochineal, carminic acid, carmines	E 120	Cochineal extract, carmine	73.100
Chlorophyll(in)s	E 140	Not allowed	-
Copper complexes of chlorophyll(in)s	E 141	Sodium copper chlorophyllin	73.125
Caramel	E 150	Caramel	73.850
Vegetable carbon	E 153	Not allowed	-
Mixed carotenes	E 160a (i)	Carrot oil	73.300
β -Carotene	E 160a (ii)	β -Carotene	73.950
Annatto, bixin, norbixin	E 160b	Annatto extract	73.300 73.340, 73.345
Paprika extract	E 160c	Paprika	73.345
Lycopene	E 160d	Not allowed	-
β -Apo-8'-catotenal	E 160e	β -Apo-8'-catotenal	73.900
Ethyle ester of β -apo-8'- carotenoic acid	E 160f	Not allowed	-
Lutein	E 161b	Not allowed	-
Canthaxanthin	E 161g	Canthaxanthin	73.750
Beetroot red	E 162	Dehydrated beets	73.400
Anthocyanins	E 163	Fruit Juice Vegetable Juice	73.250 73.260

Table 1.04: Allowed Natural and Nature-Identical Colorants in the EU and the USA
(Source: Adapted from Mortensen, 2006; Schwartz et al., 2007)

1.4.4 Natural Colorants / Pigments:

Caramel color is one of the most popular colorants in the world and is produced from the heat treatment/condensation of various sugars with salts, acids, alkalis, and heat treatments in order to produce a various colors (Mortensen, 2006). There are four types of recognized caramels: plain, sulfite, ammonia, and sulfite ammonia. They are generally seen in beverages, snacks, cereals, desserts, and confections.

Chlorophyll is a green pigment that is central in photosynthesis. It is fat soluble when fully intact and water soluble when the phytol group is lost due to heat and/or acidic conditions. The magnesium ion that aids in photosynthesis is easily displaced during cooking; resulting in an olive-brown color. Other metals can replace the lost Mg including but not limited to Zn and Cu. These metals will generally produce a bright green pigmentation; since these molecules are harder to displace from the pheophytin complex (Mortensen, 2006; Schwartz et al., 2007). Both Chlorophyll and Cu chlorine are seen in figure 1.09.

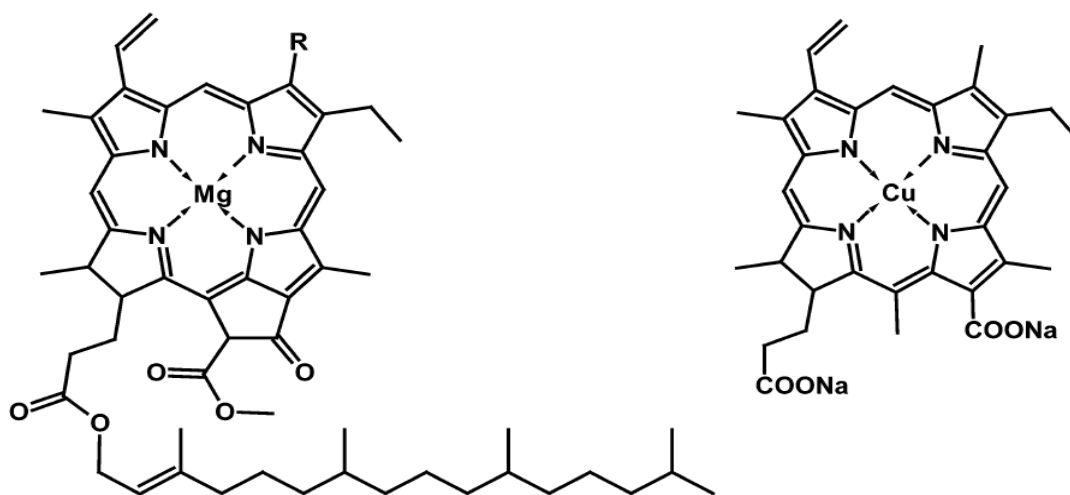


Figure 1.09: Structure of Chlorophyll *a* (R = CH₃) and *b* (R = CHO) and Cu Chlorin *e4* - as a Sodium Salt. (Source: Mortensen, 2006).

Carotenoids are lipid soluble molecules that provide yellow, orange, and red color to various organisms. In carrots, oranges, and peaches, carotene provides the yellow-orange color. In tomatoes, watermelon, guava, and pink grapefruit, lycopene provided orange-red color. They generally consist of linear conjugated double bonds, as seen in figure

1.10, and can have many different structures depending on what source it is isolated from. However, they are susceptible to oxidation and light which catalyzes this reaction.

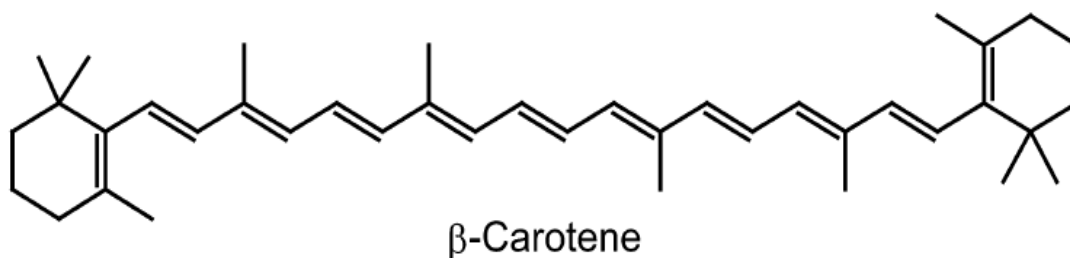


Figure 1.10: β -Carotene (Source: Mortensen, 2006)

Betalains are water soluble beet pigments that provide either red/purple (betacyanidins) or yellow (betaxanthins) (Mortensen, 2006; Schwartz et al., 2007). Betacyanidins are a conjugated form of cyclo-DOPA and betalamic acid, while betaxanthins are a conjugated form of amino acids or amines with betalamic acid (figure 1.11) (Mortensen, 2006).

Beetroot colors are generally more purple and brighter than anthocyanins and they do not vary in hue in the pH range of 4-7; most stable between pH 4.0-5.0 (Schwartz et al., 2007). However, they are readily labile to heat, oxygen, and light which limits their incorporation in systems. Therefore, they are generally incorporated into frozen pizzas, sauces, dried products, ice cream, and some yogurts.

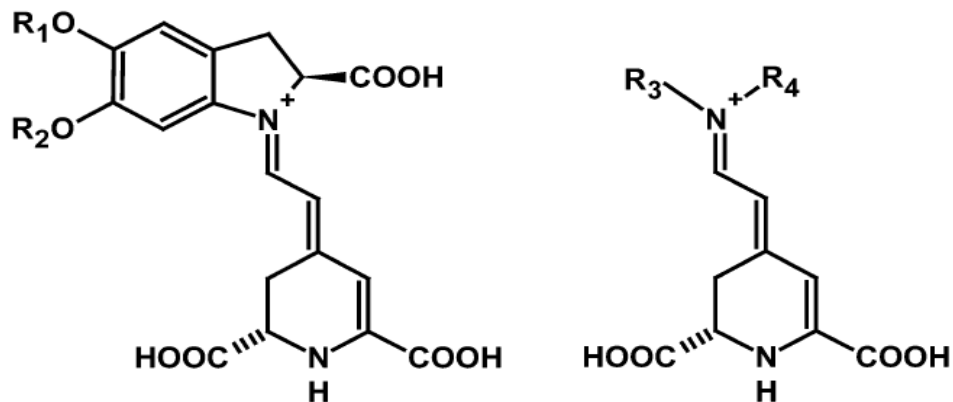


Figure 1.11: Structures of Betacyanins (left) and Betaxanthins (right). Betanidin: R₁, R₂ = H; Betanin: R₁ = Glucose, R₂ = H. (Source: Mortensen, 2006).

According to Mortensen (2006), colorants are not different from other compounds in the sense that they are susceptible to heat, light, and oxygen. Ironically, it is the coloring capacity of these molecules that leads to their demise; due to their structure that strongly absorbs light and is highly unsaturated. However, Giusti and Wrolstad (2003) believe there are more issues; since there are some limitations that restrict the incorporation of natural colorants into food systems: low stability to processing techniques, storage conditions, and they may have an undesirable aroma or flavor profile. Both radish and red cabbage extracts have great stability to heat, pH, light, and oxygen and can be incorporated into food matrices once the vegetable sensory notes are removed.

1.5 Challenges of Anthocyanin Colorants:

There are many challenges when using anthocyanins as natural colorants since they are susceptible to high temperatures, oxygen, light, and other factors (Giusti and Wrolstad, 2003) and they can contribute undesirable aromas or flavors to the final product. In addition, it may be challenging to match the color / hue characteristics that are required,

and finally, anthocyanin-based colorants are generally more expensive than their synthetic counterparts, and there could be costs related to processing modifications required to use them in a food application.

1.5.1 Temperature:

Heat treatment is common practice in food manufacturing, but it generally has a negative effect on the anthocyanins (figure 1.12). When anthocyanins are exposed to heat treatment they potentially can brown due to various forms of degradation (Giusti and Wrolstad, 1996a). However, when an anthocyanin is highly acylated, it is typically more thermo-stable as compared to that of mono and non-acylated anthocyanins (Giusti et al 1996a).

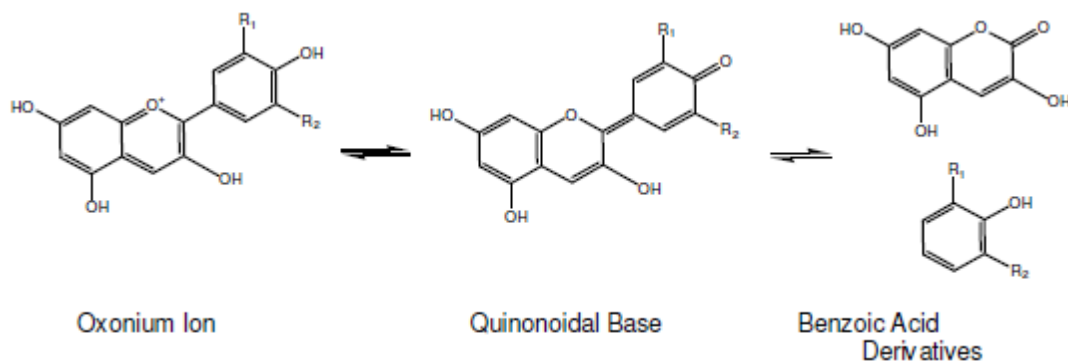


Figure 1.12: Degradation of Oxonium Ion at pH 3.7 Accelerated by Heat.
(Source: Giusti and Wallace, 2009)

1.5.2 Light:

Light accelerates degradation of anthocyanins, especially after they have been extracted and used in food as a colorant. If oxygen is present along with light, especially

fluorescent, there is an accelerated loss of anthocyanins (Palamidis and Markakis, 1978.) However, acylated anthocyanins seem to possess a greater stability when exposed to various light conditions. For instance, when acylated pelargonidin derivatives from red radishes were incorporated into maraschino cherry matrix and placed in two different locations, in the dark and exposed to light, there was only a slight increase in the rate of pigment degradation in the light sample when compared to that of the sample in the dark (Giusti and Wrolstad, 1996b). This increased stability was credited to the anthocyanin having cinnamic acid acylations.

1.5.3 Oxygen and Ascorbic Acid:

The conjugated network that is responsible for vibrant colors is also susceptible to degradation by both oxygen and ascorbic acid. Oxygen seems to have a catalytic effect on the degradation process, in the sense that it amplifies other anthocyanin degradation processes (Nebesky et al., 1949). It has been seen that when oxygen is coupled with heat there is good amount degradation on the anthocyanins present in various juices (Nebesky et al., 1949). This degraded product by oxygen creates a dull oxidized looking yellow-brown hue (Jackman et al., 1987). However, when packaging anthocyanin containing products a nitrogen purge, displacing oxygen, can increase the stability of the anthocyanins and thus the shelf life of the product.

The use of ascorbic acid, vitamin C, in food systems is to inhibit oxidation while enhancing the nutritional quality of a product. It seems that when both anthocyanins and ascorbic acid are both in solution, they have the tendency to degrade; likely due to the

formation of hydrogen peroxide, especially in the catalyzing presence of copper (Francis, 1989). Another theory is that a direct condensation of the anthocyanins with ascorbic acid destroys both of the molecules (Poei-Langston 1981). However, it seems that ascorbic acid may protect anthocyanins against enzymatic attack (Talcott et al., 2003).

1.5.4 Enzymes and Sugars:

Both enzymes and sugars are naturally occurring in both fruits and vegetables.

Sometimes these molecules are added during processing to enhance a product in some form. For instance, pectolytic enzymes are added during juice processing in order to increase both the yield and the extracted color of the juice (Iversen, 2006). However, enzymes may have the potential to degrade anthocyanins and other pigments present by hydrolysis of glycosidic bond (Wightman and Wrolstad, 1996). Naturally, these hydrolyzed anthocyanins will lose their color due to their unstable state. It has been shown that the inactivation of enzymes has a positive effect on anthocyanin stability (Garcia-Palazon et al., 2004).

Glycosidases are the most common degradation enzyme since they break glycosidic linkages between the anthocyanin and its respective sugar moiety; resulting in a more unstable molecule (Huang, 1956). Galactosides have been shown to have a similar effect and both of these enzymes can be produced by various molds (Wightman and Wrolstad, 1996).

Enzymes can also degrade other phenolics present in a product; forming quinines which combine with anthocyanins to form a brown condensation product (Kader et al., 1999). Likewise, low concentrations of sugars and their degraded byproducts decrease stability and can yield a brown polymerized complex (Daravingas and Cain, 1968). Conversely, a high concentration of sugars may lower the water activity and thus have a protective effect on the anthocyanins activity (Tsai et al., 2004). Sucrose solutions also seemed to protect anthocyanins from browning and polymeric pigments by inhibiting enzymatic reactions (Wrolstad et al., 1990).

1.5.5 Sulfur Dioxide:

Sulfur dioxide (SO₂) is used in the industry as an inhibitor of molds, yeasts, bacteria, as well as, both enzymatic and non-enzymatic browning (Wrolstad, 2009). The nucleophilic attack of the sulfite ion on the oxonium ion's (flavylium cation) 4 position makes the anthocyanin colorless possibly due to the disruption of the conjugated double bond system (Jackman et al., 1987). This can be seen in figure 1.13.

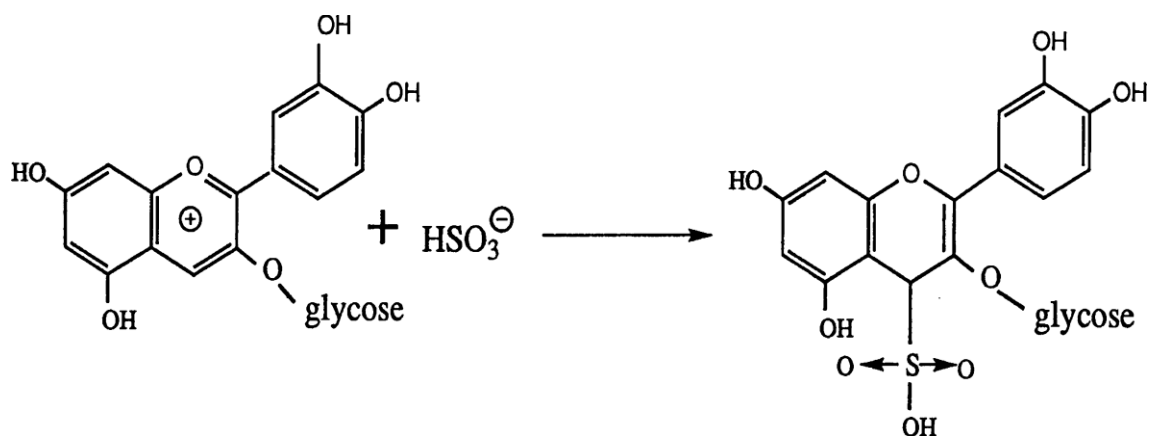


Figure 1.13: Reaction of Anthocyanin Pigments with HSO_3^- to Form a Colorless Sulfonic Acid Addition Product (Source: Wrolstad 2009).

1.6 Radish Anthocyanins

The radish (*Raphanus sativus* L.) is an edible root that has been consumed for centuries. Radishes are known to come in many colors, shapes, and sizes, as well as for their pungent flavor/aroma. Radish anthocyanins have been identified by previous workers (Harbourne, 1963; Ishikura and Hayashi, 1962, 1963; Fuleki, 1969), with the pigments being pelargonidin derivatives. (Giusti and Wrolstad, 1996a) The color comes from anthocyanins; which can be found in just the epidermal tissue or throughout the whole radish (Giusti et al., 1999 and Rodriguez-Saona et al., 2001). Radishes mainly contain the six following anthocyanins: pelargonidin-3-sophorose-5-glucoside, pelargonidin-3-sophorose-5-glucoside acylated with caffeic acid, pelargonidin-3-sophorose-5-glucoside acylated with p-coumaric acid, pelargonidin-3-sophorose-5-glucoside acylated with ferulic acid, pelargonidin-3-sophorose-5-glucoside acylated with p-coumaric and malonic acid acylation, and pelargonidin-3-sophorose-5-glucoside acylated with ferulic and malonic acid (Giusti and Wrolstad, 1996a). The common acids acylated to the sugar moieties is in figure 1.14

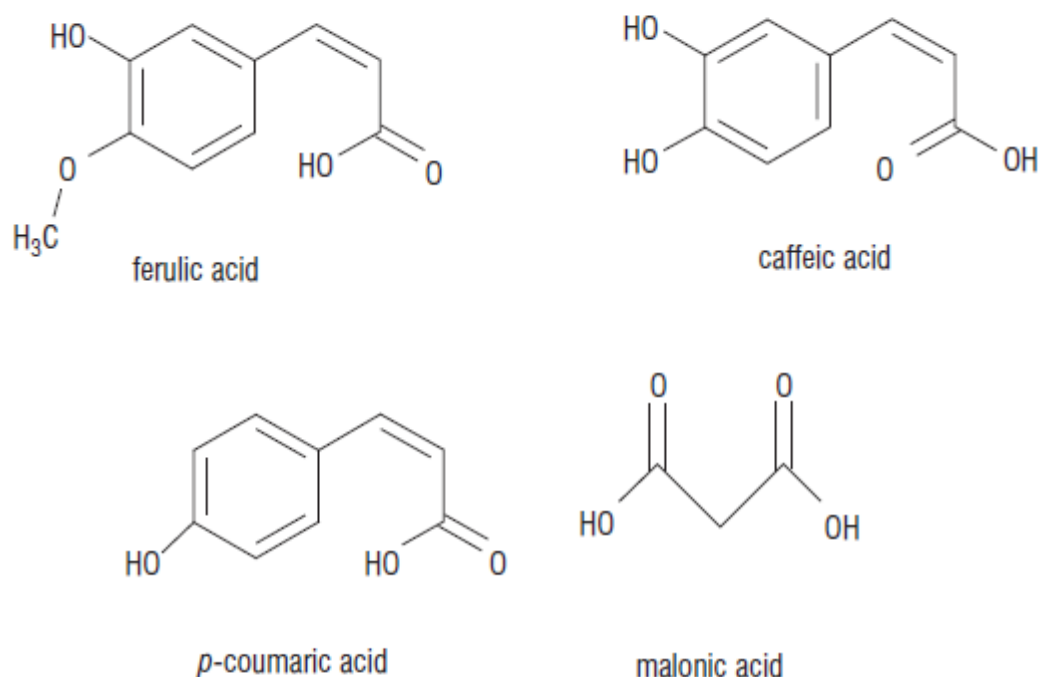


Figure 1.14: Common Cinnamic and Aliphatic Acids Acylated to Sugar Moieties of Pelargonidin from Red Radish (Source: Bakowska-Barczak, 2005)

The major anthocyanins present are pelargonidin-3-sophoroside-5-glucoside with p-coumaric, ferulic, and caffeic acid esterified to sugar substituents (Giusti and Wrolstad, 1996). Red radishes have been reported to have 11-60mg pg-3-glu/ 100g of product (Giusti et al., 1998). However, higher numbers have been reported and like many other natural products there is variability in anthocyanin concentration based on cultivar type and growing conditions. Radish anthocyanins also seem to have increased stability when compared to other anthocyanins.

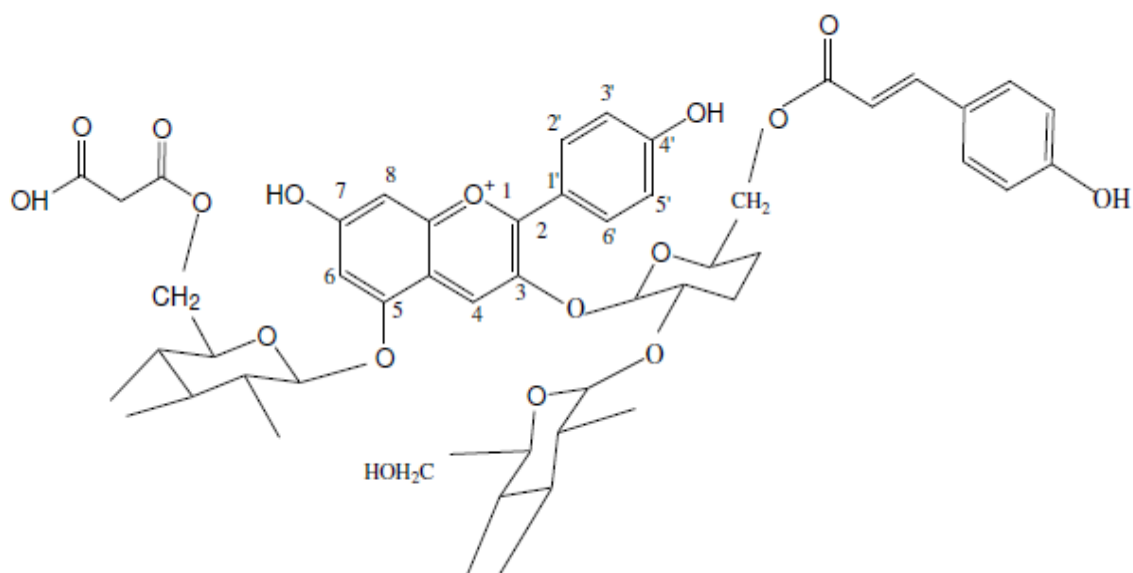


Figure 1.15: Chemical Structure of a Pelargonidin Derivative from Red Radish
(Source: Giusti et al., 1998).

Red radish anthocyanins are acylated which imparts both an attractive color and increased stability to the molecule (Giusti and Wrolstad, 2003). Acylated anthocyanins exhibit greater stability during both processing and storage (Bassa and Francis, 1987). According to Francis, it is these acylating groups that protect the oxonium ion from hydration; thus, retarding the forming of a hemiketal or chalcone forms (1989). The presence of acylation on an anthocyanin also makes the molecule more resistance to acid hydrolysis (Giusti and Wrolstad, 1996). Moreover, Matsufuji et al. reported that acylated anthocyanins, specifically from red radish extract, were more stable to light (fluorescent), heat, and hydrogen peroxide at low pH (2007). They attribute this stability to intramolecular copigmentation, which is believed to protect the flavylium chromophore from nucleophilic attack by water. They suggest that diacylated anthocyanins might be more stable than monoacylated anthocyanins due sandwich-like stacking of the

hydrophobic components between the planar acid molecules as seen in figure 1.05.

Giusti and Wrolstad (2003) believed that improved stabilization is due to intramolecular and intermolecular copigmentation, self-association, metal complexing, and the presence of inorganic salts.

Besides stability, the acylation of pelargonidin shifts the orange-red hue to an intense red hue (Giusti and Wrolstad, 1996a). Additionally, this shift makes red radish juice have a similar hue angle to that of FD&C Red No. 40, as can be seen in figure 1.16 when compared to other acylated anthocyanin extracts. Giusti and Wrolstad (1996b) used red radish anthocyanins to color brined maraschino cherries, since the hue angle (table 1.05) is very similar to that of FD&C Red No. 40. Two different anthocyanin concentrations, 600mg/L and 1200mg/L, were used and evaluated over six months. The half life of each respective material was 29 weeks for 600mg/L and 33 weeks for 1200mg/L. Additionally, they found that a higher anthocyanin concentration had a protective effect on color stability.

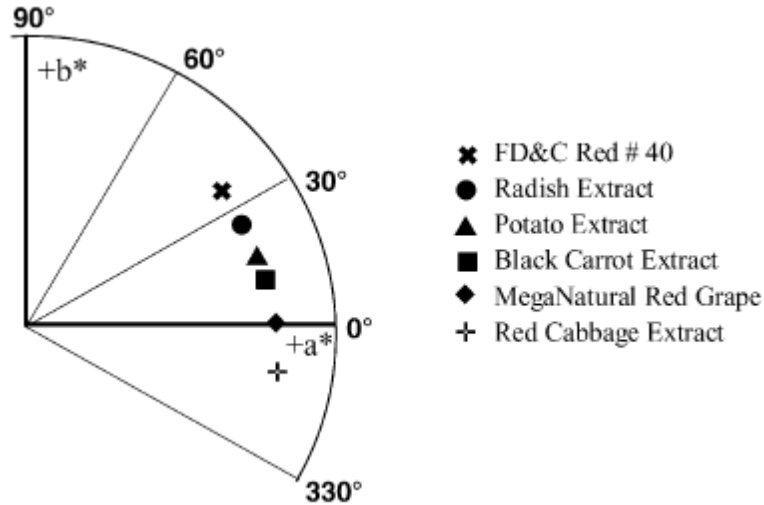


Figure 1.16: Hue Angle of Different Anthocyanin Extracts in Solution (pH 3) as Compared to Allura Red at the Same Level of Chroma (Source: Giusti and Wrolstad, 2003)

Sample	Concentration (mg/100 mL)	Hue Angle (Degrees)
Red Radish	15	16.23
Red Radish	30	26.22
Red Radish	45	37.39
Red No. 40	1.5	37.73

Table 1.05: Hue Angles of Red Radish Juice and FD&C Red No. 40 (Adapted from Rodriguez-Saona et al., 2001).

Rodriguez et al. showed that it is possible to produce a highly concentrated radish juice that would be exempt from certification (2000). They produced high anthocyanin recoveries (>90%) and reduced the aroma compounds all while maintaining the similar color to that of FD&C Red No. 40. Their sensory analysis showed that the combination of thermal evaporation and direct osmosis concentration reduced the levels of undesirable radish aroma compounds found in the samples.

1.7 Glucosinolates and Myrosinase:

Radishes are a root vegetable belonging to the *Brassicaceae* family. They come in a variety of colors including red, black, white, pink, amongst many other shades. They are considered nutritious due to various minerals, vitamins, and glucosinolates (GLS). GLS are known to provide anticancer benefits and are the precursor to their pungent aroma (Blazevic and Mastelic). The amount of glucosinolates varies between cultivars, plant parts, and plants themselves, due to genetic, environmental, and nutritional factors (Blazevic and Mastelic, 2009). It seems that there is a higher concentration of GLS in younger material (Blazevic and Mastelic, 2009). GLS are believed to be involved in plant defense, sulfur and nitrogen metabolism, and plant development (Blazevic and Mastelic, 2009). These molecules are composed glucose and sulfur containing anions that readily hydrolyzed by myrosinase (Vaughn and Berhow, 2005). This reaction cascade happens when a cell, and thus the vacuoles, of the respective plant are ruptured and thus allowing the molecules time to interact; which can happen via cutting, chewing, cooking, fermenting, and/or freezing (Blazevic and Mastelic, 2009). GLS hydrolysis is believed to be part of a defense mechanism for a plant due to their biocidal activities against insects, fungi, and bacteria (Vaughn and Berhow, 2005). This reaction which produces glucose, sulfate, isothiocyanates, thiocyanates, and nitriles can be seen in the figure 1.17.

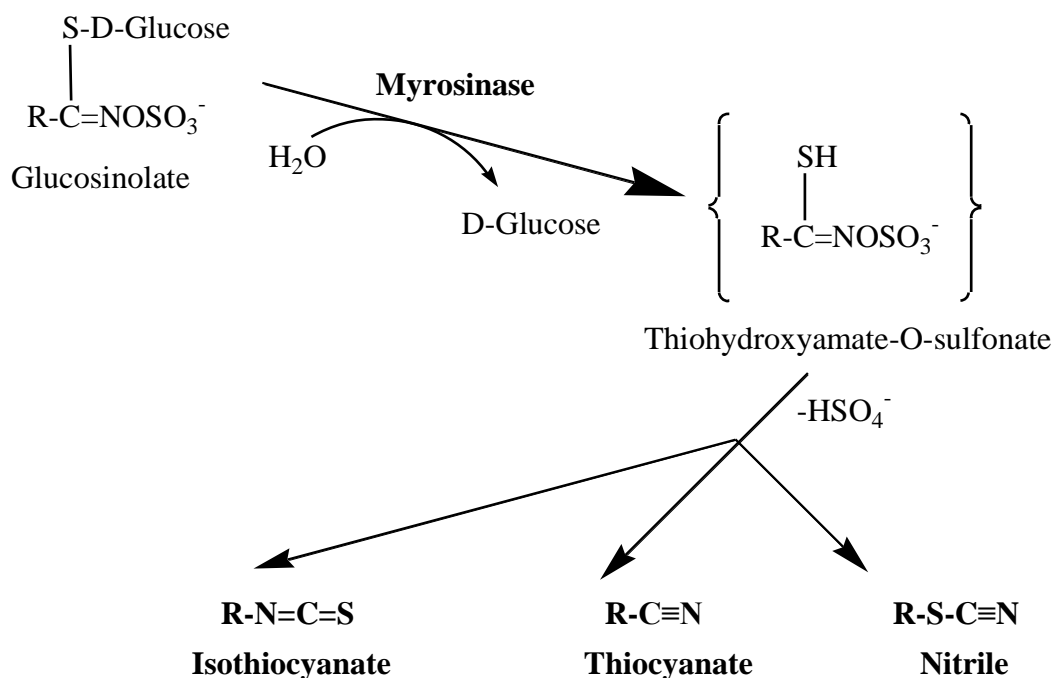


Figure 1.17: Glucosinolate and Myrosinase Cascade (Adapted from Van Eylen, 2007).

The major GLS present in the root are 4-methylthio-3-butenyl glucosinolate which is converted enzymatically to 4-methylthio-3-butenyl isothiocyanate (Blazevic and Mastelic, 2009). Cooking the material has a direct effect on GLS and their products; allowing them to solubilize in the water, as well as, thermal inactivation of myrosinase (Blazevic and Mastelic, 2009).

Myrosinase is an enzyme that is present in all glucosinolate-containing plants; it hydrolyzes glucosinolates to form isothiocyanates and other compounds. The isothiocyanates formed by this reaction produce an undesirable aroma. It has an active pH range of 5-7; however, its activity decreases as the pH leaves this bioactive range. Additionally, the enzyme is inhibited by Cl^- ; which can interact with the positive charge present in the active site by replacing other anions (Iori et al. 1996). At 0.5M Cl^- the

enzyme is inhibited by 70% and at higher concentrations of over 2M, the enzyme almost completely inhibited (Iori et al. 1996). The enzyme can be completely denatured via heating the molecule to 70°C, as can be seen in figure 1.18 (Van Eylen, 2007). They also mentioned that the cofactor epithiospecifer protein is easily denatured at 60°C in 5 to 10 minutes. Conversely, the enzyme is not easily denatured when exposed to heat and pressure; a combination that usually has an effect on molecules. Instead, a myrosinase inactivation study using pressures up to 200 MPa and temperatures above 50°C indicated that pressure actually retards thermal inactivation (Van Eylen, 2007). They also found that isothiocyanates were pressure stable and relatively heat labile.

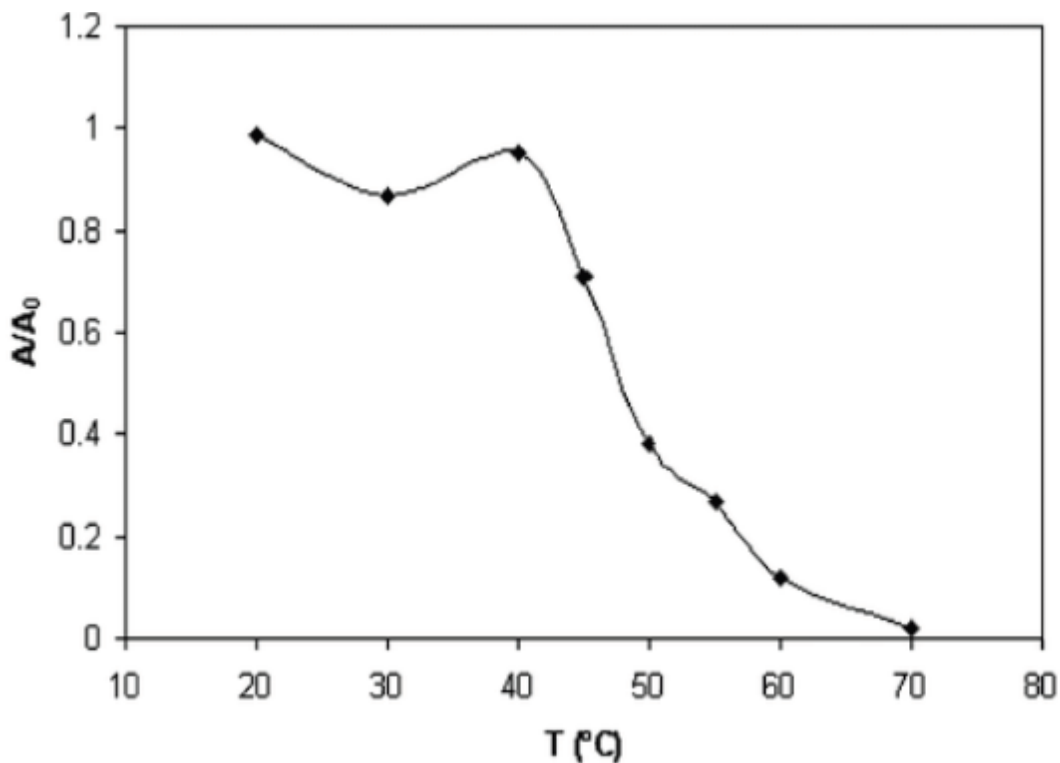


Figure 1.18: Temperature Effect on Myrosinase after a Ten Minute Period
(Source: Van Eylen et al.).

1.8 Present Study:

The goal is to produce colorant from cull radishes, a waste product, in order to add value and reduce waste with a high quality healthy product that will provide color, an acceptable aroma and flavor multiple health benefits, and stability suitable for food applications. From the skins, the color will be extracted to produce a concentrated juice; undesired aromas and flavors will be minimized or removed during processing through extraction and concentration processes. A sensory panel will evaluate the color, aroma, and flavor of the final radish juice concentrate and comment on its marketable appeal as a colorant. In addition to providing a vibrant concentrated juice colorant, potential uses for the remaining peeled radishes were explored. In essence, we will help create a value added product with potential commercial application from what would ordinarily be considered a waste product. In doing so the company should increase profit margin while giving the consumer a healthy alternative.

Our long-term goal is to provide a natural alternative to the use of synthetic dyes.

In this study we harvested natural colorant from cull radishes for a high quality, healthful product acceptable for commercial applications. From the skins, color was extracted to ultimately produce a concentrated vegetable juice. Undesirable aromas and flavors were minimized or removed during the extraction and concentration process.

Chapter 2: Material and Methods:

2.1 Plant Material:

The red radishes were provided by Meidema Produce Inc. (Hudsonville, MI). The radishes used in this study, premium and cull radishes, were grown in Michigan and Arizona and were provided directly by the grower. Various varieties, Red Silk, Crunchy Royale, Red Castle, Crunchy Crimson, were used based on those available at the time of the study.

2.2 Chemical Extraction:

The different batches of radishes received were evaluated for total anthocyanin content by using a standard chemical procedure described follows. Red radish skins (100 g) were peeled using a hand peeler and frozen using liquid nitrogen. The frozen skins were finely powered using a Waring commercial laboratory blender (Waring Laboratories, Torrington, CT) in a stainless steel container in the presence of liquid nitrogen (Giusti and Rodriguez-Soana et al., 1999).

The anthocyanins present in the powered material were extracted by blending it in the presence of 50mL of 70% acetone and 30% 0.1% HCl acidified water (v/v). The mixture

was then passed through a Buchner funnel (Fisher Scientific, Fair Lawn, NJ) coupled with Whatman No. 5 filter paper (Whatman Inc., Florham, NJ). Acidified water was then poured over the pink material until the filtrate was colorless; thus indicating that all of the pigment had been extracted from the red radish powder (Rodriguez-Soana & Wrolstad, 2001). The anthocyanin solution was then placed into a separatory funnel (Fisher Scientific, Fair Lawn, NJ) and combined with 2x the total volume chloroform. The solution was shaken and allowed to sit overnight in the refrigerator to ensure proper separation of the two phases. The bottom layer of chloroform containing fat soluble components was removed leaving the aqueous fraction; this acetone/water layer contained anthocyanins and other phenolic compounds. The residual acetone was then removed via evaporation in a Buchi rotary evaporator (Fisher Scientific, Fair Lawn, NJ) and brought up to a known level in a volumetric flask (100mL).

2.3 Anthocyanin Purification:

A Sep-Pak® C18 Vac solid cartridge (20cc, 5g sorbent; Waters Corp., Milford, MA) was activated using methanol and then the extract was passed through. A red ring developed as the anthocyanins absorbed onto the column. A 0.01% HCl acidified water mixture was passed through the column eluting the sugars, acids and other soluble components. The anthocyanins were recovered via 0.01% HCl acidified methanol. The methanol was driven off via Buchi rotary evaporator (Fisher Scientific, Fair Lawn, NJ) at 40C, to preserve molecular integrity, and the pigments were dissolved in acidified water.

2.4 Monomeric Anthocyanin Content:

The monomeric anthocyanin content was evaluated using the pH differential method described by Giusti and Wrolstad in Handbook of Food Analytical Chemistry (2005). This method uses the spectral absorbance of anthocyanins at different pH values; in other words the color will vary based on the pH of the solution. The dilution factor (DF) was determined by diluting each extract in 0.025 M potassium chloride (Fisher Scientific, Fair Lawn, NJ) buffer at pH 1.0, until the absorbance was within the appropriate spectrophotometer range; greater than 0.1 and less than 1.2. Each anthocyanin extract was then appropriately diluted using pH 1.0 and pH 4.5 sodium acetate (Fisher Scientific, Fair Lawn, NJ) buffer. At pH 1 the color appeared orange-red in color; while at pH 4.5 the color was a faint purple/colorless. Solutions were allowed to equilibrate at room temperature for 15min in the dark and then read using a UV-Visible Spectrophotometer 2450 (Shimadzu, Columbia, MD). The buffered system's absorbances were read at 510nm and 700nm. The monomeric anthocyanin content was then calculated using the following equation:

$$\text{Anthocyanin} = [(A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH}1.0} - (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH}4.5} * \text{DF} * 1000 * \text{MW}] / \epsilon$$

DF is the dilution factor; MW is the molecular weight; ϵ is the molar absorptivity coefficient. The MW and ϵ were calculated as pelargonidin-3-glucoside, where the MW = 433.2g with an extinction coefficient of $31,600 \text{ cm}^{-1} \text{ mg}^{-1}$. Absorbance was determined in two replications of each sample and then averaged.

2.5 HPLC analysis:

The extract was analyzed using a Shimadzu high performance liquid chromatography (HPLC) (Shimadzu Scientific Instruments, Inc., Columbia, MD) system outfitted with LC-20AD pumps and a SIL-20AC autosampler coupled with an SPD-M20A photodiode array (PDA) detector. LCMS Solutions Software (Version 4, Shimadzu, Columbia, MD) was used to represent the data as well as analyze it. A reverse phase Symmetry C-18 column (Waters Corp., Milford, MA) with a 4.5 micron size and a 150 x 4.6 mm column were connected to a 4.6 x 22 mm Symmetry micro guard column (Waters Corp., Milford, MA). The solvents and samples were passed/filtered through a 0.45 μ m GE Magna nylon membrane filter (Fisher Scientific, Fair Lawn, NJ). The solvents used were A, 100% acetonitrile (Fisher Scientific, Fair Lawn, NJ) and B, (4.5% (v/v) formic acid in water Fisher Scientific, Fair Lawn, NJ). The various anthocyanins were separated using a linear gradient: 0% to 30% B, 30 min. The flow rate was set at 1 ml/min with an injection volume of 20 μ L. The spectral data of the anthocyanins was collected between 250-700 nm.

2.6 Abrasive Peeler:

Premium radishes were taken to the Fruit and Vegetable Processing Pilot Plant, at the Food Industry Center, The Ohio State University. The radishes were placed into a colander and washed in cold water to remove impurities and to limit degradation. They were weighed on a scale (Mettler-Toledo, Toledo, OH) and transferred over to the abrasive peeler (Hobart, Troy, OH). The skins were removed in an abrasive action while water was poured over the radishes. A pinkish hue water, normally waste material, was

collected and the now white radish bulbs were weighed. The pink radish juice was filtered using a Buchner funnel (Fisher Scientific, Fair Lawn, NJ) equipped with Whatman No. 5 paper (Fisher Scientific, Fair Lawn, NJ) and acidified using 6M HCl (Fisher Scientific, Fair Lawn, NJ) to 3.5. The now red and clear juice was then placed into the refrigerator to encourage precipitation of slightly soluble components. The juice was once again filtered using Whatman paper (Fisher Scientific, Fair Lawn, NJ). Samples were then kept for future monomeric (above) and HPLC work as mentioned previously. This process was repeated with the cull radishes (waste) and the two samples were compared to each other.

2.7 Dehydration:

The cull radishes were passed through the abrasive peeler and filtered in a similar fashion as noted previously; resulting in a red precipitant-free colorant. The juice was placed in a round bottom flask (Fisher Scientific, Fair Lawn, NJ) and put onto a rotary evaporator (Fisher Scientific, Fair Lawn, NJ) at 40C. The juice was allowed to fully evaporate; leaving a red-ring. The water that was collected was disposed in the sink. The dry anthocyanins were then rehydrated with a known volume of distilled water and the pH was checked and adjusted to 3.5 with a Accumant Excel XL15 pH meter (Fisher Scientific, Fair Lawn, NJ). The condensed radish juice was then placed into the refrigerator to limit degradation; monomeric and HPLC were performed the next day.

2.8 Centrifugal Filtration:

The radish chemical extract and concentrated juice were passed through a series of filters to gather preliminary data on anthocyanin/filter interaction. Samples were prepared earlier and used for the study. Amicon Ultra centrifugal filters (100K, 5K, & 3K) were provided by Millipore.

The chemical extract was centrifuged through a 100K mw filter and then transferred over to a 5K filter. The chemical extract was centrifuged through a 100K mw filter, a 100K mw filter and then transferred over to a 3K filter, and then a 3K filter; schematics pictured in figure 2.01

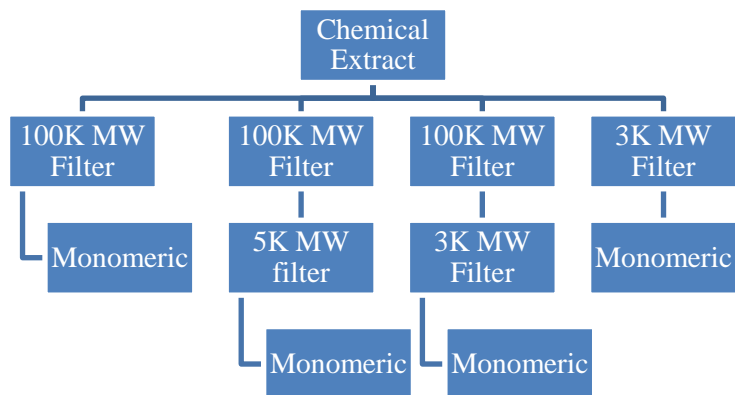


Figure 2.01: Filtration Procedure to Explore Separation of Isothiocyanates from Anthocyanins in a Radish Phenolic Chemical Extract

2.9 Nano-Filtration:

Cull radishes went through an abrasive peeler and filter in a similar fashion to that of the above; resulting in a red precipitant-free colorant. A Niro Lab 20 unit was used in order to remove the smaller isothiocyanates from the heavier anthocaynins in the juice. The

following filter papers tested were bought from GEA Process Engineering Inc. (Hudson, WI): DOW NF245, Hydranautics 7450, Hydranautics 7410. The papers were cut out to fit on both sides of the filter plate. The juice was loaded into the holding tub of the Niro Lab 20 Unit (GEA, Hudson, WI) and allowed to run through the filters. The filtrate was concentrated and kept. The waste was discharged down the drain. The filtered radish juice was then placed into the refrigerator to limit degradation; monomeric and HPLC procedures were performed the next day as described previously

2.10 High Salt and Low pH Enzyme Inhibition:

Red radishes were washed and weighed in a similar manner to that of those above. However, the water that was poured over the radishes while peeling was spiked with 2.2M pickling NaCl (Morton, Chicago, IL) and the pH was adjusted to 2.0; to inhibit myrosinase. The red-orange colored water was collected and the pulp was filtered off using Whatman No. 5 paper (Fisher Scientific, Fair Lawn, NJ). The pH was checked and adjusted using HPLC grade phosphoric acid (Fisher Scientific, Fair Lawn, NJ). The high salt and acid radish juice was then placed into the refrigerator to limit degradation; monomeric and HPLC procedures were performed the next day.

2.11 Steamed Radishes:

2.11.1 Steam Jacket Kettle Steaming:

Radishes were washed using cold water and weighed out; they were placed into a “birdcage” for later use. A “birdcage” looks like an upside down/hollowed out cage with handles. Color measurements were taken after washing the radishes. Water was

poured into the bottom portion of a steam jacketed kettle (Groen, Elk Grove Village, IL) and was heated up to boiling (100°C). Phosphoric acid HPLC grade 85% (Fisher Scientific, Fair Lawn, NJ) was added to water present in metal bowl in order to adjust it to a pH <2.0. The metal bowl containing the acidified water was placed in and floated in the steam jacketed kettle. The birdcage was then placed on top of this and the kettle was covered with aluminum foil. The boiling water produced steam which was allowed to interact with the radishes for 2.5, 5, and 10 minutes. The radishes were removed and allowed to cool before color measurements were taken with a hand held colorimeter with a 2° observer angle (Minolta, Osaka, Japan). The bowl was removed carefully and the red juice was poured into a graduated cylinder. The juice was then poured into a glass container with a screw cap and put on ice to limit degradation. The remaining radishes were then placed into the abrasive peeler as described previously. The waste was collected and used for monomeric analysis. The cooled juice was then filtered with Whatman No. 5 paper (Fisher Scientific, Fair Lawn, NJ) and placed on the Buchi rotary evaporator (Fisher Scientific, Fair Lawn, NJ) until dehydration. The dry material was then reconstituted to a known volume. The monomeric values of steaming and abrasive peeling were used to assess how much total material was extracted during the steaming process. Additionally, HPLC and monomeric content were used to assess possible degradation/loss.

2.11.2 Still Retort Steaming:

Radishes were washed using cold water and weighed; they were placed into a “birdcage” for later use. Color measurements were taken after washing the radishes. Water was let

into the bottom of a still retort (Loveless Manufacturing, Tulsa, OK) and was heated up to a desired temperature: 82.2-126°C (180-260°F). Phosphoric acid (Fisher Scientific, Fair Lawn, NJ) was added to water present in metal bowl in order to adjust it to a pH <2.0. The metal bowl containing the acidified water was placed in and floated in the still retort. The birdcage was then placed on top of this and the lid was lowered and tightened. Steam was pumped into the retort in order to heat up the water to the desired temperature and in doing so it also interacted with the radishes at intervals of 2.5 min, 5 min, and 10 min. The radishes were removed and allowed to cool before color measurements were taken with a hand held colorimeter with a 2° observer angle. The bowl was removed carefully and the red juice was poured into a graduated cylinder. The juice was then poured into a glass container with a screw cap and put on ice to limit degradation. The remaining radishes were then placed into the abrasive peeler the same manner as previously. The waste was collected and used for monomeric analysis. The cooled juice was then filtered with Whatman No. 5 paper (Fisher Scientific, Fair Lawn, NJ) and placed on the Buchi rotary evaporator (Fisher Scientific, Fair Lawn, NJ) until dehydration. The dry material was then reconstituted to a known volume. The monomeric values of steaming and abrasive peeling were used to assess how much total material was extracted during the steaming process. Additionally, HPLC and monomeric contents were used to assess possible degradation/loss.

2.12 Sensory:

A sensory evaluation was done to determine how the various treatments affected the aroma of the juice and how it compared to that of product containing FD&C Red No. 40.

Twenty random individuals were asked to assess previously prepared samples and a commercial drink mix colored with FD&C Red No. 40 (Strawberry-kiwi flavored Kool-Aid). The previous samples included control (abrasively peeled), Whatman filtrated, dehydration/rehydrated, nano-filtered, and enzyme inhibited. These samples, including the commercial sample, were poured into clean clear cups with lids and assigned a pre-assigned 3-digit random number. The samples were served randomly in a monadic order (one-at-a-time) until all 6 were evaluated. The panelists selected were asked to assess:

- 1) The Overall Visual Liking of the Juice; based on a 9 point Hedonic scale, where 1 is dislike extremely and 9 like extremely.
- 2) The Liking of the Juice Aroma; based on a 9 point Hedonic scale, where 1 is dislike extremely and 9 like extremely.
- 3) The Aroma Intensity of the Juice; based on a 15 point Intensity scale. Where 1 is no aroma (water-like) and 15 is most imaginable aroma (skunk-like).

The results were analyzed by Compusense 5 (Compusense Inc, Guelph, ON, CA).

2.13 Steamed Sensory:

A sensory evaluation was done to determine how the steam treatments compared to juice produced from an abrasive peeler (control) and FD&C Red No. 40. Twenty/Thirty random individuals were asked to assess previously prepared samples in water and colorless commercial drink mix (colorless cherry-flavored Kool-Aid). These samples were poured into clean clear cups with lids and assigned a pre-assigned 3-digit random

number. The samples were served randomly in a monadic order (one-at-a-time) until all 6 were evaluated. The panelists selected were asked to assess:

- 1) The Aroma Intensity of the Juice in water; based on a 15 point Intensity scale. Where 1 is no aroma (water-like) and 15 is most imaginable aroma (skunk-like).
- 2) The Aroma Intensity of the Juice in colorless cherry Kool-Aid; based on a 15 point Intensity scale. Where 1 is no aroma (water-like) and 15 is most imaginable aroma (skunk-like).
- 3) The Liking of the Juice taste; based on a 9 point Hedonic scale, where 1 is dislike extremely and 9 like extremely.

The results were captured and analyzed by Compusense 5.

2.14 Colored Salt:

The salted and acidified radish juice was poured inside a round bottom flask and placed on a Buchi rotary evaporator (Fisher Scientific, Fair Lawn, NJ) until all of the water was removed. The salt was then placed in a screw cap container.

2.15 Statistical Analyses

Statistical analyses were performed using one way analysis of variance (ANOVA) with an alpha level of $p > 0.05$ for the acceptance of the null hypothesis to compare chemical analyses data, using MiniTab 15.0 software (State College, PA). Sensory data was analyzed by Compusense 5 (Compusense Inc).

Chapter 3: Results and Discussion

3.1 Chemical Extraction:

A chemical extraction was performed in order to determine the total content of pigment in the radish material. The major anthocyanins present, as seen in the figure 1 below, were pelargonidin-3-sophoroside-5-glucoside with p-coumaric, ferulic, and caffeic acid esterified to sugar substituents, also seen by Giusti and Wrolstad 1996. The fact that these anthocyanins are present is good from a stability aspect. They potentially will provide a hue close to that of FD&C Red No. 40 at pH 3.5. The monomeric anthocyanins present were 100.8 ± 7.2 mg of anthocyanins / 100g of skin. These values are higher than those reported by Giusti and Wrolstad, but this could be due to the cultivar type and the growing conditions.

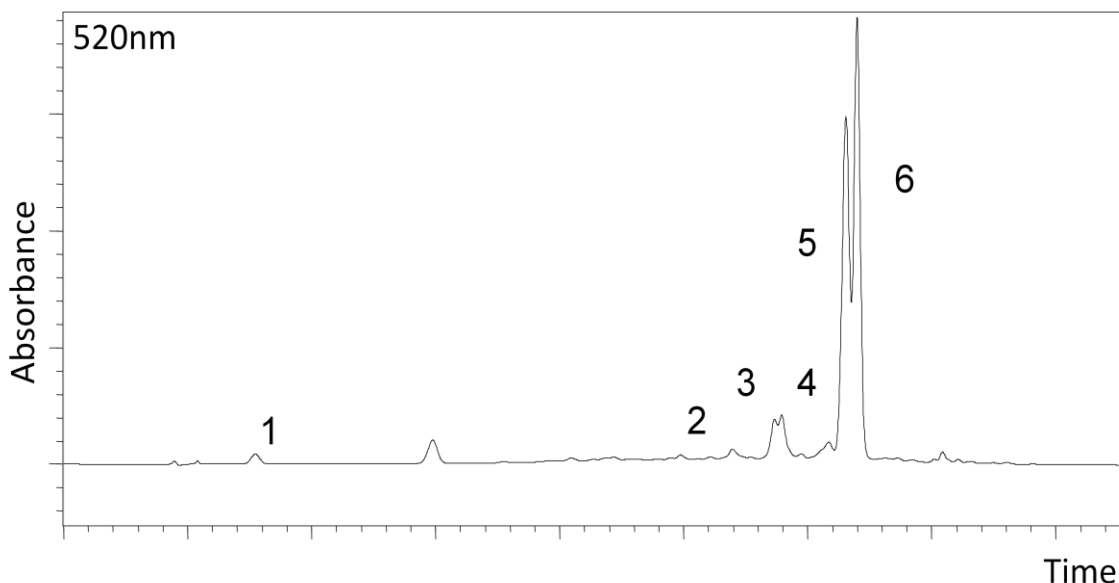


Figure 3.01: HPLC Separation of Red Radish Anthocyanins in the Chemical Extract. Waters Symmetry C-18 Column, 150 x 4.6mm Solvent A: 100% acetonitrile, B: 4% formic acid. Linear gradient 0-30% A in 30min. Flow rate: 1ml/min. Injection volume 20 uL. Peak assignment: 1. Pg-3-soph-5-glu; 2. same, acylated with caffeic acid; 3. same as 1, acylated with p-coumaric acid; 4. same as 1, acylated with ferulic acid; 5. same as 3, with additional malonic acid acylation; 6. same as 4, with additional malonic acid acylation.

3.2 Abrasive Peeler:

Anthocyanins from premium and cull radishes were extracted via abrasive peeler; water was added and the skins were removed by an abrasive and rotary action. The process was very efficient at disintegrating skins, releasing the pigments as evidenced by the white color of the remaining radishes and the magenta color of the water collected. Good pigment extraction from the skins was also obtained with this process. The solids, fragmented pieces of skin and pulp, which were removed, exhibited very little pigmentation. The juice was acidified to pH of 3.5 and produced a vibrant red color, that was enhanced after concentration in a rotary evaporator. Cull radishes produced similar results; the two juices both had a similar magenta hue when they were first extracted with

a pH of 6.5, a similar vibrant red color when acidified to a pH of 3.5, and an intense dark red color when condensed. . Furthermore, there was a “radish smell” that was collected in the condensate-collecting flask; thus, the evaporation step seemed to remove both water and volatiles.

Sample	Monomeric Anthocyanins mg pg-3-glu eq/100g of skin	L*	Chroma*	Hue Angle* (degrees)	Color
Premium	90.5	67.6	63.7	32.8	Red-orange
Cull	89.4	67.7	63.8	32.7	Red-orange

Table 3.01: Monomeric Anthocyanins and Color Characteristics (CIELab) of Premium and Cull Radishes. * = at a concentration of 20mg/100mL

Based on table 2 and the figures 1 and 2 there were subtle differences between the premium and the cull variety of radishes. Similar results were seen in the HPLC chromatograms. Therefore, the pigments (anthocyanins) were effectively removed from the radish skins from both premium and cull radishes with the use of the abrasive peeler; they also have a similar molecular make-up. Thus, cull radishes can be used instead of premium radishes in order to produce a similar natural red juice.

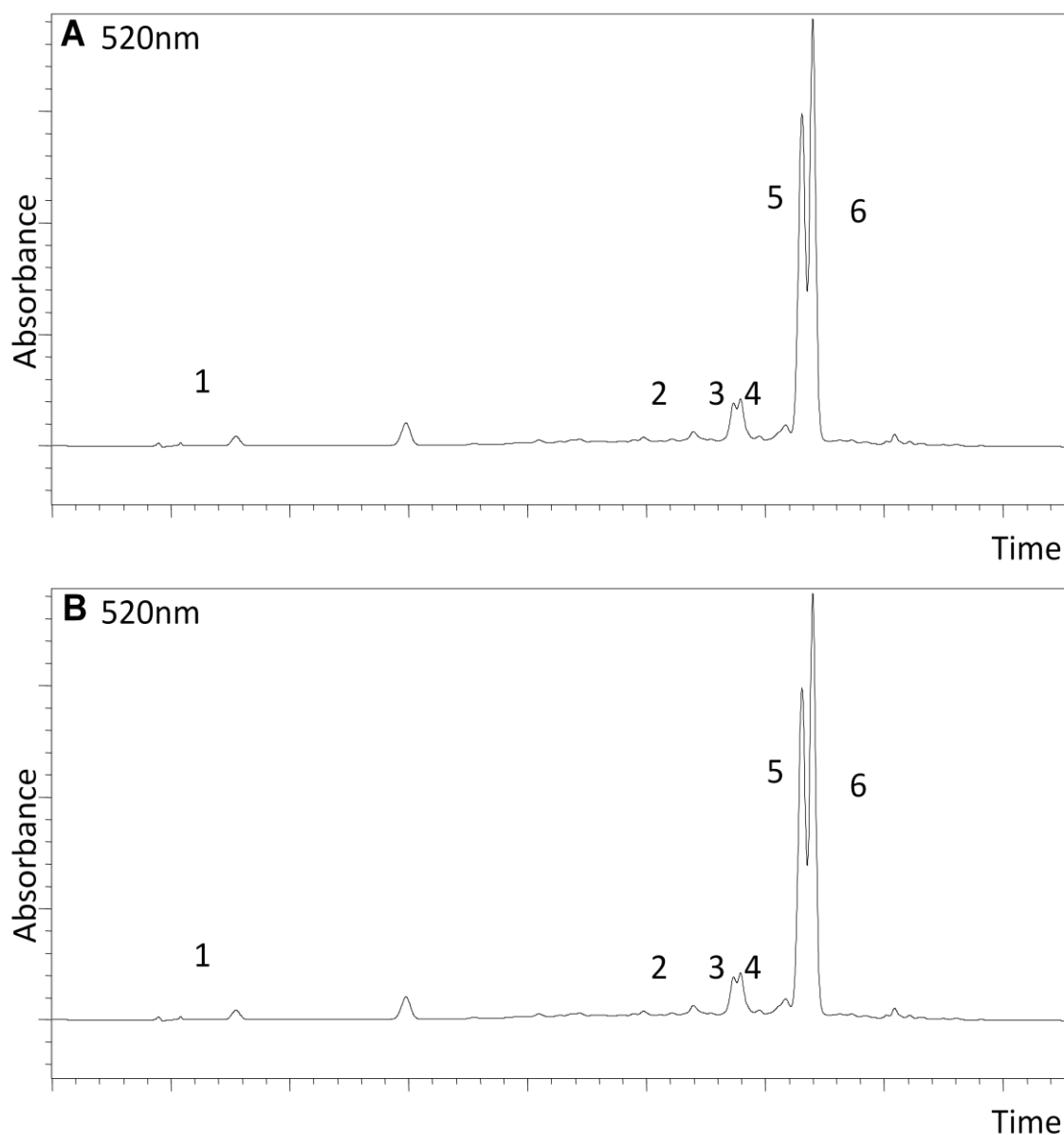


Figure 3.02: Premium (A) and Cull (B) Radish HPLC Chromatogram; Peak Assignment: same as Figure 3.01.

3.3 Dehydration:

The juice was prepared as described with abrasive peeler, filtering, and pH adjustment, followed by concentration in a rotary evaporator. Complete dehydration resulted in a red ring around the round bottom flask, easily re-dissolved in distilled water. This actually produced a vibrant extract with little to no aroma detected.

Radish	Radish concentrate**		
Juice**	1min	5min	10min
15.13	491.5±6.9	488.3±3.6	474.46±7.7

Table 3.02: Recovery or Yield of Total Monomeric Anthocyanins after Various Dehydration Times; Expressed as mg/L of Juice – Average of 2 Replications

It is encouraging that the anthocyanins made it through the excess dehydration period without polymerizing or acid hydrolysis. There were some difference in total monomeric anthocyanins between 1 and 10 minutes, but overall relatively small (table 3). This is potentially due to the enhanced stability of these molecules, as compared to other anthocyanins. The radish juice's lower monomeric number than that of its counterparts; can be explained by the fact that it was not concentrated to the same extent. Instead, it represented the entire 2.1L of juice before concentration/dehydration. By comparing the peak area percentages of the two juices (table 4) one can see some degradation.

However, the major pigments were still present and these differences may have been due to variations in the radish batches (figure 3)

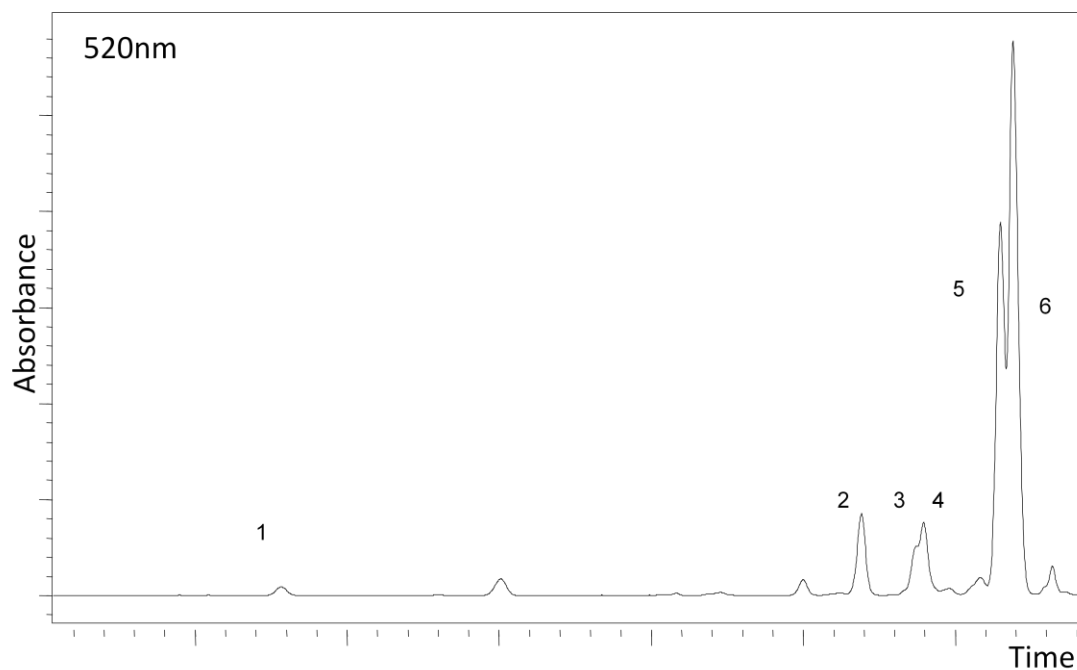


Figure 3.03: HPLC Chromatogram of Dehydrated Juice.

Peak	Regular Juice Area %	Dehydrated 1 min Area %	Name
1	1.5	1.0	Pg-3-soph-5-glu
2	1.3	6.7	Same as 1, acylated with caffeic acid
3	4.1	3.7	Same as 1, acylated with p-coumaric acid
4	4.7	5.4	Same as 1, acylated with ferulic acid
5	41.7	32.1	Same as 3, with additional malonic acid acylation
6	46.8	51.0	Same as 4, with additional malonic acid acylation

Table 3.03: Comparison of Anthocyanin Peak Area Percentage in Radish Regular Juice vs. Dehydrated Juice

3.4 Centrifugal Filtration:

The data (Table 3.04) showed that the smaller the pore size the lower the amount of monomeric anthocyanins being recovered. In other words, some of the color components have become bound/trapped by the filter; more pigment is retained by the filter as the

MW is lowered. Additionally, the filter has a brighter pink aura as the MW is decreased. Some of these can be recovered via sonication; the water turns clear to a light pink and the filters move from a bright pink to a lighter pink.

Filter + Juice	Anthocyanins (mg/100g of skin)
None	117, 139.8
100,000 MW	107.55, 126.5
100k + 3k MW	100.8
5,000 MW	100.76
3,000 MW	99.7

Table 3.04: Monomeric Anthocyanin Results of Radish Juice after Use of Centrifuge Filters

Another point of interest is the fact that one has to increase the time, 30min→1.5hr, and the G's, 5k→7k, for the smaller sized pores to get the liquid through the membrane. Additionally, the 3k still retained a good amount of the initial fluid after 1.5hr; there was a noticeable difference between the colors above the filter (dark bright red) and that below (lighter red). Moreover, as the samples were run through their respective filters there was a difference in the recovered fluid. For example, in the 100k + 3k series, one can notice the slight color changes as the progression changes from dark red to a lighter hue.

3.5 Nano-Filtration:

Nano-filtration was employed on the hope that the isothiocyanates of small MW, would move through the membrane and the anthocyanins of larger MW, would be left behind and collected; resulting in an aroma-free juice. The lab20 unit uses the concept of cross-

flow filtration as opposed to dead end filtration. In that respect, a cake layer is not produced on the membrane surface. Instead, there is a concentrate stream that will be elevated in the components that are retained by the membrane (anthocyanins) and the waste (isothiocyanates) is removed. Three different nano-filtration membranes were used; they are listed in order of increasing porosity: (1) DOW NF245, (2) Hydranautics 7450 (3) Hydranautics 7410.

The use of the Hydranautics 7410 membrane, the largest pore size, resulted in a net loss of 11mg of anthocyanins (Table 3.05). There was some loss with the use of the Whatman filter, but this can be recovered easily with acidified water. However, there seemed to be a greater loss with the use of the membrane; as can be seen by a pink presence on the membrane itself. Moreover, a quick smell test confirmed that the radish odor was still present.

Sample	Monomeric Anthocyanins mg/100g of skin
Original Liquid (No filtration)	83.6
Filtered Through Whatman Paper	78.2
Hydranautics 7410 (Nano-Filtration)	72.6

Table 3.05: Monomeric Anthocyanin Loss Throughout Hydranautics 7410 Nano-filtration Processing

The use of the Hydranautics 7450 membrane, the medium pore size, resulted in a net loss of 14.8mg of anthocyanins (table 3.06). There was some loss with the use of the

Whatman filter, but this can be recovered easily with acidified water. However, there seemed to be a greater loss with the use of the membrane; as can be seen by a pink presence on the membrane itself. Moreover, a quick smell test confirmed that the radish smell was still present despite the smaller pore size.

Sample	Monomeric Anthocyanins mg/100g of skin
Original Liquid (No filtration)	84.9
Filtered Through Whatman Paper	80.3
Hydranautics 7450 (Nano-Filtration)	70.1

Table 3.06: Monomeric Anthocyanin Loss Throughout Hydranautics 7450 Nano-filtration Processing

The use of the Dow NF245 membrane, the smallest pore size, resulted in a net loss of 14.9mg of anthocyanins (Table 3.07). This was just a little bit more than the Hydranautics 7450 membrane. There was some loss with the use of the Whatman filter, but this can be recovered easily with acidified water. However, there seemed to be a greater loss with the use of the membrane; as can be seen by a pink presence on the membrane itself. Moreover, a quick smell test confirmed that the radish aroma was still present despite the smaller pore size.

Sample	Monomeric Anthocyanins mg/100g of skin
Original Liquid (No filtration)	84.0
Filtered Through Whatman Paper	80.5
DOW NF245 (Nano-Filtration)	69.1

Table 3.07: Monomeric Anthocyanin Loss Throughout DOW NF245 Nano-filtration Processing

Overall, as the pore size of the membrane decreases so increases the “loss” of anthocyanins. It appears that the molecule would adhere to the membrane; thus, making the membrane appear like it was wearing a pink coat. This was similar to the results with the centrifuged filters. There was a net loss 11mg-14.9mg (largest pore to smallest pore); depending on the membrane selected. One would believe that if the juice was concentrated, instead of diluted, it would potentially increase the amount of anthocyanins lost. Attempting to wash the anthocyanins off the various membranes with acidified water was not particularly effective. Additionally, the pungent smell would still be present after filtration. Furthermore, these membranes are potentially quite expensive and would not be practical to use for a smaller business or one looking to cut costs and make a good aroma free red colorant.

3.6 High Salt and Low pH Enzyme Inhibition:

Enzymes are susceptible to various conditions; they can be inhibited and usually have a specific preferred environment. Myrosinase’s activity is reduced to about 40% at a pH of 3 and below 40% at a pH of 10 (Iori, 1996). Enzymatic operation is further inhibited with the presence of a negative anion at high concentrations, for instance Cl^- (Iori, 1996). Combining these two pieces of information we decided to use an acidic (below 3.5) salt solution ($>2.2\text{M}$) on the abrasive peeler instead of tap water to extract the pigments. Based on in-house sensory testing it produced a juice with low aroma and the same deep

red color obtained before. Additionally, the acid present in the water may actually stabilize the anthocyanins upon initial extraction (table 3.08)

Sample	Monomeric Anthocyanins (mg/100g of skin)
Low pH, High Salt	90.4

Table 3.08: Monomeric Anthocyanins Results of High Acid and High Salt Additions

There is some variation in the chromatogram (figure 3.04) compared to that of the regular juice. The initial peaks could be some sort of impurities present despite the use of a filter. Additionally it seems that there is a decrease in the 5th peak, Pg-3-soph-5-glu acylated with p-coumaric acid and additional malonic acid acylation, when compared to the regular juice; 24.18 (salt) vs. 34.82 (regular) % area. This could potentially be due to varietal differences in the radishes or some sort of degradation.

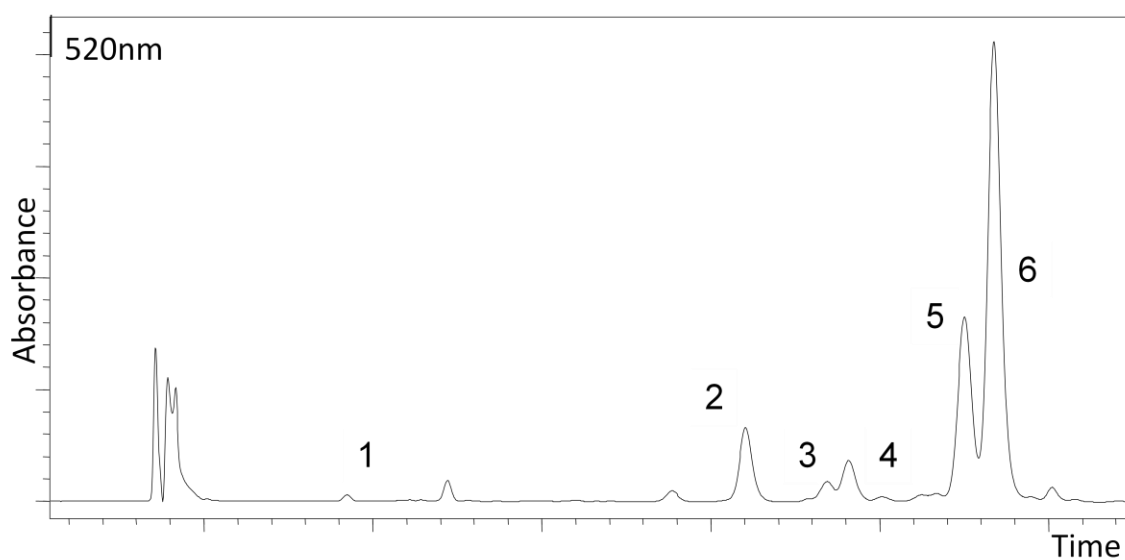


Figure 3.04: HPLC Chromatogram of High Acid and Salt Radish Juice

3.7 Sensory:

A sensory test was done to compare the effect of various treatment methods on the radish juice. Overall visual liking of the juices was similar for commercial drink mix (colored with FD&C Red No. 40) and the samples colored with radish anthocyanins. The only difference was the nano-filtrated sample; p -value <0.05 . In other words, consumers liked the radish juice as much as they liked the color of the radish juice produced with the cull radishes, indicating they all looked very similar to FD&C Red No. 40. This would be expected since the color of the juice is similar to that of FD&C Red No. 40 at a pH of 3.5 (figure 3.05).

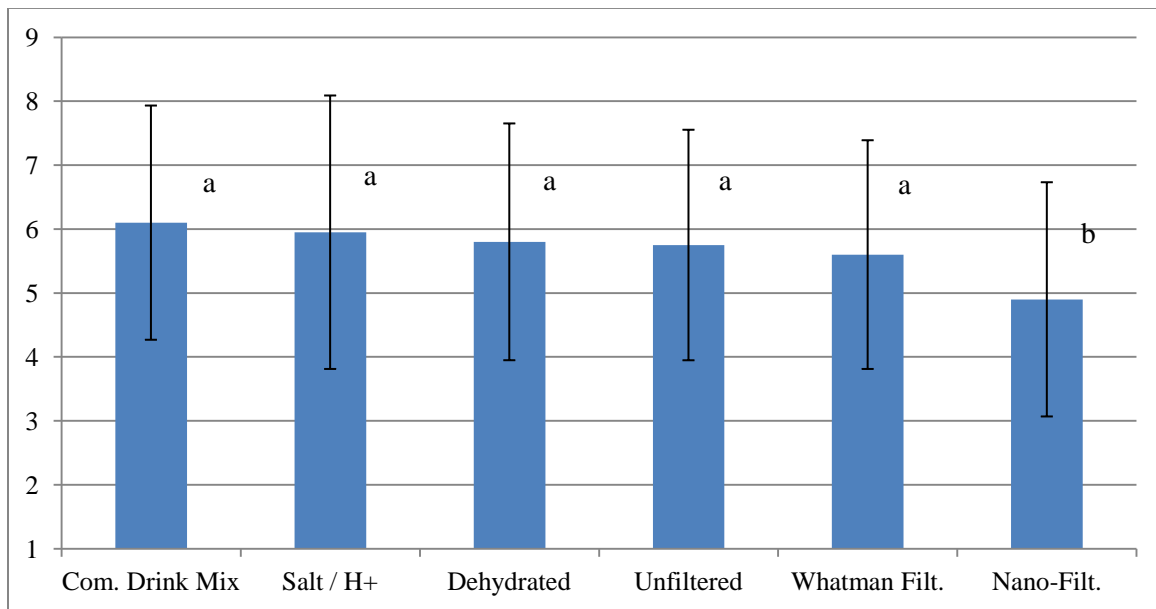


Figure 3.05: Overall Visual Liking of the Different Juices (p -value of <0.05).

The commercial drink mix performed the best (p -value <0.05). The aroma of the commercial drink mix sample was preferred by consumers, and followed by the dehydrated and the salt treated samples, with significant difference between them others

lagged behind. The commercial drink mix sample was expected to do well since it had a fruity aroma. Additionally, it shows that using salted water and/or dehydration did a good job preventing/removing the negative aroma notes from the radish juice (figure 3.06).

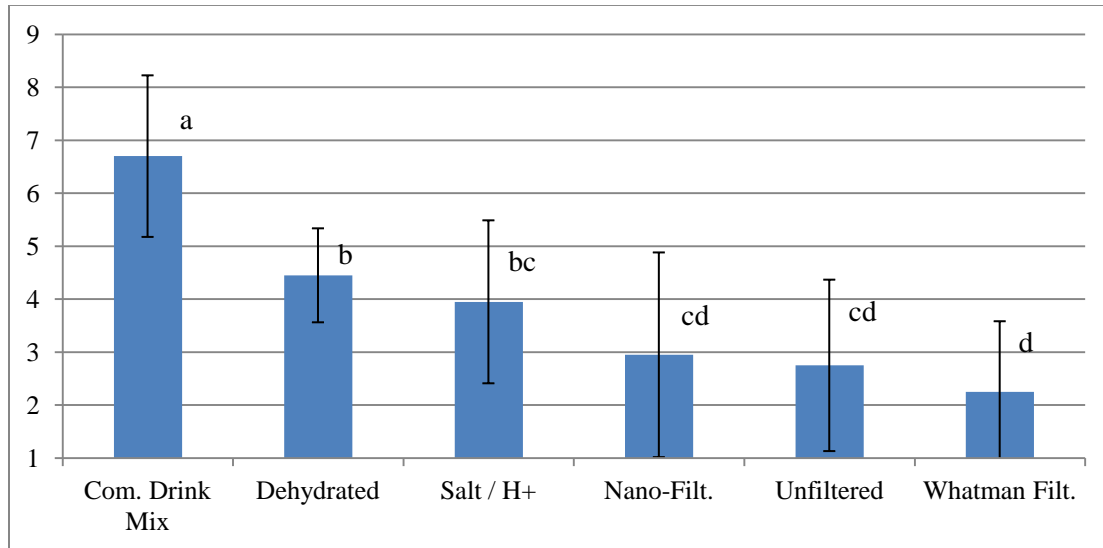


Figure 3.06: Overall Liking of the Juice Aroma of the Different Juices (p-value of <0.05).

The commercial drink mix, dehydrated, and salt inhibited samples all had relatively low intensity, p-value <0.05 . However, the dehydrated sample had the lowest intensity. The juice produced with salted water was not significantly different than the dehydrated sample and the commercial drink mix (figure 3.07). This would be expected since dehydration works well at removing the aroma as does the salt at preventing the aroma from occurring.

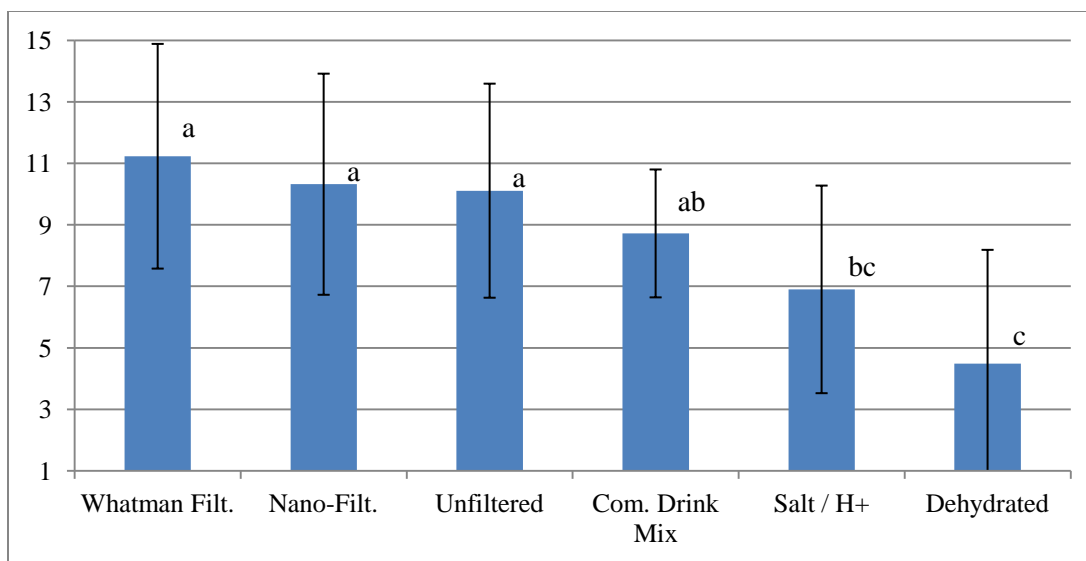


Figure 3.07: Aroma Intensity of the Different Juices (p-level of <0.05).

Overall, the color of the various juices, as compared to that of FD&C Red No. 40, is quite appealing to potential consumers. The negative aroma notes can be prevented/removed with the use of salt water and/or dehydration. Even though the use of high salt and acid is successful, based on the sensory tests, there is a problem with removing the salt; especially since it is at such a high concentration, $>2.2M$. In order to remove the salt reverse osmosis could be used, but this would add to the overall cost of the product. A radish colored salt could potentially be made from this type of product simply by removing the water present in the system. Since the dehydrated sample performed well in the sensory evaluation this would be a good route to take. It is a quick evaporation/dehydration that would potentially be more economical. Based upon the results of the sensory evaluation it would have a lower aroma as compared to other methods that were employed.

3.8 Steamed Radishes:

3.8.1 Preliminary Tests with a Steam Jacketed Kettle:

Radishes were placed inside a birdcage and into a steam jacket kettle with the goal of denaturing Myrosinase; the enzyme denatures at temperatures above 70°C / 158°F. The radishes were removed and taken to the abrasive peeler with the hope of achieving little to no aroma. After steaming the radishes seemed paler in color and the water at the bottom of the kettle was a dark purple color. The water was acidified and it changed from blue/purple to a red/orange. This indicated that some of the anthocyanins were present in the water at the bottom of the steam jacket kettle. There also appeared to be no aroma.

The chromatogram, figure 3.08, shows that there is degradation present in the sample. This is probably due to the extreme boiling conditions and slightly basic conditions that these molecules were subjected to. This hydrolysis led to an increase in the occurrence of pelargonidin-3-sophoriside-5-glucoside, Pg-3-soph-5-glu acylated with p-coumaric acid, and Pg-3-soph-5-glu acylated with ferulic acid which is seen in table 3.09. Other variations seemed to be produced as well based on the chromatogram, but their structures were not evaluated.

A few changes were made to the process in order to take advantage of the ease recovery of anthocyanins with steam. The steam condensate had a high pH level and in order to stabilize the anthocyanins, the collection water was acidified. The sample was immediately placed in an ice bath to avoid heat degradation and filtered to remove any tissue that may have contaminated the sample.

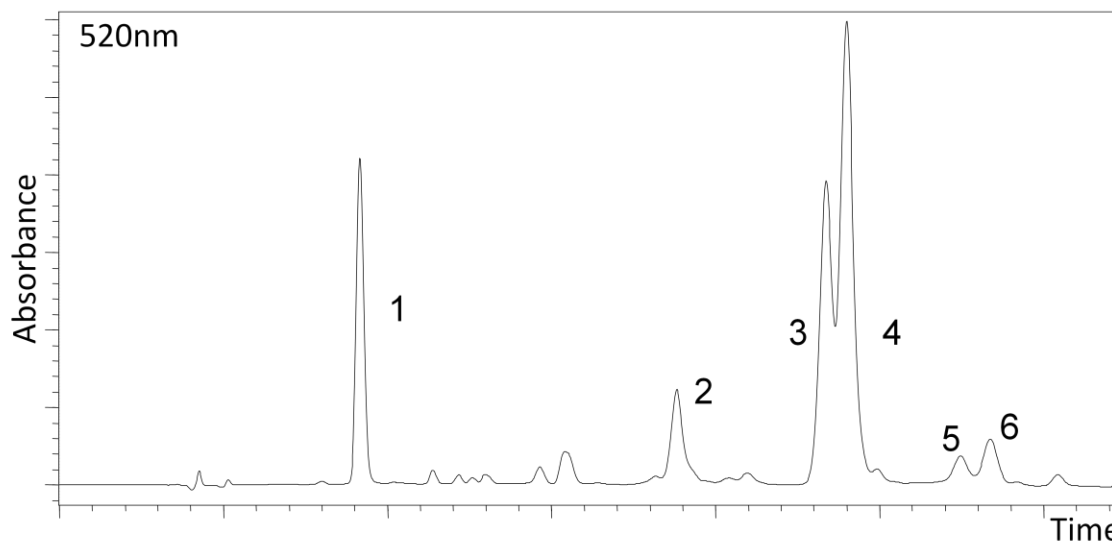


Figure 3.08: HPLC Chromatogram of Acidified Water at the Bottom of the Steam Jacket Kettle

Peak	Area %	Name
1	18.1	Pg-3-soph-5-glu
2	0.6	Same as 1 acylated with caffeic acid
3	28.6	Same as 1 acylated with p-coumaric acid
4	45.6	Same as 1 acylated with ferulic acid
5	2.8	Same as 3 with additional malonic acid acylation
6	4.3	Same as 4 with additional malonic acid acylation

Table 3.09: Percent Area of the Known Anthocyanin Peaks from the Acidified Water at the Bottom of the Steam Jacket Kettle

The steaming was repeated for 2.5 minutes and monomeric anthocyanin data (table 3.10), was collected to see the efficiency of steaming on removing the anthocyanins when coupled with the abrasive peeler. Afterwards the radishes appear to light pink in color and softer after steaming; the longer the steaming the lighter or duller the radishes appear. The water that collected from the steaming process was intensely colored or concentrated.

Sample	mg/L	mg	mg/100g	mg/kg
Steamed	92	100.94	26.18	52.25
	total %	30	30	30
Abrasive	58.35	233.4	60.54	120.82
Peeler	total %	70	70	70
	total mono	334.34	86.72	173.07

Table 3.10: Preliminary Monomeric Anthocyanin Results from Steamed Radishes

After the sample was cooled there was no aroma present; as confirmed by an in house laboratory sensory evaluation. The Brix value was also lower with the steam treatment; approximately 1.3. This is half of the normal value obtained with the abrasive peeler the steam just penetrates the outside of the radish and the skin and excess amounts of pulp are not removed. There was a recovery of 30% of the anthocyanins which appeared to be good for the short time, 2.5 minutes. It is hypothesized that an increase in steaming time would allow these percentages to increase as well. Despite the success with this method there was the need to see how higher temperatures and pressure effects anthocyanin yields. Ideally one would like to obtain a recovery greater than 50%.

3.8.2 Evaluation of the Steam Jacketed Kettle Processing:

Based on the preliminary results with the steam jacket, additional experiments were designed to determine the conditions that would recover more pigments from the radish skins.

The monomeric anthocyanin yield increased with time; from about a 30% recovery at 2.5 minutes to a 52% recovery at the ten minute mark, without any drastic changes on the anthocyanin profiles (figure 3.09 and tables 3.11 and 3.12). In essence the longer that the radishes are exposed to the steaming process the more anthocyanins can be extracted from the skins (table 3.12).

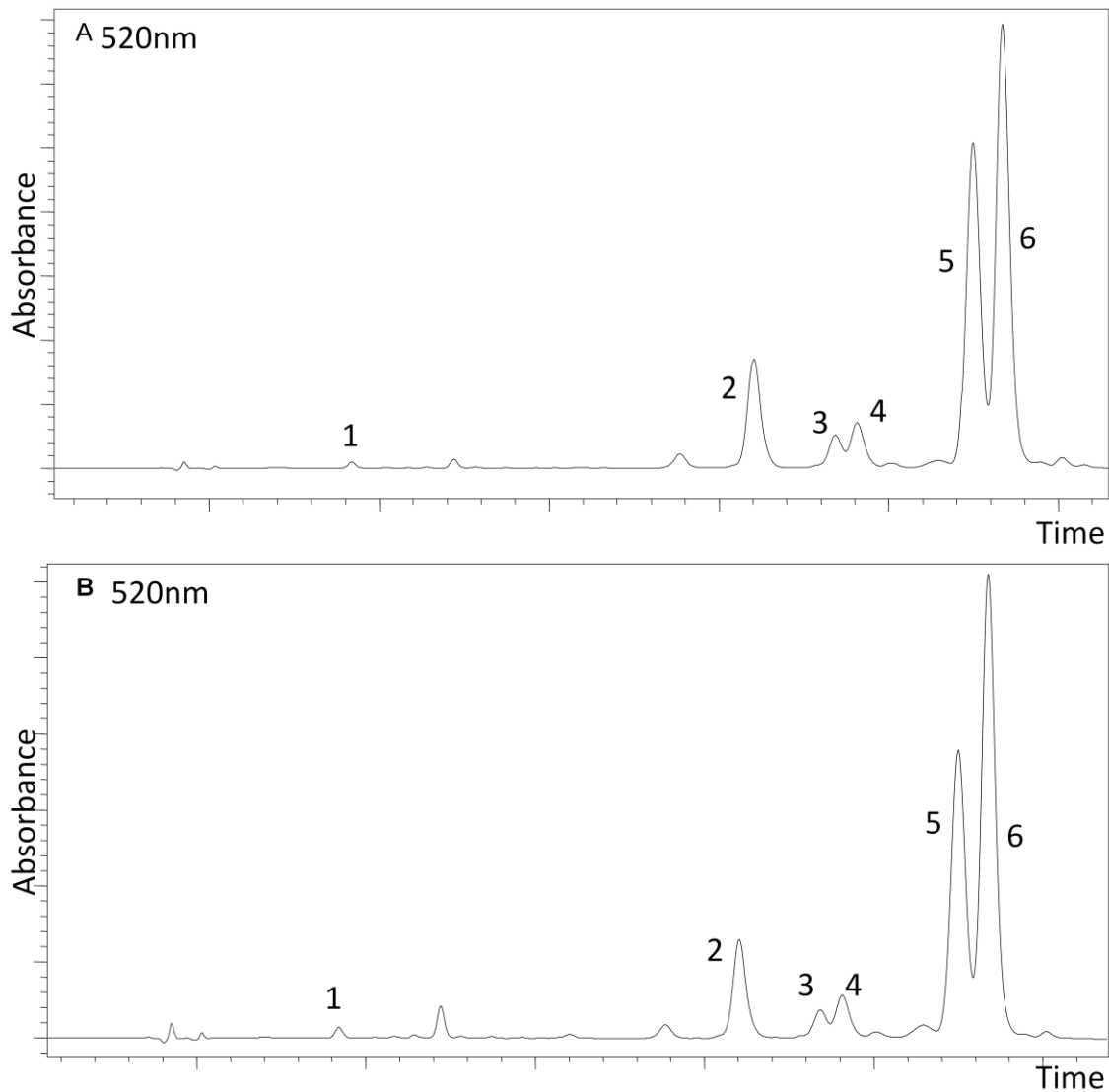


Figure 3.09: Anthocyanin Profiles of Regular Radish Juice (A) and Juice Obtained After 10 Minute of Steaming (B)

Peak	Radish Juice	2.5 Min	5 Min	10 Min	Name
1	0.3	0.5	0.5	0.6	Pg-3-soph-5-glu
2	10.7	11.1	10.7	10.1	Same, acylated with caffeic acid
3	2.8	2.2	2.5	2.6	Same as 1, acylated with p-coumaric acid
4	4.5	3.2	3.4	4.3	Same as 1, acylated with ferulic acid
5	34.8	33.5	32.9	32.9	Same as 3, with additional malonic acid acylation
6	46.8	49.6	50.1	49.4	Same as 4, with additional malonic acid acylation

Table 3.11: Comparison of Peak Area Percentage of Radish Juice and Steamed Radish Juices at Various Times.

Time	Mg/L	Mg	mg/100g	mg/kg	%
2.5 min	222.52	72.32	18.76	37.44	29.9
5 min	258.84	90.60	23.50	46.90	37.9
10 min	268.90	121.00	31.38	62.64	51.9

Table 3.12: Monomeric Anthocyanins and Percent Recovery of Steamed Staples at Various Times

The samples have a loss of less than 5% when taken dehydrated and reconstituted with the rotary-evaporator. Thus, it appeared that there was little degradation during the dehydration process (table 3.13). There was some loss, but the data does suggest that a condensing process should not affect the juice too much.

Steamed Samples	2.5 min	5 min	10 min
mg/100g after Steaming	20.52	25.49	31.47
mg/100g after Evaporation and Rehydration	19.51	24.22	30.12
Percent (%) loss	4.9	5.0	4.3

Table 3.13: Monomeric Anthocyanin Loss (%) during Evaporation and Rehydration of Steamed Samples

It appears that as the time increased all of the values change, the L-value becomes higher and thus lighter in color. The a-value decreased with increasing time and decreased by about twenty-four units; becoming less red. The b-value decreased with increasing time also; becoming less yellow. Overall, it appears that the radishes become lighter/duller color with increased time of treatment with steam (table 3.14). The increased time in steam exposure seems to allow the anthocyanins, which are responsible for the color, to migrate out of the epidermal tissue and into the water.

Time (minutes)	L	A	b
0	31.09	36.57	10.9
2.5	36.57	21.97	2.97
5	38.03	14.91	2.77
10	39.88	12.62	0.98

Table 3.14: Color Characteristics (CIELab) of Steamed Radishes after the Various Treatment Times

3.8.3 *Still Retort:*

A still retort was used to achieve higher temperatures and higher pressure in hope of removing more anthocyanins from the epidermal tissue. Various temperatures were evaluated at times of 2.5, 5, and 10 minutes (figure 3.10).

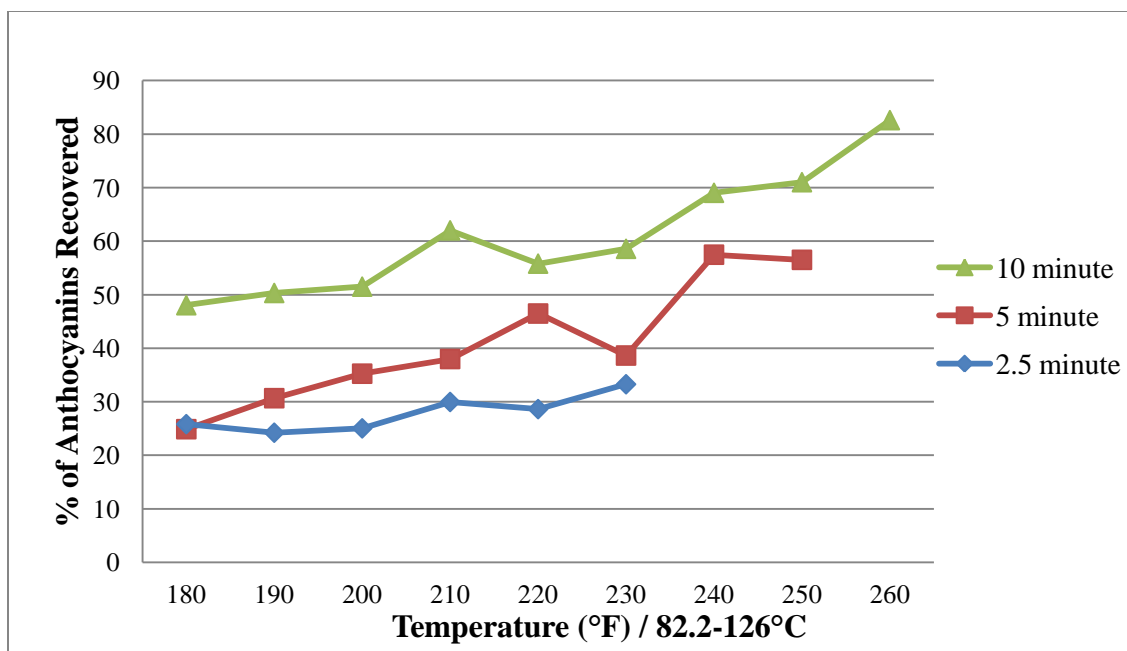


Figure 3.10: Percent of Anthocyanins Recovered During Steaming at Various Temperatures and Times (2.5, 5, and 10 Minutes).

There is a general increase in the amount of anthocyanins extracted from the radishes when they were exposed to the various temperatures. There is not a smooth line from 180 °F to 230 °F (82.2 - 110°C); this may be due to possible experimental variation from batch to batch. Further temperatures were not examined since the yield seemed low compared to that of the other times, as shown in figure 3.09. It is positive that this method and short time interaction is capable of extracting the anthocyanins. The percentage of anthocyanin recovery increases from around 50% to just past 80% when the steaming treatment lasted for 10 min.

Ten minutes at 250°F were the more extreme conditions of time and temperature tested. Under these conditions, the main anthocyanins remain present (peaks 5 and 6), with

peaks 3 and 4, also acylated pelargonidin-derivatives, becoming more abundant (figure 3.11). These molecules appeared to hold up rather well to these adverse conditions.

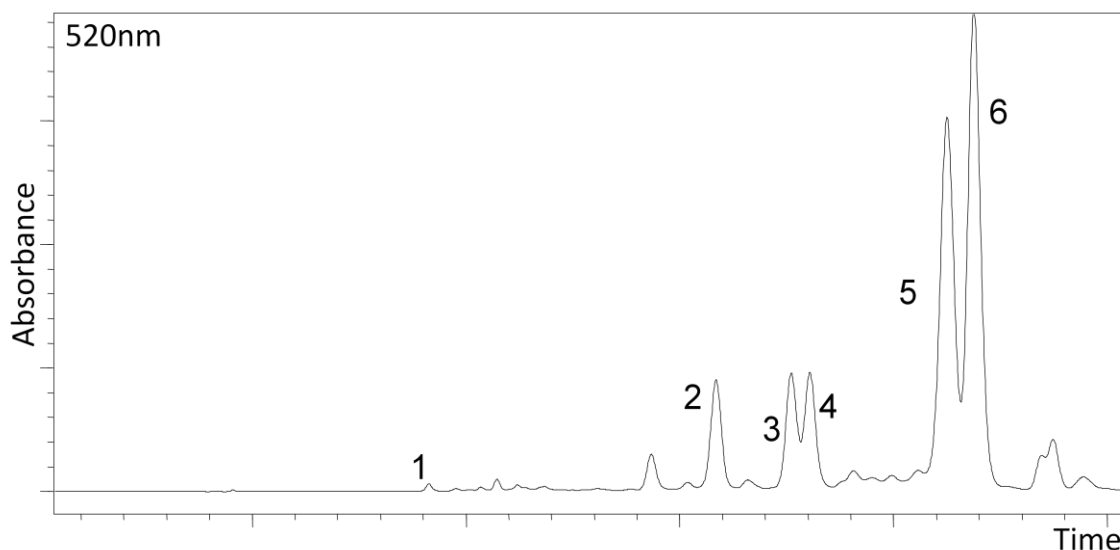


Figure 3.11: HPLC Chromatogram of Radishes in Still Retort at 250°F for 10 Minutes.

From the data, table 3.15, one can see that as temperature increases that there are minimal signs of anthocyanin degradation when compared to that of the untreated juice. This is quite encouraging since the sample was exposed to extreme conditions of 250°F for 10 minutes. All of the spectras have little bumps throughout; these may be signs of slight degradation or other organic compounds that resulted from the steaming process. As far as table 16 is concerned, there seems to be slight differences in the percentages of peak area. This may be due to slight variations in the methods used or that these anthocyanins are from different varieties of radishes.

Peak	Regular Juice	180°F 2.5 Min	220°F 5 Min	250°F 10 Min	Name
1	0.3	0.2	0.3	0.3	Pg-3-soph-5-glu
2	10.7	7.7	7.9	7.7	Same, acylated with caffeic acid
3	2.8	3.9	6.8	8.3	Same as 1, acylated with p-coumaric acid
4	4.5	2.9	5.6	8.8	Same as 1, acylated with ferulic acid
5	34.8	43.7	38.7	33.6	Same as 3, with additional malonic acid acylation
6	46.8	41.6	40.8	41.4	Same as 4, with additional malonic acid acylation

Table 3.15: Comparison of Peak Area Percentage of Radish Juice and Still Retort
Derived Radish Juices at Various Times and Temperatures

The overall color of the radish seems to be a dark red color before steaming occurs. One can tell this by just looking at the material, but the numbers make it less subjective. The L-value is based on 0-100 scale; with 0 being black and 100 being white. The a-value is red when positive (+) or green when negative (-). The b-value is yellow when positive (+) or blue when negative (-).

As the temperature increased so did the lightness (L-value) of the radishes (table 17).

This trend seems to be true even when treated at the lowest temp, 180, for a short amount of time. This lightening is most likely due to the migration of the anthocyanins from the skin to the acidified water. Additionally, the “a” value decreases with the amount of time and interesting it decreases by about two from the un-steamed to 230°F. In other words it is moving from the red sector and inches towards the origin and green sector as the temperature increases. Once again this could possibly be the loss of anthocyanins from

the skin. The “b” value decreases as well and moves more towards “0” as the temperature increase.

From table 3.16 it can be seen that all of the radishes became lighter in color after the steaming process. The time of exposure does matter in terms of all of the values. The less time and lower temperature produce a darker colored steamed radish while more time coupled with a higher temperature produced a lighter colored radish. This can be seen with the naked eye

Temperature (°C/°F)	2.5 min			5 min			10 min		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
0	31.1	36.57	10.90	31.09	36.57	10.90	31.09	36.57	10.90
82.2 /180	32.5	25.56	2.59	32.91	25.76	2.23	34.71	18.98	1.939
87.7 /190	32.6	24.7	2.15	38.02	21.72	2.69	37.54	10.66	1.51
93.3 /200	33.6	26.05	3.23	34.56	22.16	1.84	34.4	16.96	1.64
98.8 /210	36.8	23.1	2.77	37.99	14.89	2.67	34.88	16.65	0.97
104.4 /220	36.7	19.07	2.11	36.26	14.92	1.25	32.83	16.15	1.4
110 /230	35.8	17.81	3.95	36.74	18.47	3.8	37.89	11.23	-0.82
115.5 /240	--	--	--	36.35	15.02	4.15	39.04	7.14	1.28
121.1 /250	--	--	--	39.1	16.09	1.45	40.36	8.5	3.3
126.6 /260	--	--	--	--	--	--	39.23	8.19	4.04

Table 3.16: Color Characteristics (CIELab) of Radishes after Steaming at Various Temperatures for 2.5, 5, and 10 Minutes

Despite the success with the still retort there were some issues. The higher the temperature the more anthocyanins were successfully extracted, but an intense “vegetable” aroma would accompany the juice. The steam also was not culinary-grade and contained an appreciable amount of iron from the rusted pipes; this would carry over to the product. Additionally, the longer the heat exposure the more mealy the radishes

would become; at 126.6°C (260°F) they were reduced to mush once they were run through the abrasive peeler. The high heat/temperature would not allow for remaining radishes to be made into a potential commercial product. However, one would hypothesize that if these high heat extracted juice was placed on the rotary evaporator the vegetable aroma could be removed; leaving only a highly concentrated juice; yet, the iron taste may still be present.

3.9 Sensory Analysis of Radish Juice obtained with Steam:

A sensory test was done to compare the effect of various treatment methods on the radish juice. It is important to note that the steamed radish juice evaluated in this test was not concentrated, but used directly after steam processing. In water, the average intensity of the steamed sample (figure 3.12) was slightly lower but not significantly different (p-value > 0.05) than the abrasively peeled. Water colored with FD&C Red No. 40 was rated significantly lower (p-value < 0.05) than the others.

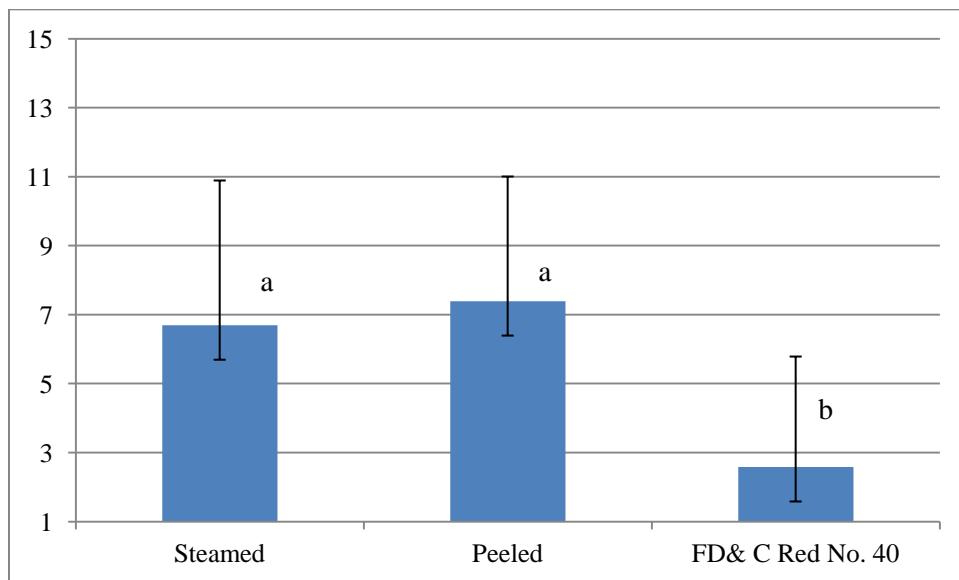


Figure 3.12: Aroma Intensity of the Juice in Water (p-value <0.05).

However, when the color was incorporated into the commercial drink mix, consumers preferred (p-value <0.05) the aroma of the sample colored with the steamed extract over the one colored with pigments obtained by abrasive peeling (figure 3.13). The aroma of the drink mix colored with FD&C Red No. 40 was still preferred over the other two methods.

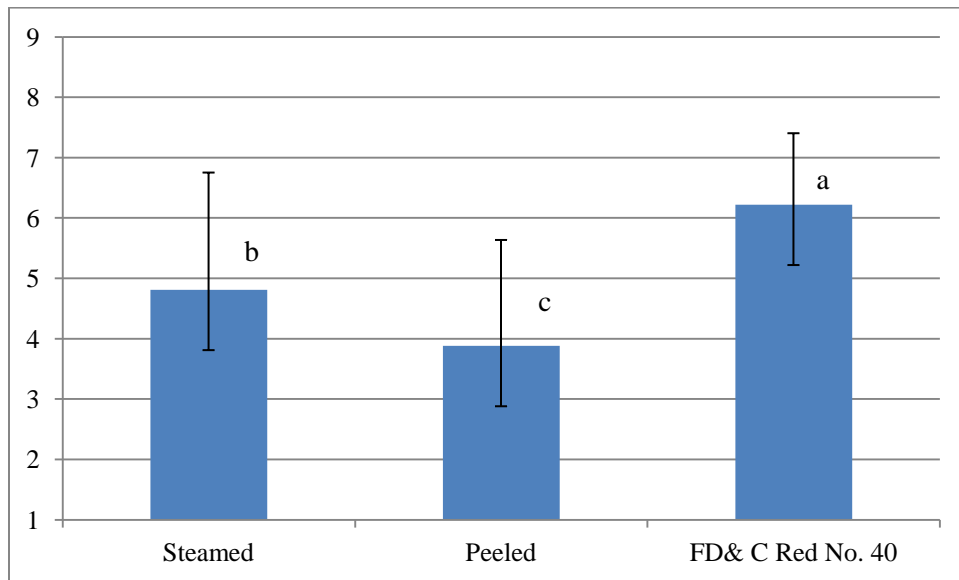


Figure 3.13: Liking of the Juice Aroma in Kool-Aid (p-value <0.05).

Even more, when asked to taste the samples, consumers did not find the flavors of the samples colored with FD&C Red No. 40 and the steamed extracts to be significantly different (p-value > 0.05). The flavor of the sample colored with radish extracts obtained by abrasive action were liked significantly less than the sample with the synthetic dye as seen in figure 3.14 (p-value <0.05). The steam may only penetrate the outside layers of the skin and may also denature myrosinase during the extraction process, resulting in potentially less formation of undesirable flavor components.

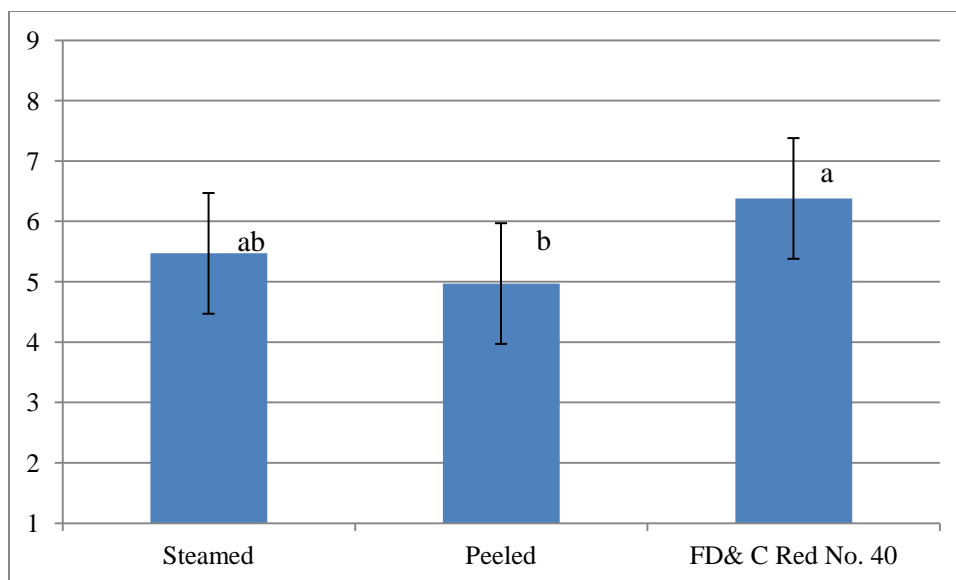


Figure 3.14: Liking of the Juice Flavor in commercial drink mix (p-value of <0.05).

Overall, the steaming process produced a better product when integrated into a food matrix than the abrasively peeled sample; in both the aroma and flavor. However, the steamed sample did not do as well as FD&C Red No. 40, but was not that different in the liking of the juice flavor category. However, combining steam with dehydration (first sensory evaluation) would potentially produce a better product.

3.10 Colored Salt:

Various colored salts were produced in order to create a potential product with the salted radish juice. Upon dehydration of the liquid a colored crystal would remain. The color was modified with simple acid and base treatment in order to obtain a desired hue.

Various shades of red/magenta were easily produced. Additionally, the salts seemed to have little aroma; especially when made from fresh juice. An in-house sensory evaluation of the product suggested that consumers may be intrigued by the product. This could

potentially be used a novelty salt and could possibly garnish a drink like a margarita or make a dish seem more interesting or appealing.

Chapter 4 Conclusion:

Based on overall goal, we were successful in attempting to make a natural colorant from cull radishes. The juice obtained with steam was of high quality. While other methods were successful for removing aromas, there were complications that were not present with the use of steam. Steaming red radishes for 10 min, at 100°C seemed to be the most efficient process for the recovery of anthocyanins from cull radishes for production of a high quality commercial product that could be directly added to foods to provide color under the already approved category of vegetable concentrate.

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