MITIGATING ANTHOCYANINS AND COLOR DEGRADATION IN PASTEURIZED CRANBERRY JUICE FORTIFIED WITH VITAMIN C

By

Sunisa Roidoung

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ABSTRACT

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Color degradation in cranberry juice during storage is the most common consumer complaint for this juice. To enhance nutritional quality, juice is typically fortified with vitamin C. Although vitamin C is an effective antioxidant, vitamin C fortification increases degradation of color in cranberry juice during storage. The color degradation is not only an appearance attribute, but also reflects the degradation of health beneficial components, anthocyanins (ACY), because ACY are natural pigments as well as antioxidant compounds. The overall goal of this study was to preserve endogenous ACY in cranberry juice with a feasible solution for the food industry. This study included two specific aims: 1) to evaluate the effectiveness of different antioxidants on ACY retention in cranberry juice and assess the effect on vitamin C retention, color intensity, and browning index (BI) during storage; and 2) to estimate the kinetic parameters and model predictive equations for color and ACY retention in cranberry juice during storage. Three natural phenolic compounds (hesperidin, catechin, and gallic acid) were tested for their protective effect against anthocyanins and color degradation. Cranberry juice was fortified with 40-80 mg/100 mL ascorbic acid and potential protective agents were added at different concentrations. The juice was then pasteurized at 85±2°C for 1 minute and stored in the dark at 23±2°C for 16 days. Juice ACY, vitamin C, color intensity, and BI were evaluated at 2-day intervals. Among the three phenolic compounds, gallic acid showed the most effective protection against ACY degradation. Addition of gallic acid significantly increased red color intensity (37%) ($p < 0.01$) and ACY concentration (41%) ($p < 0.03$) during storage, compared to control juice samples. At the end of 16-day storage,

the BI of gallic acid-added juice was significantly lower than that of the control juice (0.80 vs 1.00), confirming the protective effect of gallic acid on juice color. Therefore, the experimental data with gallic acid addition were used for the kinetic study, in order to develop predictive equations for the parameters and the dependent variables. Measurements of total monomeric anthocyanins and red color intensity were used to determine degradation rate constants (*k* values) and order of reaction (*n*) of ACY and color. Due to high correlation, *k* and *n* could not be estimated simultaneously. To overcome this difficulty, both *n* and *k* were held at different constant values in separate analyses to allow accurate estimation of each. Parameters *n* and *k* were modeled empirically as functions of vitamin C, and of vitamin C and gallic acid, respectively. Reaction order *n* ranged from 1.2 to 4.4, and decreased with increasing vitamin C concentration. The final models offer an effective tool that could be used for predicting ACYs and color retention in cranberry juice during storage. The outcome of this research not only provided a potential solution of using gallic acid to address color degradation in commercial cranberry juice, but also proposed models for predicting color and ACY retention.

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CHAPTER 1

INTRODUCTION

1.1 Overview of the dissertation

Cranberry products are widely consumed due to their potential health benefits. American cranberries (*Vaccinium macrocarpon*) are a good source of phytochemicals, which potentially provide health benefits not only in reducing risks of cancer and heart disease, but also in protecting from Alzheimer's development, diet-induced obesity, insulin resistance, and intestinal inflammation (Anhe and others 2015; Howell 2007; Zafra-Stone and others 2007). Cranberries have also gained interest among patients with urinary tract infections. Proanthocyanidins in cranberries are active compounds that help preventing bacteria adhesion to the urothelial cell wall (Mansour and others 2014; Durham and others 2015; Foxman and others 2015). Due to these benefits, American cranberries are nowadays consumed not only during traditional holidays, but also throughout the year as a regular diet in different forms (e.g., juice, sauce, dried fruits).

Commercial juice products are commonly fortified with vitamin C (ascorbic acid) in order to extend shelf-life and enhance nutritional quality. Mostly, the fortification levels follow dietary guideline for vitamin C daily intake; 75 (women) and 90 (men) mg/day (NIH, 2016). As a result of fortification, manufacturers claim 100% vitamin C of recommended daily intake on the product label. However, vitamin C fortification could cause anthocyanin (ACY) degradation via oxidation. Cranberry ACYs are natural pigments that provide a bright red color to cranberry juice, and also are well-known for their antimicrobial and antioxidant functionalities. The ACY degradation, besides lowering antioxidant compounds, results in an unpleasant brownish color in juice products, which is a major consumer complaint.

The detrimental effect of vitamin C on ACY is well-known, it is a common commercial practice to fortify fruit juices with vitamin C, which is added either to prevent oxidation in juices, or to increase nutritional quality so as to claim "100% vitamin C daily intake" on the label.

Attempts to overcome ACY degradation resulting from vitamin C fortification have been previously researched. Since oxidation plays a role in ACY and color degradation, polyphenols, as natural antioxidants, have been studied for their protective effect. The application of polyphenols in juices is rather limited due to their relatively low water solubility. Encapsulation or ACY structure modification has been suggested in order to improve stability of bioactive compounds (Matsufuji and others 2007). However, these methods may increase the cost of juice production, and hence could increase the retail price.

In fact, among the tremendous variety of phenolic compounds in nature, there are some compounds that are soluble in water (e.g., catechin and gallic acid) (Srinivas and others 2010b), or the water solubility substantially increases at elevated temperature (e.g., hesperetin) (Liu and Chen 2008). These compounds had never been investigated for their antioxidant effect in juices with fortified vitamin C. Therefore, it is desirable to investigate these potential compounds, which could give a feasible solution to the juice industry and, hence, avoid adding artificial colorants such as Red #40 to maintain the color.

1.2 Statement of problem

1. There are no reports of applicable phenolic compounds that could be practically applied in the commercial juice industry to overcome color degradation in vitamin C-fortified pigment-rich juices.
2. The effect of vitamin C fortification on degradation kinetics of ACY and color in juices is rarely discussed in most studies.

3. There are no publications that have reported the use of advanced parameter estimation techniques to determine degradation rate constant (*k* value) and order of degradation reaction (*n* value) for ACY and color in vitamin C-fortified juices.

1.3 Significance of the study

1. This work investigated natural phenolic compounds for their potential protective effect against ACY and color degradation in cranberry juice fortified with vitamin C. The measurements of ACY, color intensity, vitamin C retention, and browning index during storage were used to determine the efficacy of phenolic compounds. The data will help explain the antioxidant mechanism of phenolic compounds in protecting ACY degradation.
2. Degradation kinetics of anthocyanins and color in cranberry juice containing different concentrations of vitamin C and phenolic compounds were determined by using a nonlinear inverse method with ordinary least squares, and the effect of vitamin C levels on ACY and color degradation was determined in this study. Moreover, the predictive equations developed in this study can serve as a guideline for process design in industry.

1.4 Objectives of the study

1. To determine the ability of natural antioxidant compounds (hesperidin, catechin, and gallic acid) as protective agents against ACY and color degradation in pasteurized cranberry juice fortified with vitamin C.
2. To estimate the kinetic parameters (k, C_0, n) of ACY and of color degradation during storage using an inverse method of ordinary least squares and to develop predictive equations for color and ACY in cranberry juice during storage.

3. To demonstrate practical applications of results from objective 2 for the use in the food industry, especially by juice processors.

CHAPTER 2

LITERATURE REVIEW

2.1 Cranberries

Cranberries (Family: Ericaceae) are low bush plants, growing in fresh water and mountain soil with layers of sand, peat, gravel, and clay. There are two types of cranberries, American cranberry (*Vaccinium macrocarpon Ait.*) and European cranberry (*Vaccinium oxycoccus L.*). European cranberries are grown in Finland and Germany. Although the European variety possesses an anthocyanin profile similar to the American variety, the fruits of European cranberries are smaller in size and contain different levels of malic, citric, and quinic acid (Cape Cod Cranberry Growers' Association, 2016). American cranberries have been more widely consumed more than European cranberries, and hence have been extensively studied. Native Americans consumed cranberries as fresh and dried fruits. Owing to the large amount of benzoic acid in cranberries, the dried cranberries were used as a natural food preservative as well (Davidson, 1999). The American cranberries are mostly cultivated in North America (Wisconsin, Massachusetts, New Jersey, Oregon, Washington) during April to November. The majority of cranberries are harvested between September and October. Nutrient compositions of cranberry products are shown in **Table 2.1**. Cranberries contain a complex mixture of organic acids (~14 types, i.e., ferulic acid, vanillic acid, caffeic acid, benzoic acid), and flavonoids (~22 types; i.e., catechin, quercetin, myricetin, kaempferol, prunin), with quercetin and myricetin predominating (Guay 2009; Pappas and others 2009). The chemical profile of cranberry juice was evaluated by Hummer and others (2014) as shown in **Table 2.2**. Polyphenols in cranberries, especially anthocyanins and proanthocyanidins, provide natural defensive functions in plants against microbes, which believe to reduce risks of cancer and heart disease, to protect from Alzheimer's development, diet-induced obesity, insulin resistance, and intestinal inflammation (Anhe and others 2015; Howell 2007).

Table 2.1 Nutritional values of cranberries (per 100 g product)

Nutrient	Cranberry product				
	Frozen[a]	Concentrate	Sweetened/Dried[b]	Flavored SDC[c]	Powder
Calories (kcal)	48	198	298–367	337–342	360
Saturated fat (g)	0	0	0	0	0
Cholesterol (mg)	0	0	0	0	0
Sodium (mg)	3	14	3–4	2–3	29
Potassium (mg)	73	500	40–90	11	734
Sugar (g)	4	22	64–69	67–68	37
Total Carbohydrate (g)	10	49	82–88	83–84	89
Dietary fiber (g)	4	<0.5	6–9	5–6	6
Protein (g)	0.6	<0.5	<0.5	<0.5	<0.5
Vitamin A (IU[d])	0	0	70[e]	16,200[f]	0
Vitamin C (mg)	18	58	0	1	5
Calcium (mg)	10	39	10–18	4	184
Iron (mg)	0.6	1.7	0.5	0	4

Source: Girard and Sinha (2012)
[a]Whole or sliced.

[b]Regular, soft and moist, and glycerated forms.

[c]Orange, blueberry, cherry, or raspberry flavored sweetened dried cranberries (SDCs).

[d]As provitamin A.

[e]Value for glycated forms of sweetened dried cranberries.

[f]Value for orange flavored sweetened dried cranberries

Table 2.2 Chemical profile[1] of American cranberry juice (*Vaccinium macrocarpon Ait.*)

Parameters	**Content**
°Brix	9.58
pH	2.7
Titratable acidity (as citric) (g kg^{-1})	0.25
Sugar (g kg^{-1})	
Glucose	0.352
Fructose	0.108
Sucrose	0.002
Anthocyanins (mg kg^{-1})	
Cyanidin-3-galactoside	27.1
Cyanidin-3-glucoside	1.3
Cyanidin-3-arabinoside	14.9
Peonidin-3-galactoside	38.5
Peonidin-3-glucoside	4.4
Peonidin-3-arabinoside	13.0
Antioxidants	
Vitamin C (mg/kg)	0.930
ORAC (µmol L^{-1} Trolox equivalent kg^{-1})	86.5
FRAP (µmol L^{-1} Trolox equivalent kg^{-1})	107.6
Total Phenolics (mg gallic acid equivalent kg^{-1})	12.8

[1]Chemical analysis was done in 2009, while the results were published in 2014.
Source: Adapted from Hummer and others (2014)

In addition, cranberry extracts have been studied as a potential treatment for urinary tract infections (UTIs) in humans (Mansour, 2014; Durham 2015; Foxman 2015). Studies conducted over a five-year period (2006–2010) examining anti-bacterial properties in cranberries were summarized by Hisano and others (2012), as shown in **Table 2.3**. D-mannose and distinctive containing A-type proanthocyanidins in cranberries have been reported to exhibit significant anti-adhesion effects on P-fimbriated *Escherichia coli* bacteria to bladder cell receptors (Blumberg and others 2013; Han and others 2010; Hummer and others 2014; Tao and others 2011). Urinary tract infection is a common disease among sexually active women (20–55 year old) due to short urethras

that allow bacteria adhering to bladder cell wall (Foxman 2003). It is a long-term and recurrent disease, which could cause economic burden in UTI patients.

Cranberries have been used as an herbal medicine to treat urinary tract infections. Patients with UTIs take dry cranberry juice extract at a dose of 500 to 2,000 mg, 3 times a day, to reduce bacterial adhesion to uroepithelial cells (Jeske 2014). However, mild side effects (stomach upset, diarrhea, and occasional allergy) were reported regarding the use of cranberry concentrate.

Table 2.3 *In vitro* activity of cranberries against bacteria.

Study	Study design and patients (N)	Cranberry preparation	Micro-organism (s) and Results
Pinzon-Arango et al., (2009)	*In vitro* bacteria cultured in medium and human uroepithelial cell culture	PAC of 0, 64, 128 and 345.8 µg/ml	*E.coli*: Anti-adhesion (*E. coli*) from 50.2 to 7.9 bacteria/cell ($p<0.01$); dose dependence effect
Lee et al., (2010)	*In vitro* urine activity after cranberry consumption in volunteers. Phase 1, N=20 (16 women, 4 men); phase 2 (7 women, 2 men) N=9.	275 mg of whole, dry cranberries and 25 mg of concentrated, dry cranberries	*E. coli, K. pneumonia and C. albicans*: Phase 1: anti-adhesion activity in 35% (*E. coli*), 65% (*K. pneumoniae*) and 45% (*C. albicans).* Phase 2: anti-adhesion activity in 23% (*E. coli*), 33% (*C. albicans*) and 67% (*K. pneumoniae).*
Lavigne et al., (2007)	*In vitro* urine activity after cranberry consumption in volunteers with crossover. N=8 females	36 mg cranberry capsules of; daily dosage was 36 or 108 mg or placebo	*E. coli:* Anti-adhesion activity ($p<0.001$). Dose dependence effect
Gupta et al. (2007)	*In vitro* anti-adhesion activity against bladder and vaginal epithelial cells	Cranberry capsule with 2.7 mg of PAC diluted from 0 to 75 µg/ml	*E. coli:* Anti-adhesion activity of PAC against *E. coli* from 6.9 to 2.2 and 1.6 bacteria/cell following PAC at 0, 25 and 50 µg/ml, respectively ($p<0.001$).
Howell et al., (2010)	Multicentric, randomized, double-blind *in vitro* urine activity after cranberry consumption in volunteers. N=32 females	Cranberry capsule of 0, 18, 36 or 72 mg of PAC	*E. coli*: Anti-adhesion activity increasing with the amount of PAC. Virulence was also reduced with PAC in a dose-dependent fashion.
Valentova et al., (2007)	Double-blind, placebo-controlled *in vitro* urine. Group I (n=23) placebo; group II (n=20) 400 mg; group III (n=22) 1200 mg. N=65 females	400 mg or 1200 mg per day of dried cranberry juice	*S. aureus, E. faecalis, E. coli, P. aeruginosa, E. faecium and K. pneumoniae*: Anti-adhesion activity in a dose-dependent fashion ($p<0.05$); highest activity observed against *P. aeruginosa*.
Di Martino et al., (2006)	Double-blind, randomized, placebo-controlled *in vitro* urine activity after cranberry consumption in volunteers. N=20 (10 males, 10 females)	250 or 750 ml of 27% cranberry juice	*E. coli*: Dose-dependent decreases in bacterial adhesion to human epithelial cell line of 45% and 62% for 250 and 750 ml of cranberry juice, respectively ($p<0.05$), independent of antibiotic resistance.

Source: Hisano and others (2012)

2.2 Anthocyanins as a natural colorant

Apart from antimicrobial and antioxidant properties, anthocyanins (ACY) provide natural red, purple, and blue colors in fruits, leaves, or flowers (McGhie and Walton 2007). ACY change colors according to pH, thus ACY are somewhat a pH indicator. ACY are glycosylation of anthocyanidins, which known as color pigments. There are six types of anthocyanidins; cyanidin, delphinidin, malvidin, peonidin, petunidin, and pelargonidin. The anthocyanidins basically compose of 15 carbons of 2 aromatic rings (ring A, ring B) and one heterocyclic ring (ring C), as shown in **Figure 2.1**. In nature, anthocyanidins are not commonly found by themselves, but rather presented as ACY by glycosylation on the 3-carbon of the C-ring through an O-linkage (Glover and Martin 2012). Major ACY in cranberry juice are 3-O-galactosides and 3-O-arabinosides of cyanidin and peonidin. Monomeric ACY are major compounds that provide bright red characteristic. However, in a solution with high ACY concentration, self-aggregation of monomeric ACY could occur and results in polymeric ACY. The polymeric ACY contribute deep brownish-red color (Pappas and Schaich 2009; Brownmiller and others 2008), which is a favorable appearance in red wine (Pina and others 2015; He and others 2012).

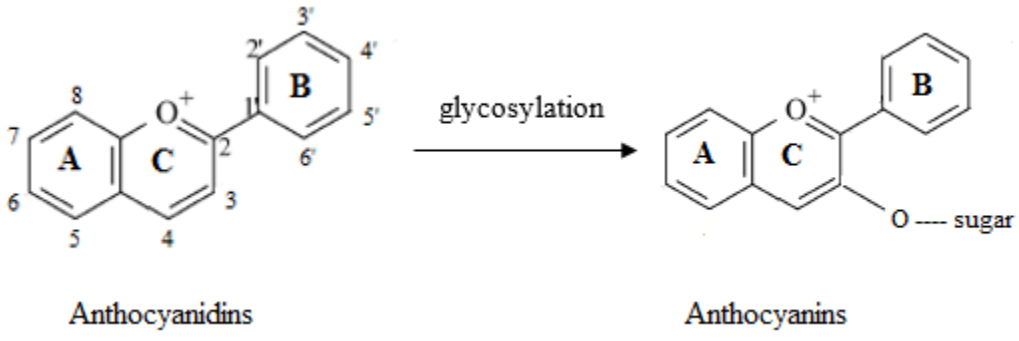


Figure 2.1 Anthocyanins formation through glycosylation of anthocyanidins.

ACY could switch among colors (red ↔ purple ↔ blue) regard to alteration of OH group position in anthocyanidins. In contrast, color degradation in juice occurs via reactions that detach sugars (deglycosylation) from ACY structures. The ACY then degrade to chalcone, which is a colorless phenolic compound (Sun and others 2011; Sadilova and others 2007). ACY are water-soluble and susceptible to degradation via oxidation by oxygen, light, and high temperatures. White and others (2011) reported that heat degraded cranberry ACY, and resulted in flavonol aglycone formation (quercetin and myricetin) that could impact color of the product.

2.3 Cranberry juice production

Due to the seasonal cultivation of cranberries from April to November, commercial cranberry juices are typically produced from juice concentrate rather than fresh fruits. Upon harvesting, fresh cranberries are ground and macerated with pectinase and /or cellulase enzyme to extract juice. The juice is then concentrated by mild evaporation, membrane filtration or reverse osmosis (Brownmiller and others 2008). The standard cranberry concentrate is 50 °Brix (Girard and Sinha, 2012). Pasteurization could be applied to juice concentrate to prevent microbial growth during storage. Even though cranberry fruits are a good source of vitamin C, cranberry juice is rarely consumed fresh owing to its extreme tart flavor. Thus, cranberry juice products are mostly diluted from juice concentrate with water, and sweetened, or blended with other juices to overcome the tart flavor. Juice production is illustrated as a flow diagram in **Figure 2.2**.

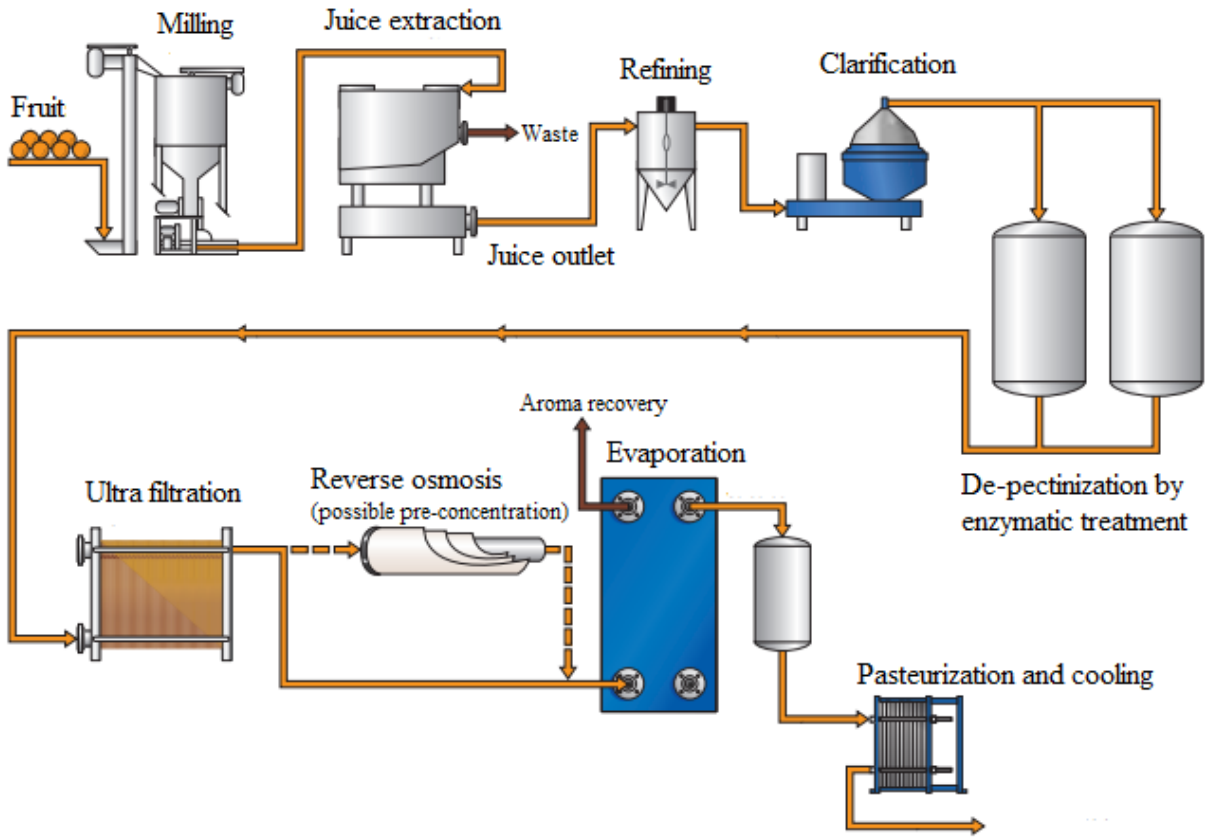


Figure 2.2 Process flow diagram for juice production. (Source: Alfa Laval membrane filtration)

2.3.1 Vitamin C fortification

Vitamin C, also known as ascorbic acid, is an effective antioxidant that prevents cell damage caused by free radicals. Vitamin C is also crucial for protein synthesis, wound healing, bone growth, and mineral absorption. Vitamin C is an essential nutrient that cannot be synthesized or stored in the human body. Therefore, it is necessary to include vitamin C in the daily diet. Examples of vitamin C-rich foods are citrus fruits and juices, berries, cantaloupe, broccoli, spinach, peppers. In addition, vitamin C is widely fortified in many food products in order to extend product shelf-life and enhance nutritional levels.

L-ascorbic acid is the form of vitamin C found in nature, which is easily converted to dehydroascorbic acid (DHA) upon oxidation. However, DHA can revert back to L-ascorbic acid with the addition of two hydrogen atoms. Meanwhile, further hydrolysis reaction will result in irreversible foundation of diketogulonic acid. Oxidation mechanism of L-ascorbic acid is shown in **Figure 2.3**.

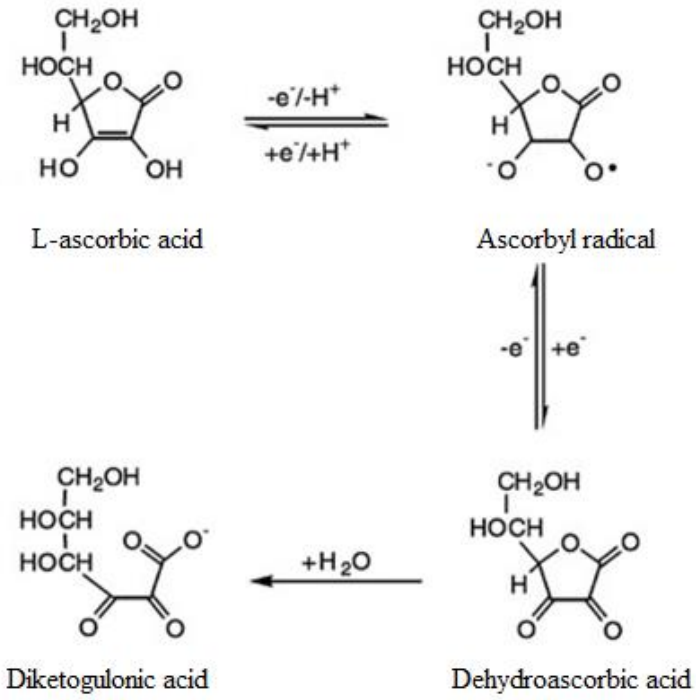


Figure 2.3 Oxidation mechanism of L-ascorbic acid (Source: Levine and others, 1996)

Despite being a potent antioxidant, fortification of vitamin C in pigment-rich juices has been found to induce free radical formation and accelerate color and ACY degradation (Starr and Francis 1968; Remini and others 2015; Li and others 2014; Choi and others 2002). The mechanism of ACY degradation as a result of vitamin C fortification was explained via two pathways (Ozkan and others 2005; Poei-Langston and Wrolstad 1981; Garcia-Viguera and Bridle 1999; Sun and others 2011):

1) Condensation of ascorbyl radical: Anion of ascorbyl radical could react with flavylium cation at carbon-4 of ACY. The reaction damages ACY by cleaving the structure.

2) Hydrogen peroxide formation during oxidation of ascorbic acid: Hydrogen atom donation from ascorbic acid could result in a formation of hydrogen peroxide, which suddenly decomposes to highly reactive compounds; peroxyl anion (HOO^-), perhydroxyl radical ($HOO^{\bullet}$), and hydroxyl radicals ($HO^{\bullet}$). Among these three, $HO^{\bullet}$ is the main reactive specie to cleave benzene ring in ACY.

It is also known that high doses of vitamin C can act as a pro-oxidant, which induces more free radical formation (Paolini and others 1999). At high doses of vitamin C, another pathway of reactive oxygen species (ROS) formation could occur via Fenton's reaction (**Figure 2.4**), where ascorbate reduces metal ions in juice, such as Fe^{3+} (Rietjens and others 2002). Owing to Fenton's reaction, Fe^{2+} is oxidized to Fe^{3+} by hydrogen peroxide (H_2O_2) and resulted in a hydroxyl radical ($HO\bullet$) and hydroxide ion (OH^-). Meanwhile, another molecule of H_2O_2 reduces Fe^{3+} to Fe^{2+} and forms a peroxide radical ($HOO\bullet$) and a proton (H^+).

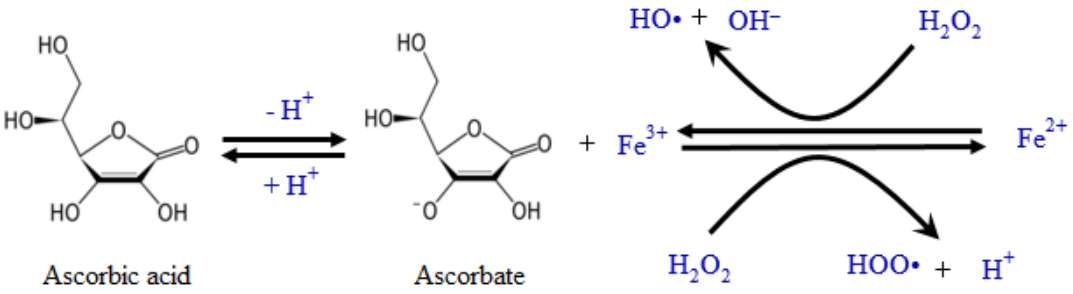


Figure 2.4 Pro-oxidant effect of ascorbic acid through Fenton's reaction.

2.3.2 Juice pasteurization (Hot-filling versus aseptic process)

Cranberry juices are available in grocery stores across the United States. Ready-to-drink juices are pasteurized to extend their shelf-life, as pasteurization is an effective method to inhibit growth of spoilage microorganisms. In the food industry, juice pasteurization can be done by two techniques; hot fill or aseptic processing. Hot filling is a low cost operation compared to the aseptic technique. In hot filling, the juice is rapidly heated to 85–95°C in a heat exchanger and held at that temperature to assure product safety from microbial growth, then hot-filled into containers. Cooling is done immediately afterward. However, temperature of the juice does not decrease rapidly, thus the heat remaining in the juice during cooling could cause damage to the juice's flavor and color. In contrast, the aseptic method comes with modern system controls that provide rapid continuous heat and cooling before packaging. The processes are also handled under sterile conditions, hence the operational cost of the aseptic system could be more than US $1,000,000 (Bates and others 2001). Therefore, hot-fill processing is mostly installed in small- and medium-scale juice businesses, which often encounter quality losses.

2.4 Protective effect of polyphenols against ACY degradation

Free radicals are produced in the presence of oxygen, radiation, sunlight, or pollution. The phenol group (or OH group) in phenolic compounds helps prevent degradation by scavenging free radicals. Flavonols are a subclass of polyphenols, and exhibit a powerful radical scavenging by donating H-atoms from their phenol groups to the radicals. Therefore, the free radicals in the food system could not attack health-beneficial components, such as ACY. Quercetin is the most abundant flavonol distributed in many plants, and hence it is the focus of interest among researchers. An early study in 1974, Shrikhan and Francis (1974) found that quercetin helped

decrease degradation of cranberry ACY in the juice fortified with vitamin C. However, the authors reported the limitation of quercetin as it precipitated out immediately after pasteurization. Protective effects of different polyphenols on ACY degradation are summarized in **Table 2.4**.

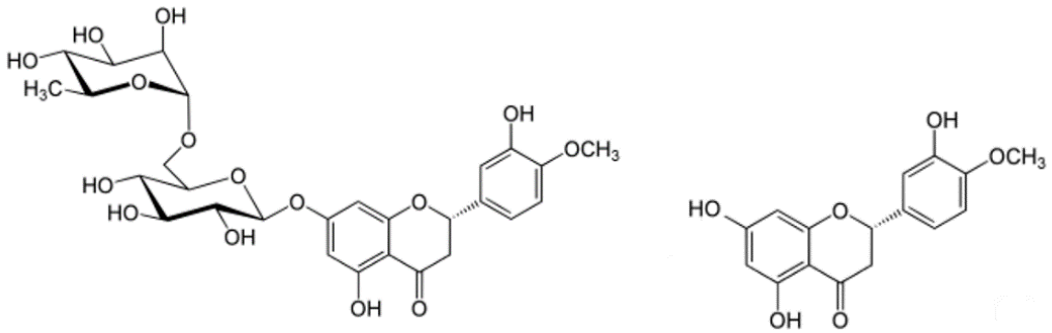
Table 2.4 Summary of protective effect of polyphenol on stability of anthocyanins.

Study	**Polyphenols**	**Degradation factors**
Blackcurrant juice (Clegg and Morton 1968)	Quercetin Quercitrin Flavonol aglycone	Ascorbic acid
Cranberry juice (Shrikhan and Francis 1974)	Quercetin Quercitrin	Ascorbic acid
Strawberry and blackcurrant juice (Skrede and others 1992)	Strawberry flavonoid extract	Ascorbic acid
Grape juice (Brenes and others 2005)	Rosemary extract	Ascorbic acid
Blood orange juice (Cao and others 2009)	Hesperidin Narirutin Naringin Neohesperidin	Ascorbic acid Glucose Sucrose Fructose
Grape skin anthocyanins (Yan and others 2013)	Enzymatically modified isoquercitrin	Heat and light
Plum juice (Hernandez-Herrero and Frutos 2015)	Rutin	Ascorbic acid

Overall, polyphenols exhibited a protective effect on ACY against degradation caused by ascorbic acid, sugar, heat, and light. Therefore, phenolic compounds are potential agents to protect ACY from degradation via scavenging activity, and copigmentation, which occurs via chelation between ACY and other non-colored phenolic compounds (Hernandez-Herrero and Frutos 2015; Boulton 2001). Although the studies by Brenes and others (2005) and Cao and others (2009), as shown in Table 2.4, were done by pasteurizing juices, the process durations were at 30 minutes, and 3 hours, respectively, which are not a regular practice for commercially pasteurized juices.

2.4.1 Hesperidin

A common limitation of most polyphenols is low water solubility, which limits their use in juice processing. Thus, water-soluble phenolic compounds were considered in this study. Liu and Chen (2008) reported an increase of water solubility in hesperetin with increasing temperature (15 to 50 °C). Thus, hesperetin possibly dissolves in aqueous solutions under pasteurization conditions (85 °C). Hesperidin is mostly found in nature rather than hesperetin, since the glycosylated hesperidin is a flavonoid in citrus fruits (**Figure 2.5**). The natural form of flavonoids are mostly glycosylated; sugar linkage. Glycosides help increase water solubility of the flavonoids. Hesperidin is a flavonol-diglycoside, which is composed of rhamnose and glucose. The aglycone form (no sugar) of hesperidin is then called hesperetin. Therefore, hesperidin could be a potential water soluble polyphenol that might be used in juice processing.



(a) (b)

Figure 2.5 Chemical structures of hesperidin (a) and hesperetin (b)

2.4.2 Catechin

Catechin is similar to quercetin, in both natural availability and chemical structure (**Figure 2.6**). Therefore, catechin is also a potent antioxidant, and has been extensively studied for its copigmentation ability to stabilize ACY (Hidalgo and others 2010; Gordillo and others 2015; Kopjar and Pilizota 2009). Unlike quercetin, catechin possesses higher water solubility (2.26 g/L at 25°C (Srinivas and others 2010b), than quercetin (0.00215 g/L at 25°C, (Srinivas and others 2010a), which makes catechin more feasible to apply directly in juice production. Although structures of catechin and quercetin look very similar (Figure 2.6), the difference in structural orientations could possibly affect chemical properties, including water solubility of these two compounds. The stick notation represents plane bonding, wedged notation means one bond is coming out of the plane, and dashed line shows the bond is going below the plane.

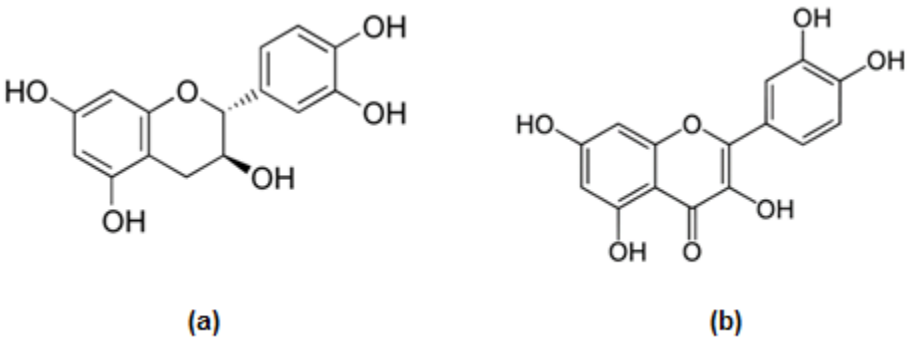


Figure 2.6. Chemical structure of catechin (a) and quercetin (b).

2.4.3 Gallic acid

Gallic acid, an organic acid with a benzene ring as a core structure (**Figure 2.7**), is a phenolic compound, which is commonly available in many plant parts such as leaf, bark, wood, root, fruit, and seed. Besides antifungal and antiviral properties, gallic acid exhibits remarkable antioxidant activities. Gallic acid has been reported to possess anti-allergic, anti-inflammatory, anti-mutagenic and anti-carcinogenic activities (Gali and others 1992). Gallic acid is not only a potent water-soluble antioxidant, but also exhibits effective antioxidant in emulsions (Cholbi and others 1991). Gallic acid has been studied in many applications, including juice production due to sweetness inducer.

The evidence supporting feasibility and benefits of using gallic acid in commercial juice production is well documented. First of all, gallic acid is soluble in water (up to 20 g/L) and considered a non-toxic substance to humans (Rajalakshmi and others 2001), and humans seem to be able to absorb gallic acid better than polyphenols (Daglia and others 2014). There is no upper limit of gallic acid usage in the diet, as it is safe (Rajalakshmi and others 2001). Secondly, food grade gallic acid is relatively inexpensive ($26–29/kg), as compared some other antioxidants. Lastly, gallic acid induces long-lasting and non-caloric sweetness, and a mildly sour taste is developed at concentrations greater than 0.05 M, or 850 mg/100 mL (Srinivas and others 2010b; Verhagen and others 2002).

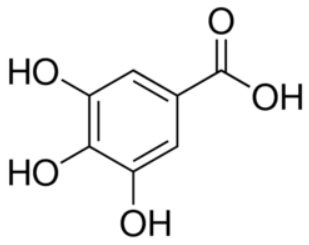


Figure 2.7. Chemical structure of gallic acid

2.5 Kinetics of ACY and color degradation

Kinetics are often studied to determine food quality and food safety during processing or storage. Degradation kinetics are also a basic requirement for shelf-life prediction. The n^{th}-order degradation kinetic model, Eq. (2.1), consists of three parameters: degradation rate constant (k, $[\text{conc}]^{1-n}$ time^{-1}), initial concentration (C_0, conc), and reaction order (n, dimensionless). Concentration at any time t is given by $C(t)$. The n value is mostly varied in a range from 0 to 2, and typically assumed to be 0 or 1 for nutritional degradation reactions, including degradation of color and ACY (Ozkan and others 2005; Wang and Xu 2007).

$$\frac{dC}{dt} = -kC^n \tag{2.1}$$

In addition, Peleg and others (2015) reported experimental evidence that a 1st-order reaction represented thermal degradation of ACY at various studied conditions, i.e., processing and storage. Examples of n-order determination in previous studies are presented in **Table 2.5**. As summarized in Table 2.5, n = 1 has been widely reported and assigned in many studies, thus most researchers assumed 1st-order without proving it from the data. According to the literature review, determining actual n value of degradation reaction is not a common practice among researchers, even though n could play significant impact on k and C_0 values. When n = 0 or 1, the integrated kinetic model (Eq. (2.1)) becomes a linear model (n = 0) or can be log-transformed to a linear model (n = 1), hence it is easy to estimate parameters k and C_0 by linear regression. Some studies, without initially assigning n = 1, determined the most suitable n using a nonlinear method by fixing n at 0, 0.5, 1, and 2 (Remini and others 2015; Wibowo and others 2015), or varying n from 1 to 1.8 in 0.05 increments (Buckow and others 2010), then chose the n that gave the lowest

Root Mean Square Error (RMSE) between predicted and experimental values to represent the reactions.

Table 2.5 Examples of *n*-value for degradation kinetics of anthocyanins and color

Kinetics	***n*-order**	***n* determining method**	**Reference**
Anthocyanins:			
Pomegranate juice	1	assigned 1st order	Ozkan and others (2005)
Strawberry juice	1	assigned 1st order	Ozkan and others (2005); Garzon and Wrolstad (2002)
Cherry nectar	1	assigned 1st order	Ozkan and others (2005)
Blackberry juice	1	assigned 1st order	Wang and Xu (2007)
Blueberry juice	1.4	varied from 1 to 1.8	Buckow and others (2010)
Blueberries	1	assigned 1st order	Martynenko and Chen (2016)
Color:			
Blood orange juice	1	compared 0, 0.5, 1, 2	Remini and others (2015)
Chestnuts	1	compared 0, and 1	Hou and others (2015)
Bayberry juice	1	assigned 1st order	Guangming and others (2016)
Apple slices	0 and 1	compared 0, and 1	Qian-yu and others (2015)

2.5.1 Estimation of parameters

One of the most common methods of estimating kinetic parameters (k, C_0, n) is fixing n as an integer (0, 1, and 2), and then estimating parameters k and C_0 through curve-fitting or optimization. However, this approach does not provide sufficient statistical information that could interpret physical meaning of estimated parameters, especially parameter errors (van Boekel 1996). Unlike curve-fitting or optimization, parameter estimation considers parameter errors, scaled sensitivity coefficients, the sensitivity matrix, and confidence intervals (CIs), which inform whether parameters are accurate, can be estimated, are correlated, or are significantly different

from zero and can be removed from model (Dolan and Mishra 2013). Linear and nonlinear models can be evaluated by examining the sensitivity coefficient (X_i) (Eq. (2.2)):

$$X_i = \frac{\partial \eta}{\partial \beta_i} \tag{2.2}$$

where η is the dependent variable, and β are the true values of the parameters, and the i^{th} parameter is β_i.

The model is linear if **all** the sensitivity coefficients (X_i) are not a functions of any parameter(s) β_i, i.e., if the model's first derivative $\frac{\partial \eta_i}{\partial \beta_j} \neq f(\beta_j)$. For example, the explicit form of the kinetic model (Eq. (2.1)) is $C = \left((n-1)kt + C_0^{1-n}\right)^{1/1-n}$, the first derivatives of $\frac{\partial C}{\partial n}, \frac{\partial C}{\partial k}, and\ \frac{\partial C}{\partial C_0}$ are a function of parameter n, k, and C_0, respectively. Therefore, the degradation kinetic model is a nonlinear model. Due to the complication of a non-linear method, non-linear equations are generally transformed to linear models. The transformation impacts error structure, which could result in incorrect estimated parameters (Chowdhury and Das Saha 2011). Therefore, in recent years, non-linear methods have gained interest in food quality research (Wibowo and others 2015).

In order to estimate parameters in nonlinear models, an initial guess of parameter(s) and iteration are required. Solver® in Excel can also be used to estimate parameters in nonlinear models from the nonlinear algorithm, while the sensitivity matrix **X** (Eq. (2.3)) is needed to compute errors of parameters by matrix multiplication (Dolan 2003).

$$\boldsymbol{X} = \begin{pmatrix} \left(\frac{\partial \eta_1}{\partial \beta_1}\right) & \cdots & \left(\frac{\partial \eta_1}{\partial \beta_p}\right) \\ \vdots & \ddots & \vdots \\ \left(\frac{\partial \eta_n}{\partial \beta_1}\right) & \cdots & \left(\frac{\partial \eta_n}{\partial \beta_p}\right) \end{pmatrix} \tag{2.3}$$

where **X** is an *n*-by-*p* matrix of the sensitivity coefficients; *n* is number of data; *p* is number of parameters.

2.5.2 Scaled sensitivity coefficients

Scaled sensitivity coefficients, SSC (X_i'), are a useful statistical criteria because they not only provide information regarding the ease and accuracy of estimating parameters, but also illustrate parameter correlation. The X_i' of each parameter can be calculated by multiplying β_i by its X_i (Eq. (2.2)). The parameter with large X_i' means the small change of parameter creates a large response, thus such parameter can be estimated easily with a small relative error. The estimation of a parameter with a small size of X_i' would be very difficult, and hence could give huge error. Correlation of parameters is determined by identical shape of X_i' plots, and verified by a constant ratio of X_i'/X_j', meaning those correlated parameters cannot be estimated simultaneously (Dolan and Mishra 2013).

CHAPTER 3

OBJECTIVE ONE

Determination of protective effect of selected phenolic compounds against degradation of anthocyanins and color in vitamin C-fortified cranberry juice

3.1 Materials and Methods

3.1.1 Juice preparation

Cranberry juice concentrate, processed in August 2014, was purchased from Dynamic health Laboratories Inc. (Brooklyn, NY, USA), and kept frozen at –18 °C until used, within about six months. The juice concentrate was diluted with HPLC-grade water at a ratio 1:14 (v:v) (dilution factor, DF= 15) to obtain 3.8 °Brix, which is typical of the commercial cranberry juice. The diluted juice was centrifuged at 7,600 g for 10 minutes, and the supernatant was filtered through Whatman® No.1 filter paper. A preliminary study was conducted at 80 mg/100 mL vitamin C fortification, with varying concentration of hesperidin, catechin, and gallic acid. The juice (100 mL) was fortified with 80 mg of L-ascorbic acid (CAS no. 50-81-7, Sigma Aldrich, St. Louis, MO, USA). Hesperidin (CAS no. 520263, Sigma Aldrich) was added to the fortified juice. The concentration of hesperidin was varied from 5 to 18 mg/100 mL. Catechin hydrate (C1251, CAS no. 225937-10-0, Sigma Aldrich, St. Louis, MO, USA) and gallic acid (CAS no. 149917, Sigma Aldrich, St. Louis, MO, USA) were added at 5, 15 mg/100 mL and 0, 80, 160, or 320 mg/100 mL, respectively. Juice was mixed until well-dissolved using a magnetic stirrer. Samples were prepared in two replicates. A final selection was conducted with regard to result from preliminary study. The juice (100 mL) was fortified with 40, 60, or 80 mg of L-ascorbic acid. Then, gallic acid was added to the juice at 0, 80, 160, or 320 mg/100 mL. Samples were prepared in four replicates. **Figure 3.1** is a diagram illustrating sample preparation for vitamin C and phenolic compound addition in juice.

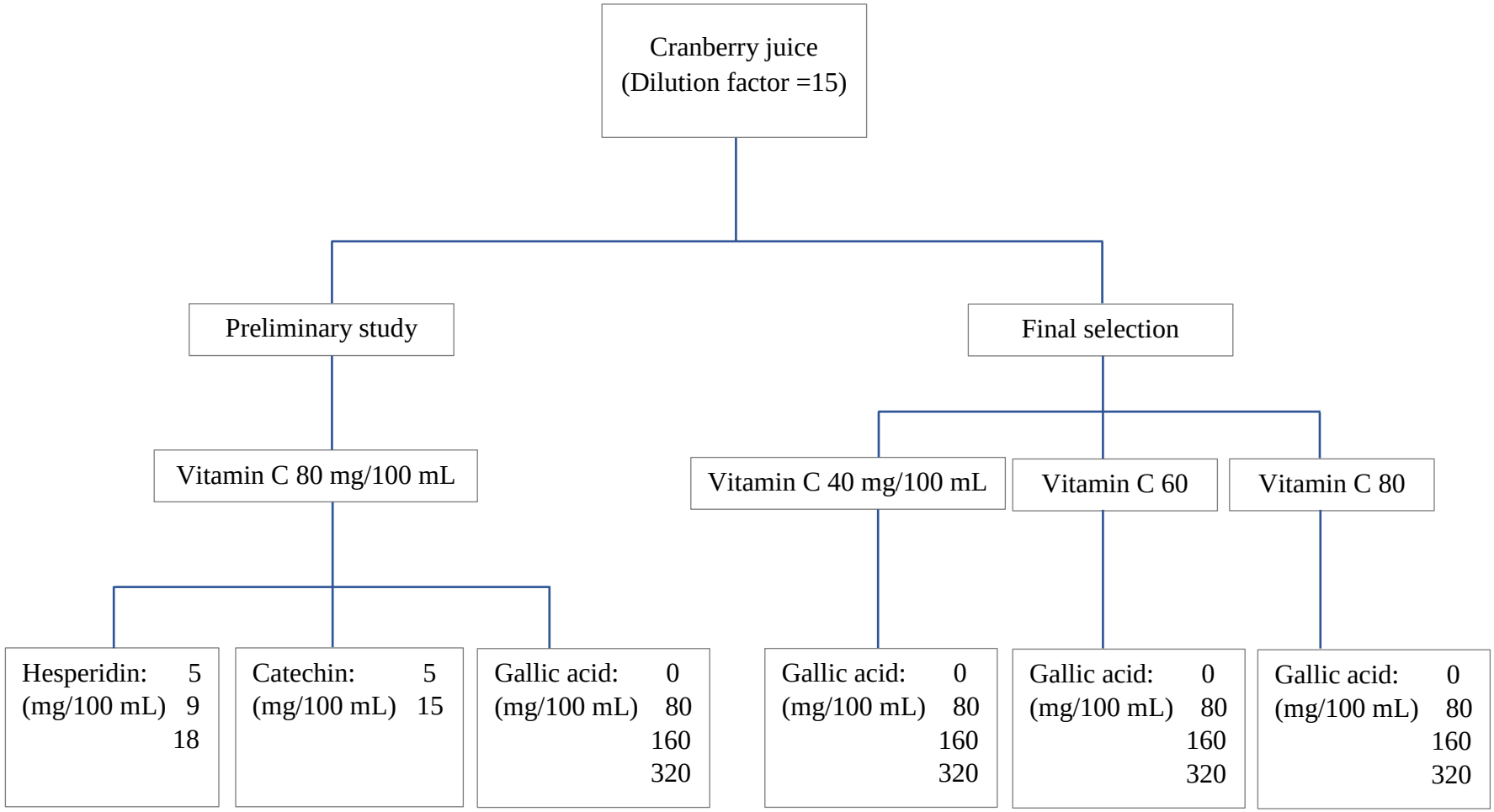


Figure 3.1 Diagram of sample preparation with addition of vitamin C and selected phenolic compounds.

3.1.2 Juice pasteurization and storage

Eight milliliters of juice were pipetted into each 15-mL glass test tube and capped (polyethylene cap). One tube was fitted with a Type-K thermocouple (Digi-Sense Type-K, Std Pen Probe, Cole-Parmer®, Vernon Hills, IL, USA), by puncturing the cap to monitor juice temperature during pasteurization. All juice samples were pasteurized at 85±2 °C for 1 minute. Pasteurization was performed in a thermostatic water bath equipped with a shaker (**Figure 3.2**). Temperatures of sample and water bath were monitored using a thermocouple meter (Digi-Sense Dual J-T-E-K, Model 91100-40, Cole-Parmer®, Vernon Hills, IL, USA). Pasteurizing time started when the thermocouple inside an assigned tube reached 85 °C. The come up time was 1–1.5 minutes. After one minutes of pasteurizing, the juice was then hot-filled into a 5-mL polypropylene vial, screw-capped and cooled in ice bath for 2 hours. Samples were kept in the dark at room temperature (23±2 °C) for 16 days. Samples were drawn every 2 days to analyze for ascorbic acid content, red color intensity, browning index, and ACY content.

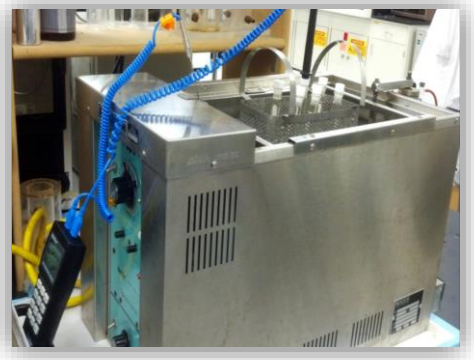


Figure 3.2 Juice pasteurization in water bath equipped with a shaker, and a hand-held thermometer for monitoring temperature of water bath and cranberry juice.

3.1.3 Anthocyanin content

Monomeric ACY are major compounds that provide bright red color in cranberry juice (Pappas and Schaich 2009). The total monomeric ACY content were determined by the pH differential method of Lee and others (2005), which is a rapid and simple assay. This study determined total ACY content as peonidin-3-galactoside equivalent, rather than individual anthocyanins since a total of 13 ACY in cranberry juice have been reported (Blumberg and others 2013, Milbury and others 2010), and the three major ACY are peonidin-3-O-galactosides (~32%), cyanidin-3-O-galactosides (23%), and peonidin-3-O-arabinosides (18%).

Cranberry juice (1 mL) was diluted separately with 1 mL each of pH 1.0 and pH 4.5 buffers. The absorbance values of the solution were determined spectrophotometrically at 520 and 700 nm (Genesys10S UV-vis spectrophotometer, Thermo Scientific, Waltham, MA, USA). ACY content was calculated by following equation:

$$Anthocyanin\ content(mg/L) = \frac{\mathrm{A} \times \mathrm{MW} \times \mathrm{DF} \times 10^3}{\varepsilon \times \mathrm{l}}$$

Where $A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$;

MW (molecular weight) of *peonidin-3-galactoside* = 463.41 g/mol;

DF = dilution factor;

L = pathlength in cm;

ε = molar extinction coefficient (26,900 $L \times mol^{-1} \times cm^{-1}$);

10^3 = factor for conversion of g to mg.

3.1.4 Color intensity and browning index (BI)

Two milliliters of each sample were pipetted into a 2-mL polycarbonate cuvette. A dual beam Genesys 10S UV-vis spectrophotometer was used to measure absorbance values (AU) at 520 nm (AU_{520}), which is the maximum absorbance of red color from monomeric ACY (Vegara and others 2013). The absorbance of juice was also measured at 430 nm (AU_{430}) and the browning index was expressed as a ratio of AU_{430}/AU_{520}.

3.1.5 L-ascorbic acid quantification

L-ascorbic acid content was quantified using high performance liquid chromatography assay and Breeze^{TM} software (Waters Corporation, Milford, MA, USA), with mobile phase of 65:35 mixture of 30 mM KH_2PO_4 buffer pH 2.5 (adjusted by adding H_3PO_4) and acetronitrile. Cranberry juice samples (1 mL) were filtered through a 25-mm diameter nylon syringe filter with 0.2μm pore size (Water Corporation). The 20 μL of each juice sample was injected into the HPLC and separated was conducted using a C18 SORBAX column (dimension 4.6 x 250 mm, 5μm pore size) equipped with a guard column (dimension 4.6 x 12.5 mm). The mobile phase was pumped through the columns at flow rate of 1 mL/min. Absorbance was measured at 245 nm was set for Waters 2487 dual λ absorbance detector.

L-ascorbic acid standard stock was prepared by dissolving 10 mg L-ascorbic acid in HPLC-grade water and the volume was adjusted to 10 mL. The 100, 200, 400, 600, 800, and 1000 μL of stock solution was diluted in 10 mL HPLC-grade water to obtain final concentrations of 10, 20, 40, 60, 80, and 100 mg/100 mL. All samples were prepared in duplicate to prepare a standard curve. The regression equation according to the standard curve, was as follows:

$$Area\ under\ curve = 513{,}000\left(ascorbic\ acid\ \left(\frac{mg}{100\ mL}\right)\right) + 1{,}400{,}000$$

3.1.6 Statistical analysis

All data were analyzed using SAS software (SAS Institute, Inc., Cary, NC, USA). Effect of vitamin C and antioxidant treatments were analyzed using one-way analysis of variance (ANOVA). The significant difference was determined by Tukey's HSD test, and the statistical significant level was defined as $\alpha = 0.05$.

3.2 Results and Discussion

3.2.1 Anthocyanins retention

The use of antioxidants (hesperidin, catechin, gallic acid) had variable effect on the retention of anthocyanins (ACY) in cranberry juice, stored 16 days at 23 °C (**Figure 3.3**). Control juice (80 mg/100 mL vitamin C, with no added antioxidants) had average ACY content of 9.63 mg/L during storage. Generally, increasing levels of different antioxidants showed an increasing protective effect on ACY. Hesperidin was found to be the most effective in ACY retention (13.76–20.56 mg/L) as compared to catechin (11.72–12.32 mg/L) and gallic acid (12.09–14.74 mg/L).

The relatively high protective effect of hesperidin was possibly due to its copigmentation with ACY, as has been reported previously (Mazza and Brouillard 1990; Sari 2015; Türkyılmaz and Ozkan 2014). Copigmentation is a result of hydrophobic interactions among phenols and ACY (Mazza and Brouillard 1990). However, hesperidin was found to precipitate somewhat in the juice during storage. Catechin addition at both concentrations (5 and 15 mg/100mL) showed no significant difference ($p > 0.05$) on ACY retention. In contrast to catechin, addition of gallic acid significantly increased ACY retention.

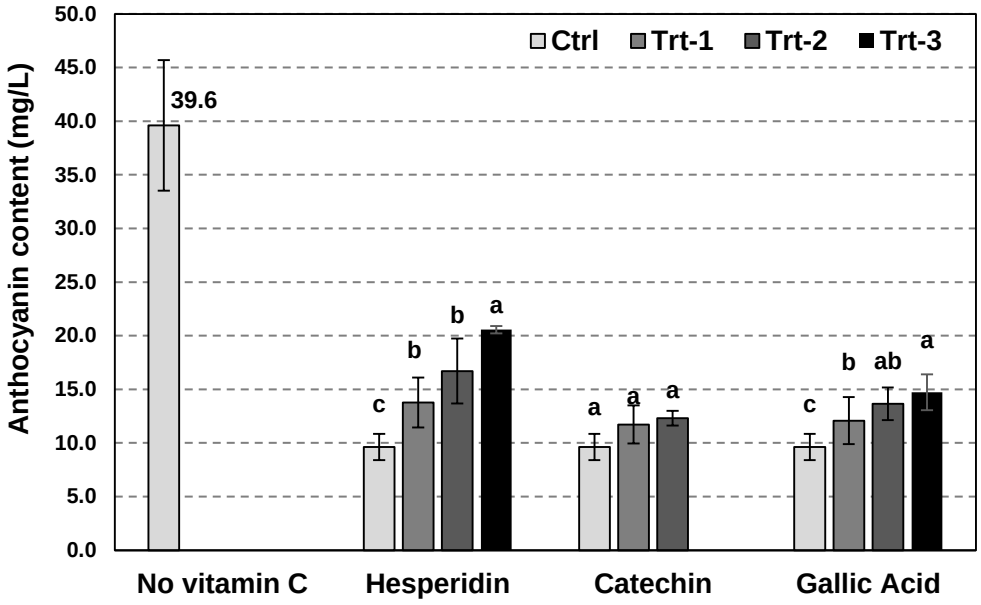


Figure 3.3 Average anthocyanin content during 16-day storage, at 23 °C. Treatment levels per 100 mL were: vitamin C fortification (80 mg) without adding antioxidant compounds (Ctrl); Hesperidin Trt-1 (5 mg), Trt-2 (9 mg), and Trt-3 (18 mg); Catechin Trt-1 (5 mg) and Trt-2 (15 mg); and Gallic Acid Trt-1 (80 mg), Trt-2 (160 mg), and Trt-3 (320 mg).
Treatments sharing same letters (a, b, c) are not significantly different from each other within the same antioxidant (Tukey's HSD test, $\alpha = 0.05$).

Hesperidin showed better ACY retention than catechin and gallic acid; however, its rather poor solubility in water limits its commercial application. Based on the results of this experiment, gallic acid was selected to further evaluate its protective effect on ACY in vitamin C fortified cranberry juice. Increasing levels of gallic acid from 0 to 320 mg/100 mL showed a consistent positive effect on ACY retention at all vitamin C fortification levels (**Figure 3.4**). ACYs were protected by scavenging activity of gallic acid. Gallic acid scavenges radicals by donating H-atoms from its phenol groups. The proton donation neutralizes free radicals to be less active, and thus limits the interaction with ACY (Daglia and others 2014; Yen and others 2002). However, increasing the level of vitamin C fortification had a negative impact on ACY, thereby negating the

protective effect of gallic acid. Ascorbic acid showed a detrimental effect on ACY in cranberry juice, as was evidenced by decreasing ACY values from 40 mg vitamin C juice to 80 mg vitamin C juice.

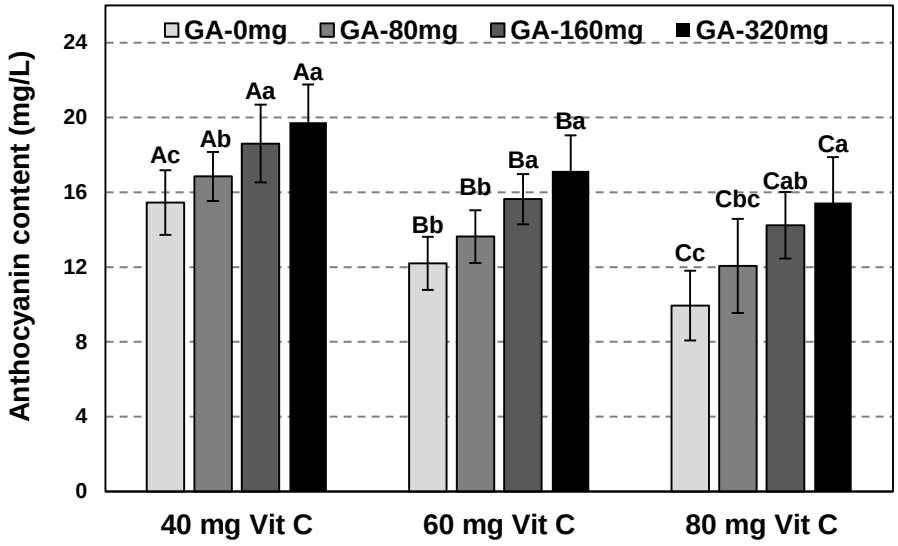


Figure 3.4 Effect of gallic acid (0-320 mg/100 mL) on the average anthocyanin content of cranberry juice, fortified with 40-80 mg/100 mL vitamin C, during 16-day storage at 23 °C. *Treatments sharing same letters (a, b, c) and (A, B, C) are not significantly different from each other within the same vitamin C fortification level and across different levels, respectively (Tukey's HSD test, $\alpha = 0.05$).*

Starr and Francis (1968) showed that addition of vitamin C reduced ACY in cranberry juice by 9–25% depending on oxygen level in the headspace. Similarly, Li and others (2014) reported that adding ascorbic acid at 360 mg/L increased the degradation rate constant of ACY in purple sweet potatoes from 7.24×10^{-2} to 8.57×10^{-2} (hr^{-1}). Ozkan and others (2005) and Sun and others (2011) explained that oxidation of vitamin C-induced ACY loss occurs via two mechanisms. The first mechanism is a direct condensation of ascorbate radicals to ACY structure. The second mechanism is the reaction between highly reactive radicals (•OH, •OOH) and ACY. The radicals

are byproducts from H_2O_2 decomposition upon vitamin C oxidation. The radicals are known as reactive oxygen species (ROS), which are damaging to ACY.

3.2.2 Color intensity

Juice color was determined to correlate with ACY retention. Control juice had average red color intensity of 0.93 AU during storage. Catechin and gallic acid were shown to have significant ($p < 0.05$) protective effect on red color retention in cranberry juice during 16-day storage at 23 °C (**Figure 3.5**). Gallic acid was found to be the most effective treatment for color retention (1.05–1.32 AU) as compared to catechin (1.12–1.25 AU) and hesperidin (0.93–1.12 AU). Hesperidin at all concentrations (5, 9, and 18 mg/100 mL) showed no effect on color intensity. The possible reason for this inverse effect is that co-pigmentation of hesperidin with anthocyanins might have resulted in polymeric anthocyanins, which contributed dark red color in the juice, and hence lower the bright red color intensity. Although Liu and Chen (2008) reported an increase in the water solubility of hesperetin on heating, the time and temperature used during pasteurization in the present study might have been insufficient to significantly increase the solubility. In contrast to the results for ACY retention, catechin was found to have a significant positive effect on the color intensity at both concentrations (5 and 15 mg/100 mL). However, the red color intensity at both catechin concentrations were not significantly different. In addition, measurement of color intensity with catechin addition could be misleading due to yellow color formation regarding oxidized catechin. Bark and others (2011) reported yellow color formation by catechin upon dissolving in aqueous solution due to catechin oxidation. This study confirmed yellow color formation by dissolving catechin (5 and 15 mg/100 mL) in water at ambient temperature for 2 hours, and found that the higher the catechin concentration, the more intense the yellow color in solution (visual observation). In contrast to hesperidin and catechin, we observed that gallic acid

solubilized well in water and did not form yellow compound upon dissolving in water. Moreover, gallic acid significantly ($p < 0.05$) increased red color retention in cranberry juice. The higher color intensity during storage represented better retention of ACY.

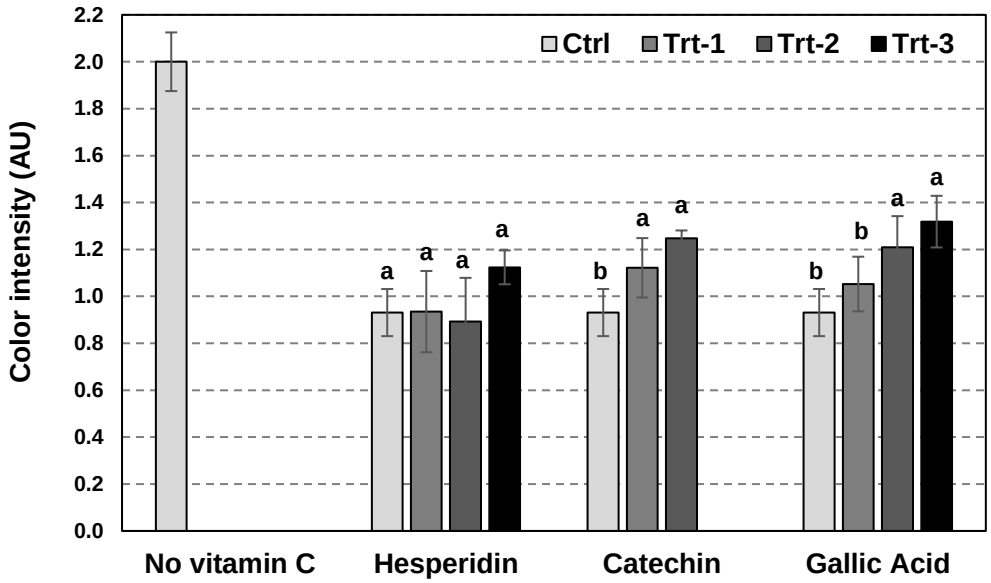


Figure 3.5 Average red color intensity during 16-day storage, at 23 °C. Treatment levels per 100 mL were: vitamin C fortification (80 mg) without adding antioxidant compounds (Ctrl); Hesperidin Trt-1 (5 mg), Trt-2 (9 mg), and Trt-3 (18 mg); Catechin Trt-1 (5 mg) and Trt-2 (15 mg); and Gallic Acid Trt-1 (80 mg), Trt-2 (160 mg), and Trt-3 (320 mg).
Treatments sharing same letters (a, b, c) are not significantly different from each other within the same antioxidant (Tukey's HSD test, $\alpha = 0.05$).

The effect of gallic acid was also assessed in cranberry juice at various concentration levels of vitamin C fortification. The negative impact of vitamin C on color retention (**Figure 3.6**) was found to be similar to the effect that was observed in the case of ACY retention. With an increase of vitamin C fortification level from 40 to 80 mg/100 mL, the color intensity of cranberry juice kept decreasing. As the fortification of vitamin C was increased to 60 and 80 mg/100 mL, the color

intensity decreased by 15.7% and 29.6%, respectively. Even though gallic acid showed significant increase in color intensity, as its concentration was increased up to 320 mg/100 mL, such increases could not bring back the original red color intensity (2 AU) due to the corresponding detrimental effect of added vitamin C. These results were consistent with the negative impact of vitamin C addition on ACY, as reported by Li and others (2014) and Sadilova and others (2007). Since the characteristic red color of cranberry juice is due to presence of ACY, any detrimental effect on ACY would have directly translated to a corresponding decrease in color intensity.

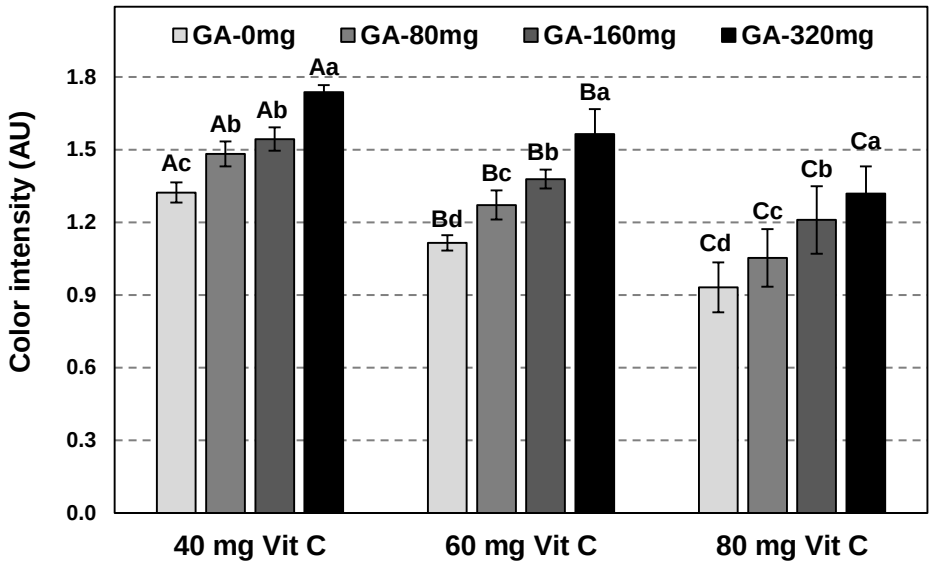


Figure 3.6 Effect of gallic acid (0-320 mg/100 mL) on the average color intensity of cranberry juice, fortified with 40-80 mg/100 mL vitamin C, during 16-day storage at 23 °C.
Treatments sharing same letters (a, b, c) and (A, B, C) are not significantly different from each other within the same vitamin C fortification level and across different levels, respectively (Tukey's HSD test, $\alpha = 0.05$).

3.2.3 Browning index

Degradation of polyphenols, including ACY, could result in brownish color in juices. The browning color represents chalcone formation, which is a byproduct regarding ACY degradation. Browning index (BI) represents color changes from reddish to yellowish or brownish. The BI greater than 1.0 is unacceptable, since brownish (AU_{430}) is predominant over reddish (AU_{520}) shades, according to BI evaluation criteria in pomegranate juices (Vegara and others 2013). Due to the poor solubility, BI regarding hesperidin addition was not determined. The BI observed with catechin and gallic acid addition are shown in **Figure 3.7**. BI consistently increased over 16-day storage at 23 °C. Compared to the control juice (vitamin C 80 mg/100 mL, no antioxidant), addition of gallic acid, at 80–320 mg/100 mL, showed protective effect as exhibited by lower BI, whereas catechin had no protective effect as evidenced by higher BI. On day 16, gallic acid at 80, 160, and 320 mg/100 mL showed 7.44%, 14.68%, and 20.49% decrease in the BI of cranberry juice. This demonstrated that gallic acid addition had a protective effect on color, as was exhibited by BI of <1.0.

It was also found that vitamin C fortification tended to increase BI, as shown in **Figure 3.8**, whereas gallic acid addition tended to decrease BI. Although gallic acid addition showed no significant difference in BI of juices at 40 and 60 mg/100 mL vitamin C levels, addition of gallic acid significantly ($p < 0.0001$) decreased BI at 80 mg/100 mL vitamin C level. However, the maximum gallic acid concentration at 320 mg/100 mL could not bring BI to be equal to juice without vitamin C fortification (BI = 0.57).

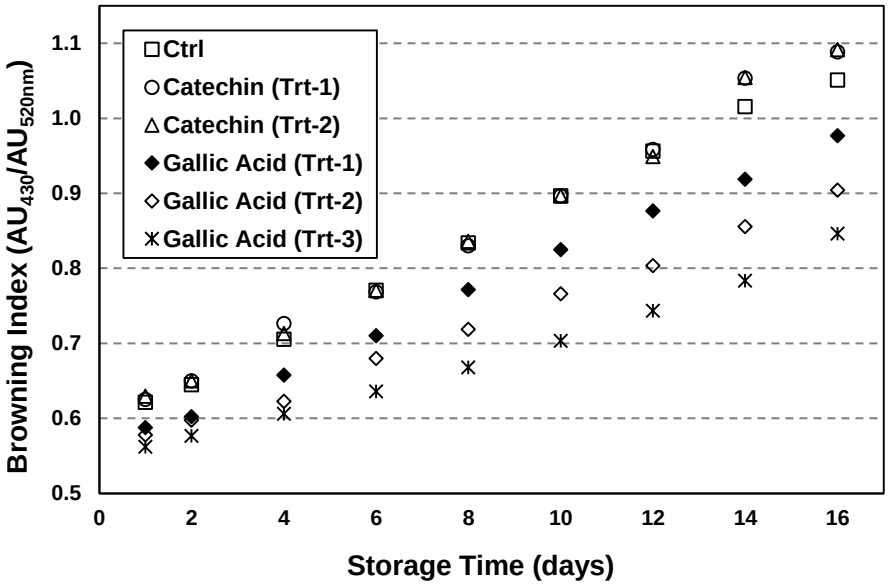


Figure 3.7 Effect of antioxidants on the browning index of cranberry juice fortified with vitamin C (80 mg/100 mL) during 16 day storage at 23 °C. Treatment levels per 100 mL: Catechin Trt-1 (5 mg) and Trt-2 (15 mg); and Gallic Acid Trt-1 (80 mg), Trt-2 (160 mg), and Trt-3 (320 mg). Standard deviation of browning index varied from 0.00016 to 0.053.

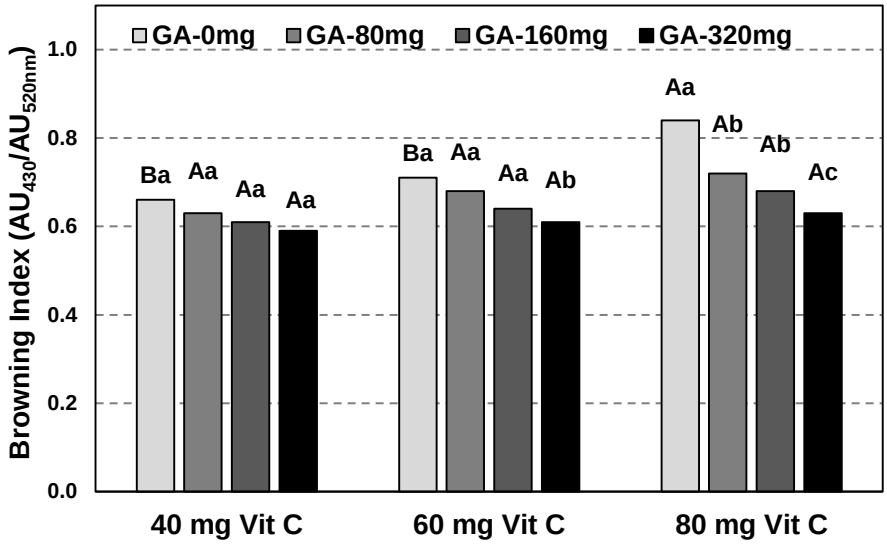


Figure 3.8 Effect of gallic acid (0-320 mg/100 mL) on browning index of cranberry juice, fortified with 40-80 mg/100 mL vitamin C, during 16-day storage at 23 °C.
Treatments sharing different letters (a, b, c) and (A, B, C) are significantly different from each other within the same vitamin C fortification level and across different levels, respectively (Tukey's HSD test, $\alpha = 0.05$).

3.2.4 Vitamin C retention

With reference to the above reported results, gallic acid was found to exhibit protective effect on both ACY and color retention during storage. Since fortification with vitamin C plays a role in ACY degradation, vitamin C retention was measured to gain better understanding on antioxidant mechanisms of gallic acid in cranberry juice. The results showed that gallic acid significantly ($p < 0.05$) increased vitamin C retention as compared to control juice (**Figure 3.9**). In order to increase vitamin C retention, ascorbate radicals possibly take protons from gallic acid, and then could convert back to L-ascorbic acid. However, in contrast to ACY and color retention, the level of gallic acid (80 to 320 mg/100 mL) showed no significant effect on vitamin C retention. It could be explained that gallic acid at 80 mg/100 mL possibly reached its maximum capacity to inhibit vitamin C oxidation. Moreover, vitamin C is a powerful antioxidant, which is susceptible to donate protons, and thus the oxidation reaction might occur more rapidly than the ability of gallic acid to inhibit the reaction of vitamin C (Yen and others 2002). This findings supported the hypothesis that the ability of gallic acid to stabilize reactive oxygen species (•OH, •OOH) was predominant over the ability to decrease vitamin C oxidation.

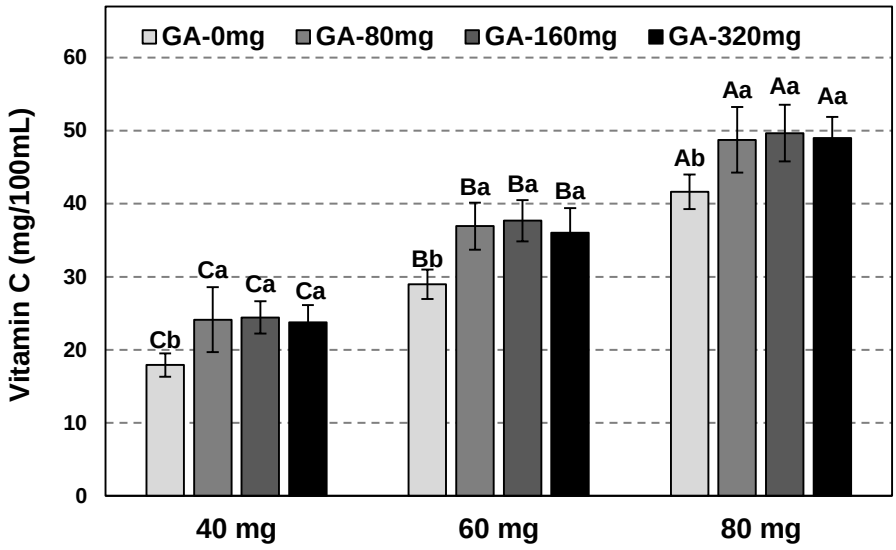


Figure 3.9 Effect of gallic acid (0-320 mg/100 mL) on the vitamin C content of cranberry juice, fortified with 40-80 mg/100 mL vitamin C, during 16-day storage at 23 °C.
Treatments sharing same letters (a, b, c) and (A, B, C) are not significantly different from each other within the same vitamin C fortification level and across different levels, respectively (Tukey's HSD test, $\alpha = 0.05$).

The results further showed that without vitamin C fortification, endogenous vitamin C in the juice stayed unchanged during 16 days of storage (7.1 mg/100 mL) (APPENDIX A, Table A2), and so did anthocyanin content (39.6 mg/L) and red color intensity (2 AU) (shown as a first bar in *Figure 3.3* and *Figure 3.5*). Vitamin C fortification from 40 to 80 mg/100mL significantly accelerated color and ACY degradation. It is known that high doses of vitamin C can act as a pro-oxidant, which induces more free radical formation (Paolini and others 1999). At higher doses of vitamin C, another pathway of reactive oxygen species (ROS) formation could occur by chelating of ascorbate and metal ions in juice, such as Cu^{3+}, Fe^{3+} (Rietjens and others 2002). The pro-oxidant effect may explain why fortified vitamin C caused color degradation, while endogenous vitamin C did not.

3.3 Conclusions

Gallic acid showed significant protective effect on ACY and color retention in cranberry juice. The higher the concentration of gallic acid, the higher ACY content and color retention were found. Generally, ACY and red color intensity of cranberry juice were effectively preserved at 320 mg/100 mL gallic acid, as it was the maximum gallic acid level in this study. Although gallic acid did not prevent vitamin C from oxidation, byproducts from the oxidation were neutralized by gallic acid, and hence gallic acid increased ACY and color retention. However, the addition of gallic acid could not completely overcome ACY and color degradation due to the effect from fortified vitamin C was very powerful. Therefore, concentration of fortified vitamin C needs to be taken into account to avoid or minimize a pro-oxidant effect.

The results demonstrated that gallic acid is a potential natural antioxidant compound that could be used in commercial cranberry juice. The high gallic acid concentration used in this study (320 mg/100 mL) would not develop astringent taste, but rather induce sweetness, as discussed in *Chapter 2*. These findings are not limited to cranberry juice but could be applied to other pigment-rich juices in order to preserve their endogenous ACY and natural color. Gallic acid is feasible to use in commercial juice production with regard to water solubility, and price. Cost of food-grade gallic acid is about $30/kg; adding gallic acid at 320 mg/100 mL would cost approximately $0.03 per 240 mL serving size. The use of gallic acid also offers a number of advantages for the juice industry in that it has no adverse effects, is colorless, and has the ability to induce non-caloric sweetness.

3.4 Limitations of the study

Referring to ACY, color, and vitamin C retention, we believe that major pathway of ACY degradation in cranberry juice is through cleavage by reactive oxygen species (ROS; •OH, •OOH). We also believe that gallic acid preserved ACY by deactivating the ROS because ACY retention increased with gallic acid concentration. The understanding of mechanisms was interpreted from retention of vitamin C and ACY. However, chemical analysis for H_2O_2 and ROS formation in juice was not performed in this study due to rapid decomposition of H_2O_2 and highly unstable ROS.

CHAPTER 4

OBJECTIVE TWO

Estimating kinetic parameters of anthocyanins and color degradation during storage by using an inverse method of ordinary least squares

4.1 Materials and Methods

4.1.1 Mathematical Modeling

4.1.1.1 Estimation of the kinetic parameters in the primary model

Data from all four replicates of ACY and color measurements during storage were used to estimate parameters *k, C_0,* and *n* in an n^{th}-order kinetic model which will be referred to as the primary model, Eq. (4.1) by the ordinary least squares inverse method in MATLAB (codes are shown in APPENDIX B). Parameter C_0 is initial red color intensity (AU), or initial ACY content (mg/L), at time = day 1. The *k* is degradation rate constant ($conc^{(1-n)}$ day^{-1}). The *n* is reaction order, dimensionless.

Table 4.1 shows three parameters that were estimated for all 12 treatments of both color and ACY. Significance of parameters was determined by noting if 95% confidence intervals (CIs) did not contain zero. The R-matrix (correlation) was used to determine correlation between parameters, correlation value greater than 0.99 refers to high correlation. Relative error indicated accuracy of estimates. The ease of estimating parameters was evaluated via the size of scaled sensitivity coefficients (SSC) (Dolan and Mishra 2013). The best model was determined by corrected Akaike Information Criteria (AIC_c). AIC_c (Eq. 4.2) is calculated based on the change of SS in regard to an increase or decrease in number of estimated parameters. Adding a parameter would always decrease SS; however, if the decrease is insufficient to justify the addition of a parameter, then AIC_c will be higher. Therefore, lower AIC_c indicates a better model.

$$\frac{dC}{dt} = -kC^n \tag{4.1}$$

$$AIC_c = \mathrm{N} \times \ln\left(\frac{SS}{N}\right) + 2K + \frac{2K(K+1)}{N-K-1} \quad (4.2)$$

where, *N* is number of data, *p* is number of parameters, *K* = p+1, *SS* is sum square of errors.

Table 4.1 Data analysis design for parameter estimation (*k*, C_0, *n*) for color and anthocyanins at different concentration of vitamin C and gallic acid (12 treatments*).

	Vitamin C		
	40 mg/100mL	60 mg/100mL	80 mg/100mL
Color			
Gallic acid (0 mg/100 mL)	*k*, C_0, *n* (Trt-1)	*k*, C_0, *n* (Trt-5)	*k*, C_0, *n* (Trt-9)
Gallic acid (80 mg/100 mL)	*k*, C_0, *n* (Trt-2)	*k*, C_0, *n* (Trt-6)	*k*, C_0, *n* (Trt-10)
Gallic acid (160 mg/100 mL)	*k*, C_0, *n* (Trt-3)	*k*, C_0, *n* (Trt-7)	*k*, C_0, *n* (Trt-11)
Gallic acid (320 mg/100 mL)	*k*, C_0, *n* (Trt-4)	*k*, C_0, *n* (Trt-8)	*k*, C_0, *n* (Trt-12)
Anthocyanins			
Gallic acid (0 mg/100 mL)	*k*, C_0, *n* (Trt-1)	*k*, C_0, *n* (Trt-5)	*k*, C_0, *n* (Trt-9)
Gallic acid (80 mg/100 mL)	*k*, C_0, *n* (Trt-2)	*k*, C_0, *n* (Trt-6)	*k*, C_0, *n* (Trt-10)
Gallic acid (160 mg/100 mL)	*k*, C_0, *n* (Trt-3)	*k*, C_0, *n* (Trt-7)	*k*, C_0, *n* (Trt-11)
Gallic acid (320 mg/100 mL)	*k*, C_0, *n* (Trt-4)	*k*, C_0, *n* (Trt-8)	*k*, C_0, *n* (Trt-12)

*4 replications per treatment

4.1.1.2 Parameter correlation

If parameters were highly correlated, they could not be estimated simultaneously, and therefore, one parameter was fixed as a constant. Other researchers frequently fixed parameter *n* at 1 for both color and ACY degradation during storage; otherwise, *n* was assumed to be 0.5, or an integer (0, 1, 2) (Polydera and others 2003; Harbourne and others 2008; Wibowo and others 2015; Remini and others 2015). However, this study determined actual n^{th}-order by varying *n* from 0.05 to 5 in 0.05 increments. The best fitted *n*-value for each treatment was the *n* that gave the smallest root mean square error (RMSE). Parameters *k* and C_0 were then estimated simultaneously by 2-parameter estimation, holding the actual *n* constant. A similar procedure was performed by

Buckow and others (2010) to determine the *n*-value for ACY degradation in blueberry juice during 40 to 121 °C pasteurization at pressures from 0.1 to 700 MPa.

Upon determining the actual *n*-value of each treatment, parameter *n* from all 12 treatments of ACY and color was determined whether the *n* values were significant different ($p < 0.05$) as changing concentration of vitamin C and gallic acid concentration. The secondary equations for n_{ACY} and n_{color} were modeled by multiple linear regression. The significant difference and modeling were done using JMP software, version 9.0.2 (SAS Institute, Inc., Cary, NC, USA).

4.1.1.3 Error of parameters

Error of estimates was determined by percent relative error, which was calculated from standard error of each estimate. Although parameter *n* was fixed as a constant in order to estimate parameters *k* and C_0, errors of n_{ACY} and n_{color} were determined by doing the reverse procedure, i.e., fixing different *k* values as previously estimated from the 2-parameter estimation shown in *Section 4.2.4.2*. Upon fixing *k* values, 2-parameter estimation was performed to estimate parameters *n* and C_0, and the asymptotic parameter standard error was computed as the square root of the diagonal of the parameter variance-covariance matrix cov(a) (Dolan 2003):

$$\mathbf{cov(a)} = \mathbf{(X^T X)^{-1}} MSE = \begin{pmatrix} \sigma^2_{b_1} & \sigma_{b_1 b_2} & \sigma_{b_1 b_3} \\ & \sigma^2_{b_2} & \sigma_{b_2 b_3} \\ \text{symmetric} & & \sigma^2_{b_3} \end{pmatrix} \qquad (4.3)$$

where; **X** is the sensitivity matrix, and MSE is the mean square error = SS/(N-p), *N* is number of data, *p* is number of parameters,

4.1.2 Developing secondary models for parameters as a function of vitamin C and gallic acid

Degradation rate constants (k_{ACY}, k_{color}) were functions of vitamin C and gallic acid (Eq. (4.4)). Empirical linear relationships between *k* and independent variables were determined by plotting *k* versus vitamin C, and *k* versus gallic acid, and conducting multiple linear regression. Because the variance of *k* values was not constant over vitamin C and gallic acid ranges, the *k* values were logarithmically transformed. In Eq. (4.4), parameters β_1 to β_6 were determined to be significant if the 95% CI (confidence interval) did not contain zero.

$$logk = \beta_1 + \beta_2 vitC + \beta_3 Gallic + \beta_4 (vitC \times Gallic) + \beta_5 (vitC)^2 + \beta_6 (Gallic)^2 \quad (4.4)$$

AIC_c also indicated whether an additional parameter was necessary in the model. Residual plots were used to determine goodness of fit of the models with experimental values, by the following standard statistical assumptions: constant variance, additive errors, zero mean, uncorrelated errors (Dolan and Mishra 2013). Multiple linear regression was also used to model polynomial equations for C_o and *n*, which are a function of vitamin C and/or gallic acid.

$$n = \beta_1 + \beta_2 vitC + \beta_3 Gallic + \beta_4 (vitC \times Gallic) + \beta_5 (vitC)^2 + \beta_6 (Gallic)^2 \quad (4.5)$$

$$C_0 = \beta_1 + \beta_2 vitC + \beta_3 Gallic + \beta_4 (vitC \times Gallic) + \beta_5 (vitC)^2 + \beta_6 (Gallic)^2 \quad (4.6)$$

4.2 Results and Discussion

4.2.1 Parameter estimation

According to a kinetic model, shown in Eq. (4.1), parameters k, C_0, n were initially estimated by three-parameter estimation. Among 12 treatments, the estimates at vitamin C 60 mg/100 mL with gallic acid 80 mg/100 mL (Trt-6 according to *Table 4.1*) was chosen to present the results in **Table 4.2**. From three-parameter estimation, it was found that confidence interval (CI) of the degradation rate constant of ACY (k_{ACY}) contained zero, showing that the k_{ACY} was not significant from zero ($p > 0.05$), and could be removed from the model. However, ACY were shown to decrease significantly ($p < 0.05$) during 16-day storage, which demonstrated that the k_{ACY} cannot be zero. According to R-matrix in Table 4.2, parameters k_{ACY} and n_{ACY} were highly correlated (correlation coefficient = $\rho_{k,n} = 0.9986$), and therefore, should not be estimated simultaneously. The estimates of k_{ACY} and n_{ACY} were found to have large error of 118.42% and 24.23%, respectively. Therefore, three-parameter estimation was not appropriate to estimate k_{ACY} and n_{ACY}. For color, although k_{color} did not contain zero, and its $\rho_{k,n} = 0.9322$ was low enough to allow simultaneous estimation; still, k_{color} and n_{color} were estimated separately to obtain better parameter accuracy and lower AIC_c (discussed later).

Table 4.2 Three parameter (*k*, C_0, *n*) estimation for anthocyanins and color in cranberry juice fortified with vitamin C (60 mg/100 mL) and gallic acid (80 mg/100 mL), during 16-day storage at 23 °C.

Kinetics	**Parameters**	**CIs**	**R-matrix**	**Error (%)**	**AIC_c**
Anthocyanins	*Co* = 21.72	20.42 - 23.03	1.0000 –0.4546 0.4825	2.94	
	k = 0.0077	–0.011 - 0.026	–0.4546 1.0000 –0.9986	118.52	34.27
	n = 1.84	0.93 - 2.74	0.4825 –0.9986 1.0000	24.23	
Color	*Co* = 1.88	1.82 - 1.93	1.0000 –0.2608 0.4589	1.36	
	k = 0.034	0.027 - 0.040	–0.2608 1.0000 –0.9322	9.56	–200.25
	n = 2.80	2.17 - 3.44	0.4589 –0.9322 1.0000	11.13	

4.2.1.1 Determination of parameter correlation

The scaled sensitivity coefficient (SSC) plots are helpful in determining which parameters can be estimated most accurately. As shown in **Figure 4.1**, SSC of all parameters *k*, C_0, *n* were large enough to be estimated. SSC plots of C_0 for both ACY and color were found to be larger than the size of *n* and *k*, which indicated that parameter C_0 can be estimated more accurately and easier than *n* and *k*. In addition, SSC plots can also illustrate parameter correlation. In **Figure 4.1A**, shape of SSC plots for k_{ACY} and n_{ACY} were nearly identical, which indicated the high correlation between those two parameters; corresponded to $\rho_{k,n} = 0.9986$ as shown in Table 4.2. In contrast to SSC_{ACY}, SSC_{color} (**Figure 4.1B**) showed that all three parameters were not highly correlated and could be estimated simultaneously. Owing to the high correlation (0.9986), k_{ACY} and n_{ACY} could not be estimated simultaneously. One parameter, either *k* or *n*, needed to be fixed as a constant. The parameter *n* is commonly fixed rather than *k*. However, prior to fixing *n*, this study estimated *n* by using an innovative statistical procedure to assure that it was the best *n* that represented degradation reactions.

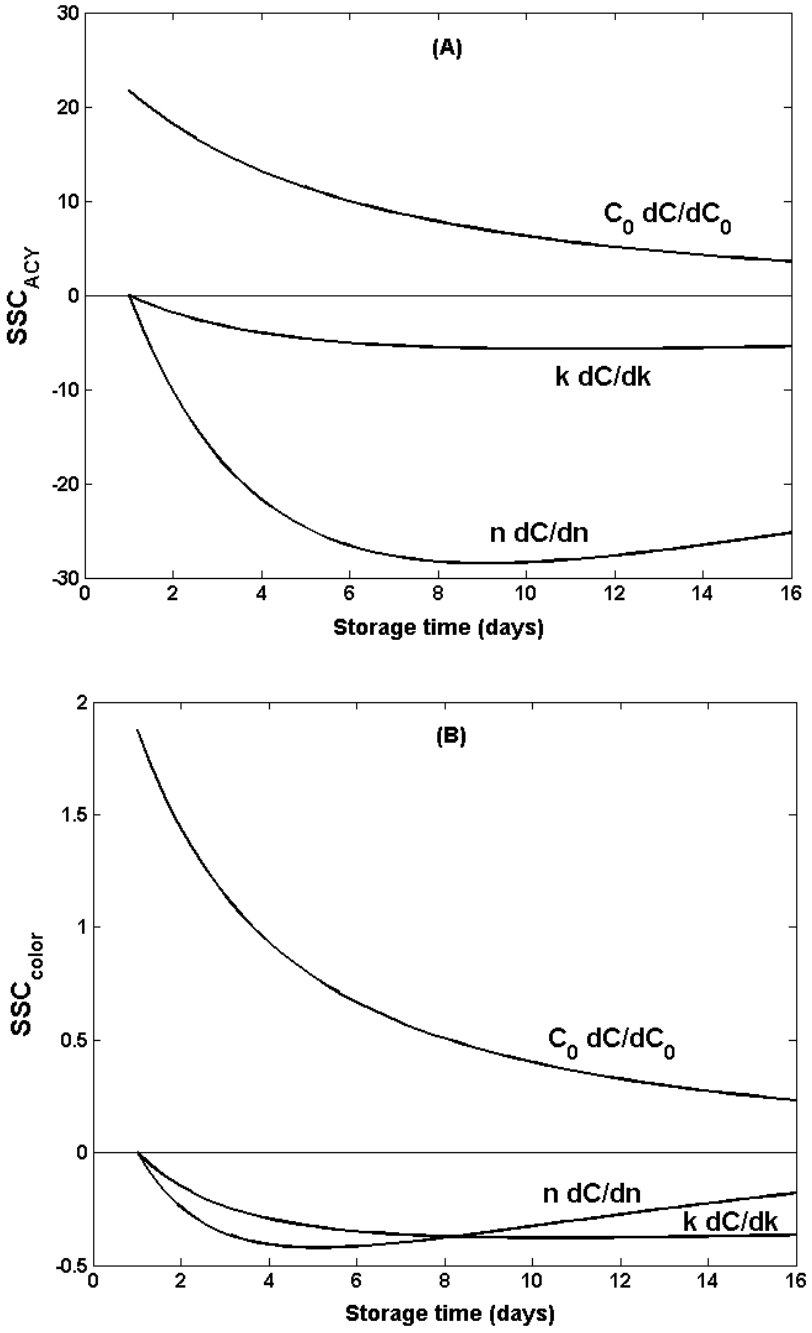


Figure 4.1 Representative SSC plots from three-parameter estimation of anthocyanins (A) and color (B) in cranberry juice fortified with vitamin C (60 mg/100 mL) and gallic acid (80 mg/100 mL), during 16-day storage at 23 °C.

Instead of fixing *n* as an integer (i.e., 0, 1, 2), this study let MATLAB vary *n* from 0.05 to 5 with 0.05 increments; total 100 *n*-values. Among 100 *n*-values, the *n* with the smallest RMSE was assigned as the best *n*, which would be later fixed in order to estimate for *k* and C_0. This study found that *n*-value of reaction was not constant for all 12 treatments. The *n* significantly decreased with an increase in vitamin C concentration in fortification. **Figure 4.2** shows representative plots of the RMSE versus *n* (reaction order) when C_0 and *k* were estimated simultaneously. These plots are for 40–80 mg/100 mL vitamin C fortification with no gallic acid addition, while other 3 pairs of plot (gallic acid 80–320 mg/100 mL) were in Appendix B, Figure B1–B3. As presented in **Figure 4.2A**, the smallest RMSE, n_{ACY} was 3.65, 1.75, and 1.45 at 40, 60, and 80 mg/100 mL vitamin C fortification, respectively. In contrast, **Figure 4.2B** shows that n_{color} was 4.25, 2.55, and 2.35 at 40, 60, and 80 mg/100 mL vitamin C fortification, respectively.

From all the 12 treatments, this study found that although *n*-order altered with respect to vitamin C concentration, it did not change ($p > 0.05$) with gallic acid concentration, as presented in **Figure 4.3**. Therefore, this implied that *n-value* was a function of vitamin C only. Linear regression was performed to model predictive equations for n_{ACY} and n_{color}, and resulted in Eq. (4.7) and Eq. (4.8), respectively. The empirical polynomial models to predict n_{ACY} and n_{color} showed an RMSE of 0.386 and 0.247, while total range of n_{ACY} and n_{color} were 3.1 and 2.5, respectively. According to Eq. (4.7) and Eq. (4.8), n_{ACY} and n_{color} at vitamin C fortification of 40, 60, and 80 mg/100 mL were 3.3, 2.2, and 1.2 for ACY, and 4.4, 2.9, and 2.2 for color, respectively.

This is the first study reporting that n^{th}-order for ACY and color degradation were different from 0 or 1, and changed with vitamin C concentration in fortification. Previous studies mostly estimated k_{ACY} by assuming first-order reaction ($n = 1$), without determining whether it was the

actual *n* for the degradation reaction (Bosch and others 2013; Ozkan and others 2005; Wang and Xu 2007). In addition, some studies have evaluated the best fitted *n* by comparing RMSE of various *n* at 0, 0.5, 1, 2, whereas the *n* greater than 2 was never even considered (Remini and others 2015; Buckow and others 2010; Wibowo and others 2015). None of the previous studies had reported that reaction order of ACY and color degradation could vary in such a wide range, i.e., from 1.2 to 4.4, as found in this study. In the present study, *n*-values were not assumed, but rather allowed to change freely with experimental measurements. Therefore, the *n*-values were the best fitted *n* of each treatment.

$$n_{ACY} = 5.31 - 0.0513(vitC) \tag{4.7}$$

$$n_{color} = 6.2 - 0.055(vitC) + 0.001(vitC - 60)^2 \tag{4.8}$$

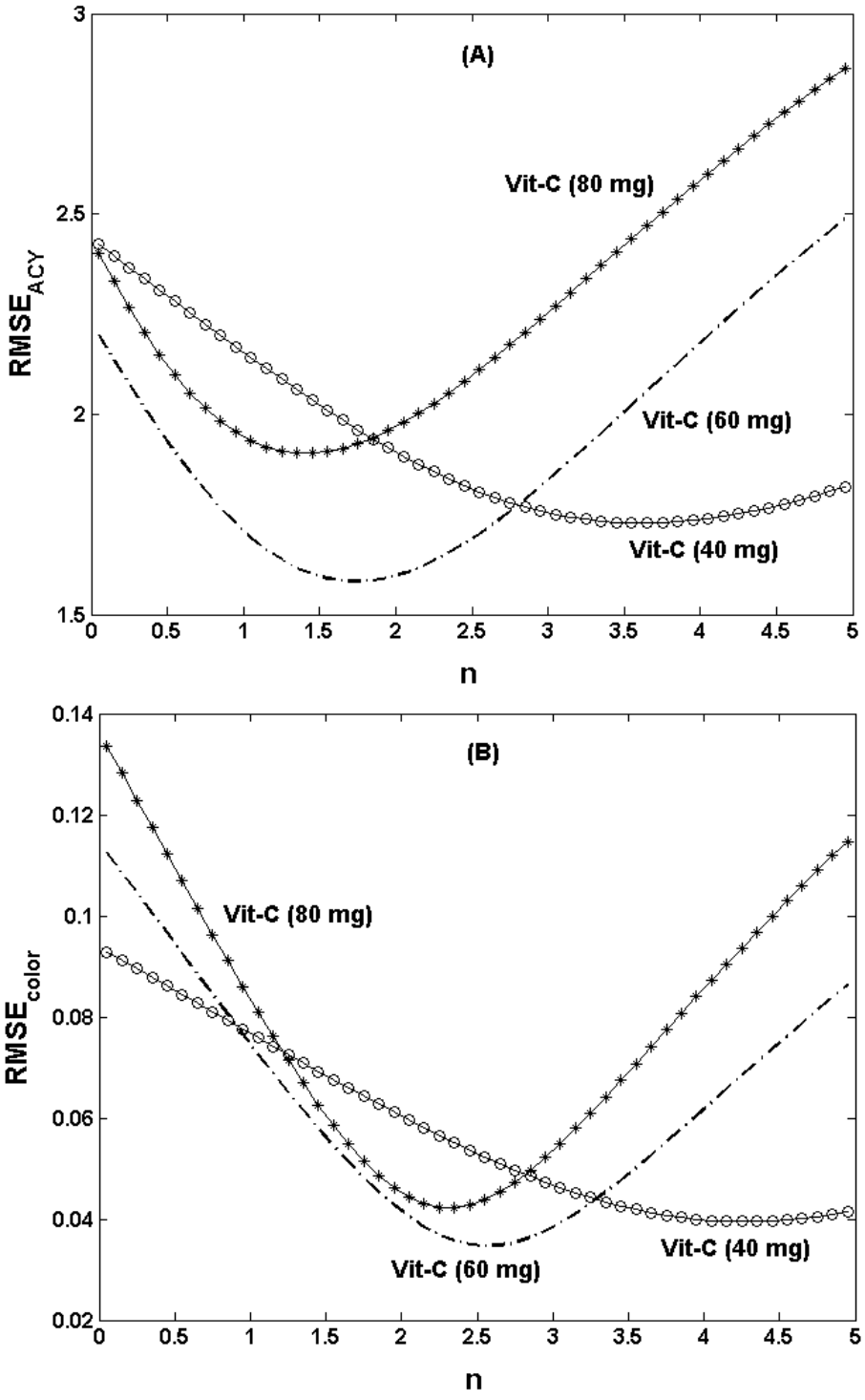


Figure 4.2 Representative plots of RMSE versus *n* (reaction order) of anthocyanins (A) and color (B) in cranberry juice fortified with vitamin C (40, 60, 80 mg/100 mL) and gallic acid (0 mg/100 mL), during 16-day storage at 23 °C.

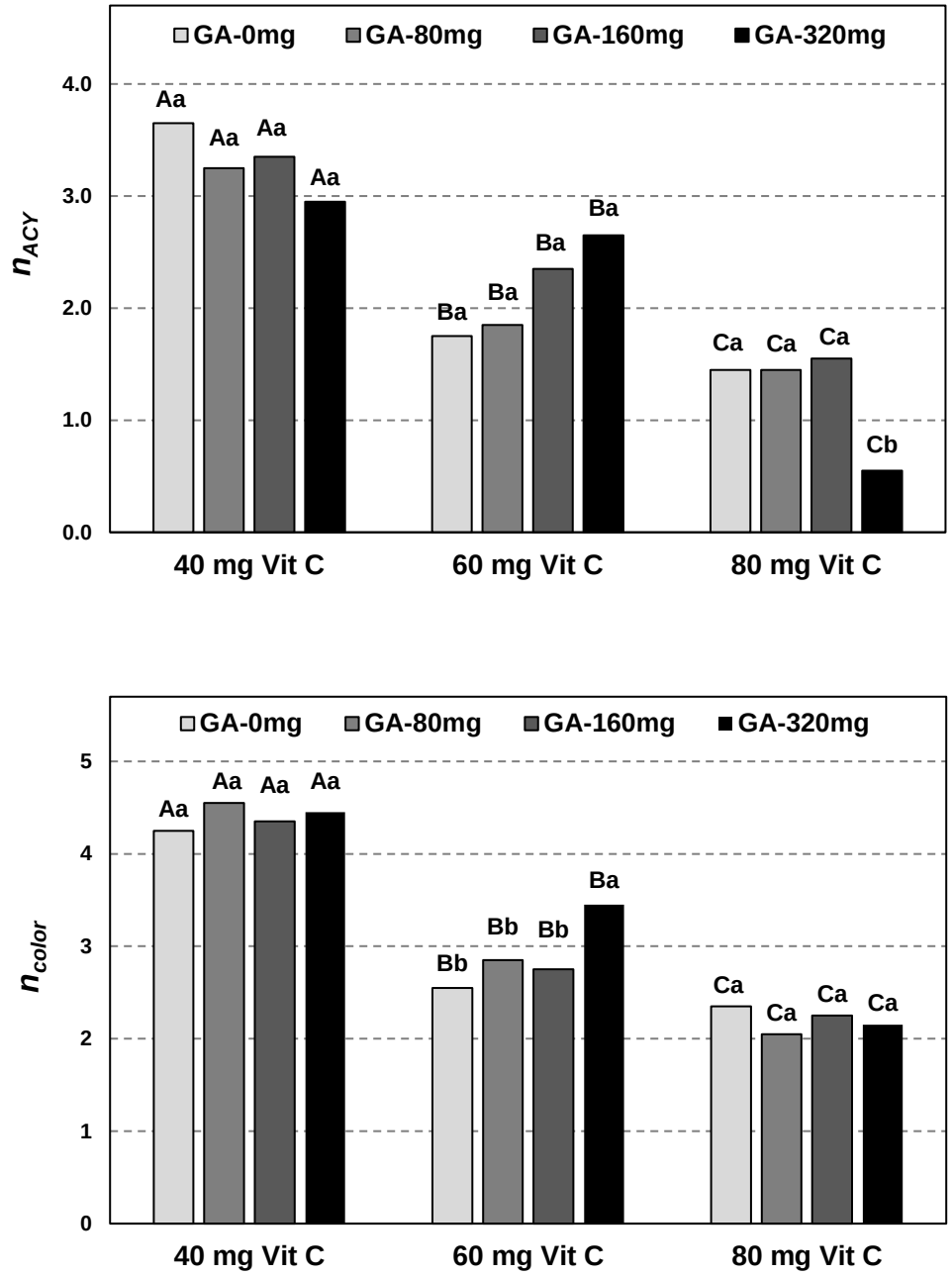


Figure 4.3 Effect of gallic acid (0-320 mg/100 mL) on order of degradation reaction of anthocyanins (n_{ACY}) and color (n_{color}) in cranberry juice, fortified with 40-80 mg/100 mL vitamin C, during 16-day storage at 23 °C.

Treatments sharing the same letters (a, b, c) and (A, B, C) are not significantly different from each other within the same vitamin C fortification level and across different levels, respectively (Tukey's HSD test, $\alpha = 0.05$).

4.2.1.2 Determining error of parameters

The actual n^{th}-orders were fixed accordingly with amount of fortified vitamin C, so that k and C_0 were estimated by two-parameter estimation. **Table 4.3** showed an example of the estimated parameters in cranberry juice fortified with vitamin C (60 mg/100 mL) and gallic acid (80 mg/100 mL), results for other treatments were in APPENDIX B, Table B3–B4. Parameters k and C_0 for both ACY and color were significantly different from zero, as the CIs did not contain zero. Correlation coefficients were lower than 0.95 ($\rho_{Co,k}$ = 0.530 for ACY, $\rho_{Co,k}$ = 0.509 for color), which indicated no correlation between parameters. Moreover, errors of parameters k and C_0 from two-parameter estimation were lower than the three-parameter estimation, i.e., errors of k_{color} and Co_{color} decreased from 9.56 to 3.45% and from 1.36 to 1.20%, respectively. Also, AIC_c slightly decreased from 34.27 to 32.31 (ACY) and –200.25 to –202.67 (color). Therefore, the two-parameter estimation was appropriate for both ACY and color models.

Table 4.3 Two parameter (k, C_0) estimation for anthocyanins and color in cranberry juice fortified with vitamin C (60 mg/100 mL) and gallic acid (80 mg/100 mL), during 16-day storage at 23 °C.

Kinetics	**Parameters**	**CIs**	**R-matrix**	**Error (%)**	**AIC_c**
Anthocyanins	C_o = 21.97	20.79 - 23.15	1.0000 0.5296	2.65	
	k = 0.0029	0.0025 - 0.0033	0.5296 1.0000	6.61	32.31
	n = 2.2 (fixed)			-	
Color	C_o = 1.88	1.83 - 1.93	1.0000 0.5086	1.20	
	k = 0.033	0.031 - 0.035	0.5086 1.0000	3.45	–202.67
	n = 2.9 (fixed)			-	

Errors of parameters n_{ACY} and n_{color} were determined by fixing previously estimated k_{ACY} and k_{color} (in Table 4.3) as a constant. **Table 4.4** indicated that n^{th}-*order* for both ACY and color were significantly different from zero and they were uncorrelated with parameter C_0, ($\rho_{Co,n}$ = 0.549 for ACY, ρ = 0.609 for color). Errors of n_{ACY} and n_{color} from all 12 treatments were lower than 10%, whereas the maximum errors of n_{ACY} and n_{color} were 3% and 6%, respectively.

Table 4.4 Two parameter (n, C_0) estimation for anthocyanins and color in cranberry juice fortified with vitamin C (60 mg/100 mL) and gallic acid (80 mg/100 mL), during 16-day storage at 23 °C.

Kinetics	**Parameters**	**CIs**	**R-matrix**	**Error (%)**
Anthocyanins	C_0 = 21.96	20.76 - 23.16	1.0000 0.5485	2.69
	k = 0.0029 (fixed)			
	n = 2.2	2.1 - 2.3	0.5485 1.0000	1.12
Color	C_0 = 1.88	1.83 - 1.93	1.0000 0.6086	1.30
	k = 0.033 (fixed)			
	n = 2.9	2.6 - 3.1	0.6086 1.0000	3.87

4.2.2 Developing secondary models

Secondary equations were modeled using parameters from all 12 treatments, while the parameters were estimated by two-parameter estimation. Since initial content of ACY and color differed due to vitamin C and gallic acid addition, empirical polynomial models for Co_{ACY} and Co_{color} were proposed as Eq. (4.9) and Eq. (4.10). Error of the models were 1.18 of 13% of the range (Co_{ACY}), and 5.26% of the range (Co_{color}). These equations were selected due to the lowest AICc (APPENDIX B, Table B5).

$$Co_{ACY} = \beta_1 + \beta_2(vitC) + \beta_3(gallic) + \beta_4(gallic - 140)^2$$

$\beta_1 = 28.24$, $\beta_2 = -8.71\times10^{-2}$, $\beta_3 = 1.06\times10^{-2}$, $\beta_4 = -8.08\times10^{-5}$ (4.9)

$$Co_{color} = \beta_1 + \beta_2(vitC) + \beta_3(gallic) + \beta_4(gallic - 140)^2$$

$\beta_1 = 1.96$, $\beta_2 = -2.49\times10^{-3}$, $\beta_3 = 8.91\times10^{-4}$, $\beta_4 = -1.96\times10^{-6}$ (4.10)

As illustrated in **Figure 4.4**, k_{ACY} and k_{color} changed regarding vitamin C fortification with added gallic acid. Vitamin C fortification significantly ($p < 0.05$) increased k_{ACY} and k_{color}, whereas gallic acid had significant positive impact on ACY and color, as exhibited by lower degradation rate constants. The increasing levels of gallic acid showed an increasing protective effect of ACY and color. This corresponded to findings in *Chapter 3* that ACY and characteristic red color intensity of cranberry juice were preserved by adding gallic acid as an antioxidant. The curvatures of k_{ACY} versus vitamin C are shown in **Figure 4.4A**. Even though the curvatures was not visually seen in **Figure 4.4B**, the curvature affected error in modeling predictive equation (data not shown). Therefore, log transformation was applied to both k_{color} and k_{ACY} in order to make the error variance more constant.

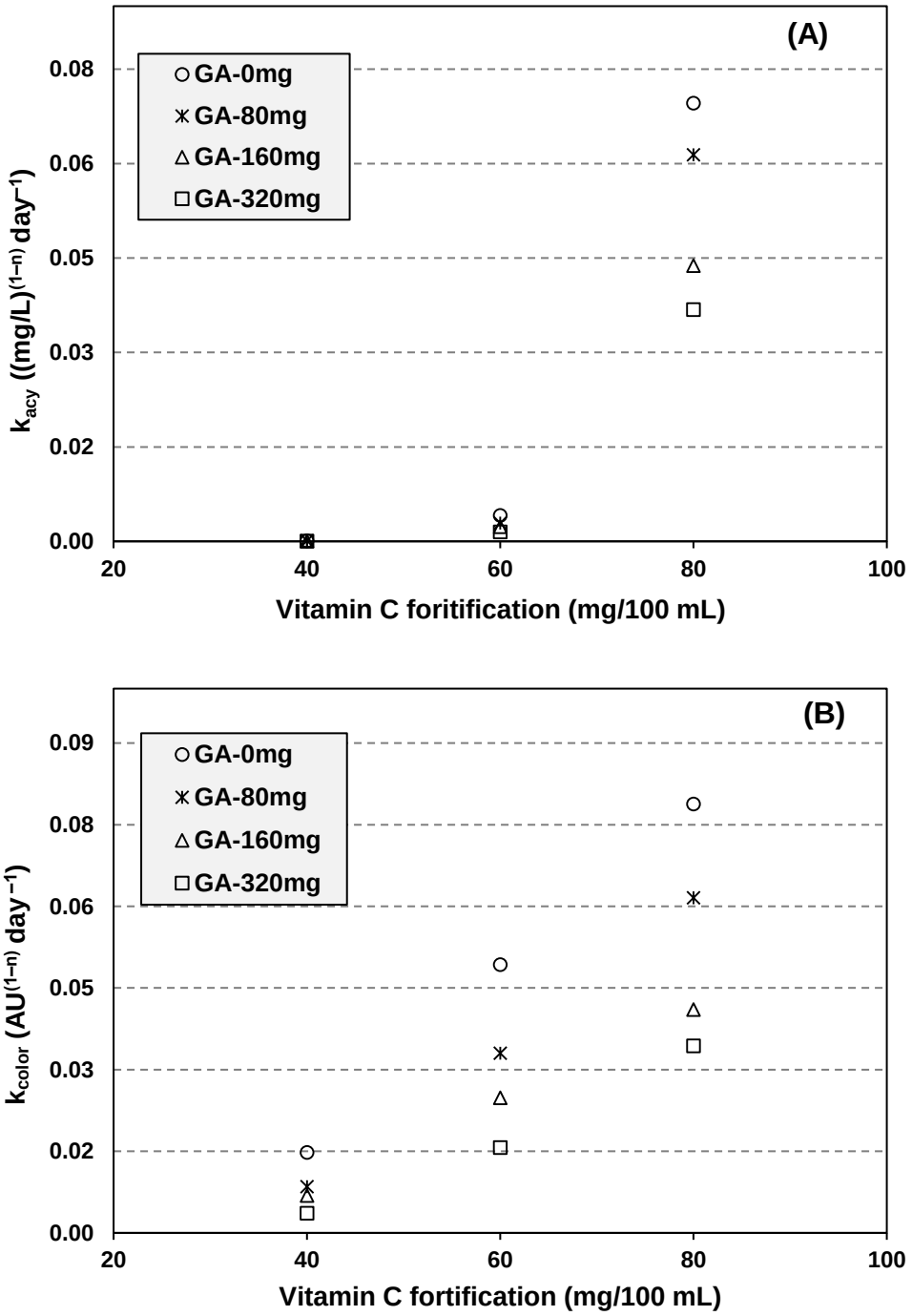


Figure 4.4 Degradation rate constant of anthocyanins (A) and color (B) in cranberry juice fortified with vitamin C (40–80 mg/100 mL) and gallic acid (0–320 mg/100 mL), during 16-day storage at 23 °C.

Thus, predictive equations of degradation rate constant (k_{ACY} and k_{color}) were modeled by multiple linear regression method. As a result, logk_{ACY} and logk_{color} could be calculated from Eq. (4.11) and Eq. (4.12), respectively.

$$logk_{ACY} = \beta_1 + \beta_2(vitC) + \beta_3(gallic) + \beta_4((vitC - 60) \times (gallic - 140)) + \beta_5(vitC - 60)^2$$

$\beta_1 = -6.7882$, $\beta_2 = 7.2875\times10^{-2}$, $\beta_3 = -1.2934\times10^{-3}$, $\beta_4 = 1.8544\times10^{-5}$, $\beta_5 = -3.7809\times10^{-4}$ (4.11)

$$logk_{color} = \beta_1 + \beta_2(vitC) + \beta_3(gallic) + \beta_4((vitC - 60) \times (gallic - 140)) + \beta_5(vitC - 60)^2 + \beta_6(gallic - 140)^2$$

$\beta_1 = -2.6121$, $\beta_2 = 2.0902\times10^{-2}$, $\beta_3 = -1.6303\times10^{-3}$, $\beta_4 = 1.6511\times10^{-5}$, $\beta_5 = -3.9769\times10^{-4}$, $\beta_6 = 2.3897\times10^{-6}$ (4.12)

The resulting models were the best models with regard to the lowest AIC_c, and all parameters β_1 to β_6 were significantly different from zero ($p < 0.05$). Errors of the models were 0.95% of total range (logk_{ACY}) and 2.24% of total range (logk_{color}). The 3D plots in **Figure 4.5** illustrated goodness of fit between experimental log*k* (dot) and surface of predicted log*k* from models.

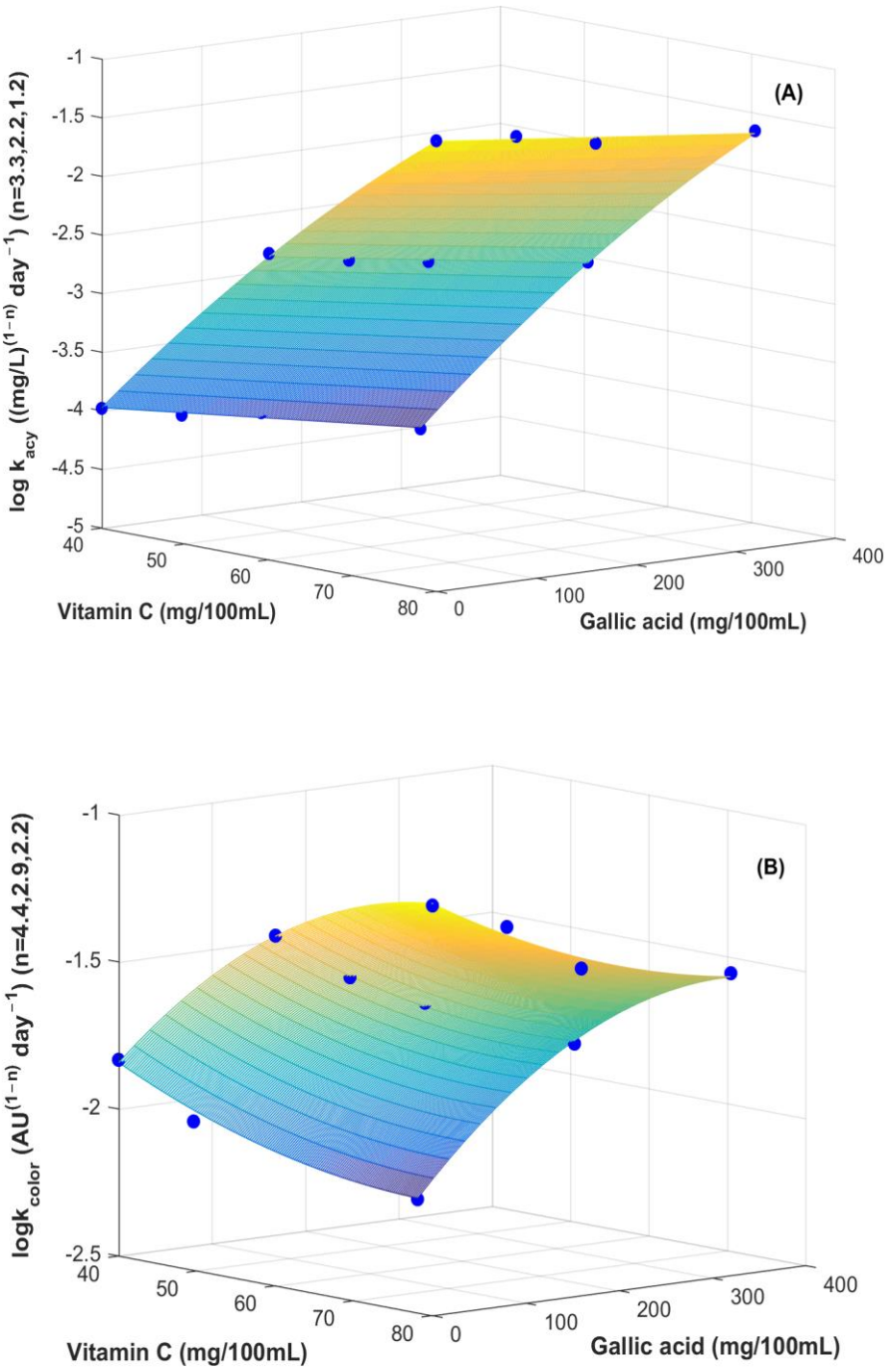


Figure 4.5 The 3D plots of logk_{ACY} and logk_{color} from experiment (•) and predictive surface calculated from Eq. 4.11 ($R^2_{adjusted}$ = 0.9994) and Eq. 4.12 ($R^2_{adjusted}$ = 0.9948), respectively.

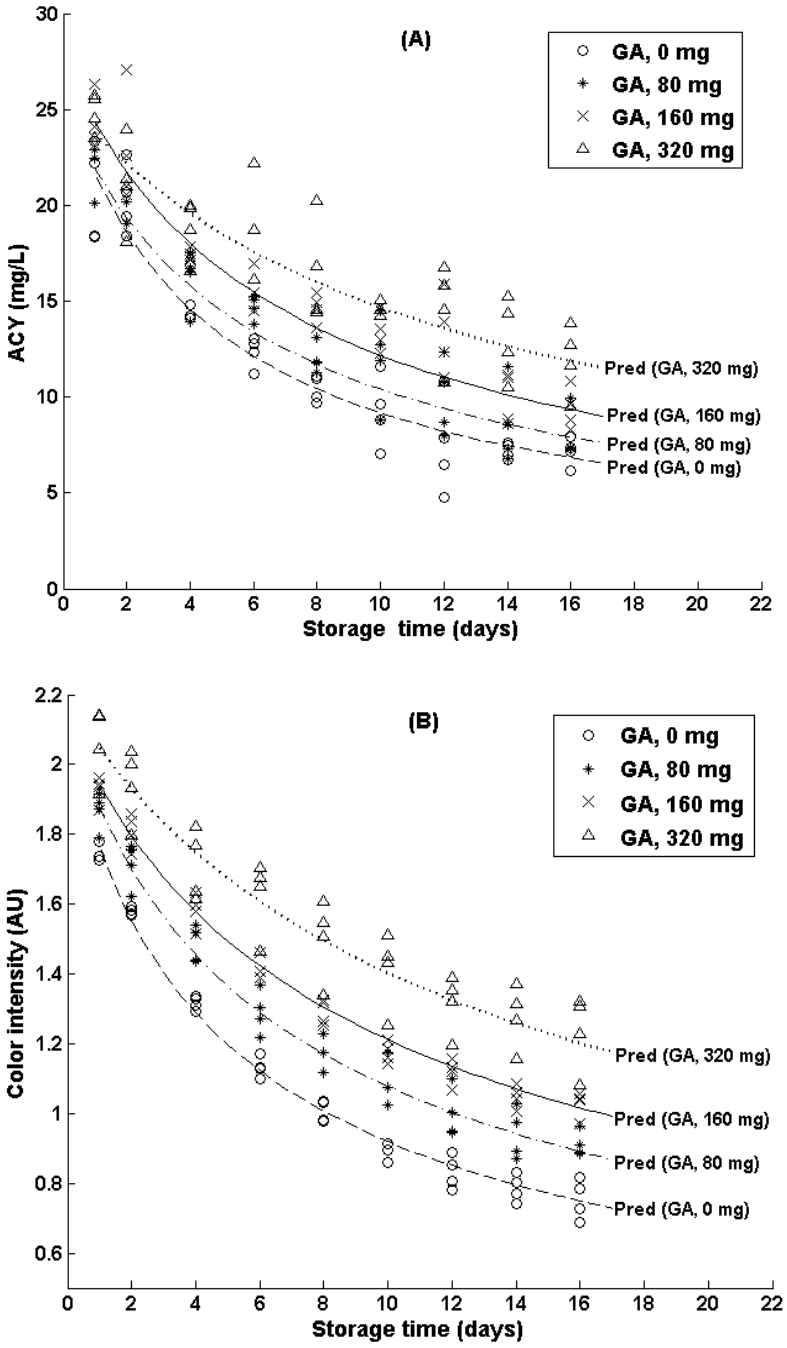


Figure 4.6 Representative model fittings of anthocyanins (A) and color (B) retention in cranberry juice fortified with vitamin C (60 mg/100 mL) and gallic acid (0–320 mg/100 mL), during 16-day storage at 23 °C, while GA and Pred refer to gallic acid, and predicted values, respectively.

Model fitting results are presented in **Figure 4.6** by plotting predictive lines with experimental measurement data. Predicted ACY and color retention (C_{ACY} and C_{color}) were calculated from differential equation, Eq. (4.1), by using ode45 in MATLAB, whereas *n*-values were calculated from Eq. (4.5) and Eq. (4.6), C_0-values were calculated from Eq. (4.7) and Eq. (4.8), and *k*-values were calculated from Eq. (4.9) and Eq. (4.10).

Residual plots are more useful than R^2 in order to determine goodness of fit of the models, since R^2 can be misleading especially with curvature data. Residual plots can tell not only how close predicted and experimental measurements are, but also present characteristics of the errors, which help determine whether it follows standard statistical assumptions. The residual plots for Figure 4.6 are shown in **Figure 4.7**. Goodness of fit was determined from constant bandwidth of the plot, which indicates constant variance. Other assumptions, such as additive errors and zero mean, were also met, Normal distribution of residuals illustrated by histograms are shown in **Figure 4.8**. For all 12 treatments of both ACY and color, there were mixed trends of the normality, which intepreted that some treatments did not show strong normality of residuals. However, except the normality, residual plots met most of statistical assumptions (constant variance, additive error, zero mean, uncorrelation). Therefore, these models could be used to predict ACY and red color retention during storage at any concentration of vitamin C and gallic acid, within the range of 40-80 mg/100 mL, and 0-320 mg/100 mL, respectively.

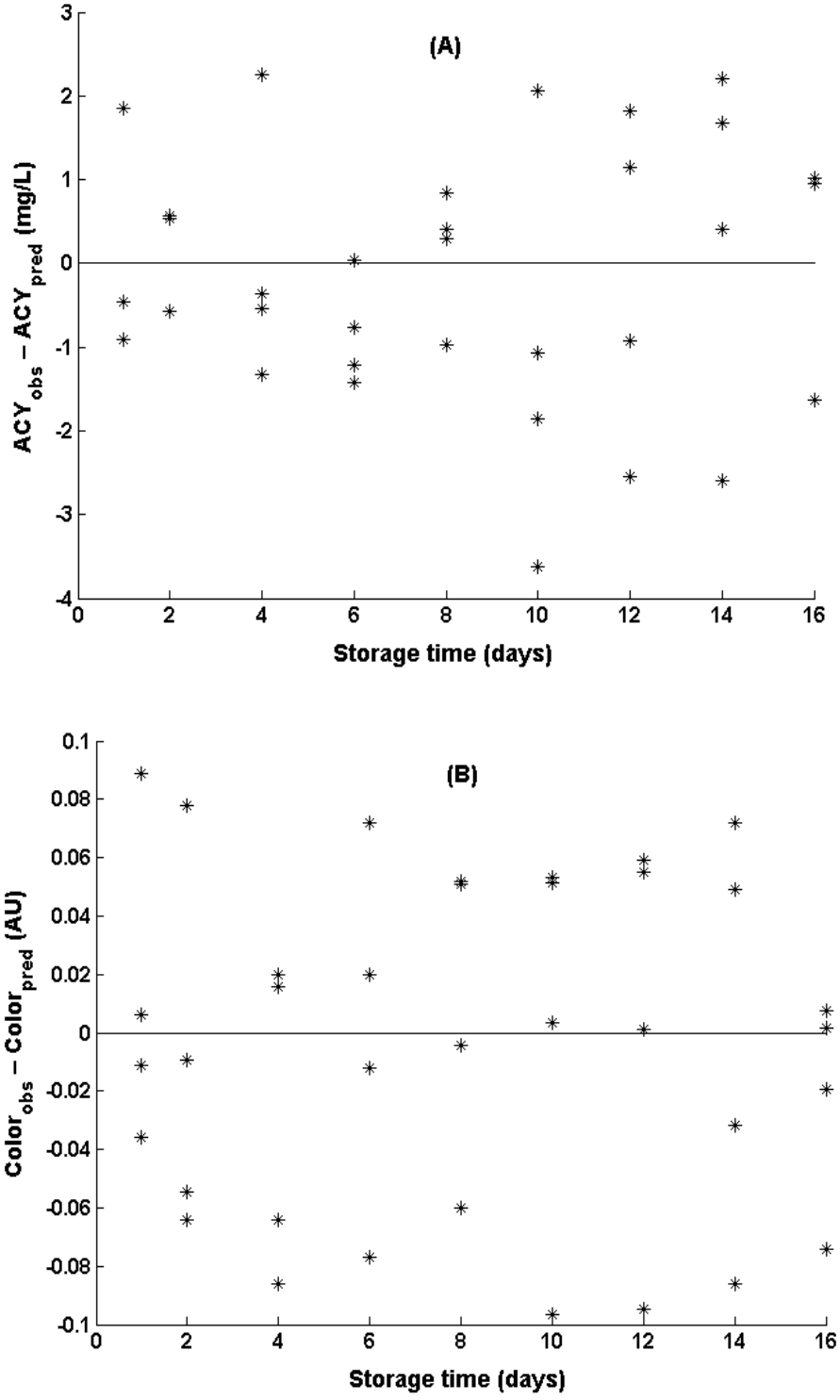


Figure 4.7 Representative residual plots showing difference between observed and predicted values of anthocyanins (A) and color (B) in cranberry juice fortified with vitamin C (60 mg/100 mL) and gallic acid (80 mg/100 mL), during 16-day storage at 23 °C.

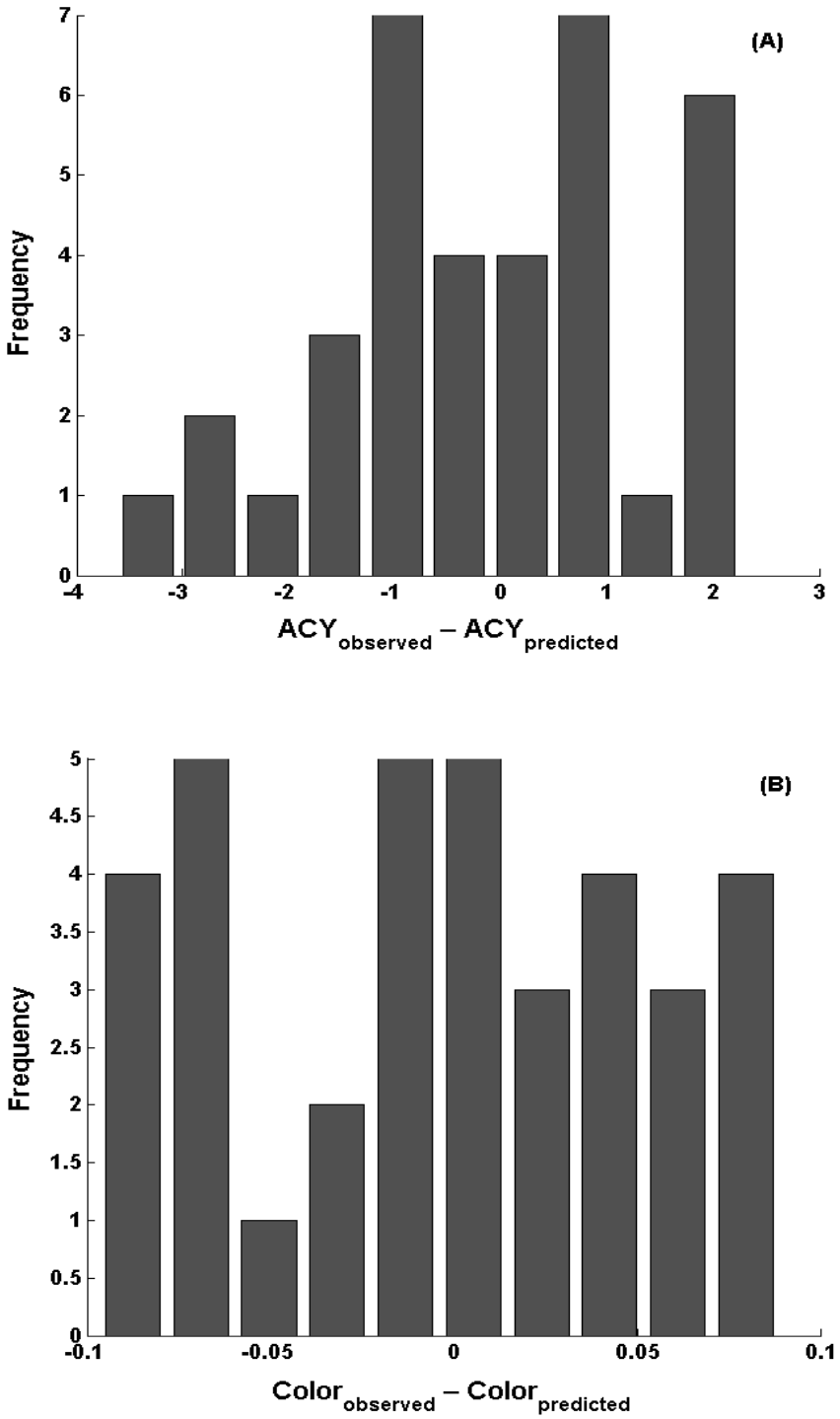


Figure 4.8 Representative histograms, plotted by dfittool in MATLAB, showing normal distribution of residuals in prediction of anthocyanins (A) and color (B) retention in cranberry juice fortified with vitamin C (60 mg/100 mL) and gallic acid (80 mg/100 mL), during 16-day storage at 23 °C.

4.3 Conclusions

Vitamin C fortification typically accerelates degradation of color and ACY in cranberry juice. Gallic acid was used as a natural antioxidant to mitigate the degradation of ACY and color. The nonlinear inverse method of ordinary least squares is an appropriate tool for estimating parameters k, C_0, n to avoid errors regarding model transformation, and also obtain physical meanings of estimates, i.e., correlation and error of parameters. Even though parameters k_{ACY} and n_{ACY} were found to be higly correlated, an innovative statistical technique was performed to obtain accurate estimates. The results showed that order of degradation reaction (n_{ACY} and n_{color}) decreased with increasing vitamin C fortification level and varied from 1.2 to 4.4, while previous studies assumed n = 1, a first order reaction. Degradation rate constants (k_{ACY} and k_{color}) were a function of both vitamin C and gallic acid, as they increased with vitamin C, but decreased with gallic acid. Statistical results from parameter estimation supported that the estimates of k, C_0, and n in this study were accurate with respect to small error, significance from zero, and uncorrelation. Predictive equations modeled from estimated parameters showed good fit with experimental data as residual plots met most of statistical assumptions.

The outcomes from this study have practical significance in that they not only introduced innovative statistical techniques to overcome highly correlation of parameters, but also proposed predictive empirical linear models for n-order (Eq. (4.7) and Eq. (4.8)), and for k (Eq. (4.11) and Eq. (4.12)) with comprehensive statistical information that could benefit to the juice industry for processing design with gallic acid implementation.

4.4 Limitations of the study

The models proposed in this study are applicable for certain ranges of vitamin C and gallic acid concentration, which are 40–80, and 0–320 mg/100 mL, respectively. The models might not give an accurate predicted values if vitamin C and gallic acid concentration are beyond the ranges used in this study. In addition, cranberry juice in this study was prepared from only one source of commercial cranberry juice concentrate. Anthocyanin content and color intensity might differ according to different sources of cranberry juice, and some variations might be expected regarding application of proposed models in this study. However, these predictive models should give an idea of percent retention of ACY and color during storage, in which could be a guideline and thus reduce work in setting up new experiments.

CHAPTER 5

OBJECTIVE THREE

Demonstrating practical application of results for the use in food industry, especially juice processor

5.1 Introduction

Research findings would be very useful if they are applicable in real practice. The study in *Chapter 3* revealed that gallic acid was an effective compound to mitigate color and anthocyanin degradation in cranberry juice fortified with vitamin C, and the models proposed in *Chapter 4* are useful for predicting retention of anthocyanins and color in cranberry juice during storage. This Chapter provides examples of model application to illustrate the implementing of predictive models in process design. Efficacy of processing is commonly determined by the increase/decrease of percent retention. Therefore, in this study, protective ability of gallic acid over detrimental effect of vitamin C fortification is determined through an increase in retention of red color intensity and anthocyanin content in the cranberry juice. Retention ratio $\left({}^{C_t}/_{C_0}\right)$ of ACY and color in the juice can be calculated using Eq. (5.2), which is derived from differential equation of kinetic model (Eq. (5.1)). The explicit-model derivation is as follows:

$$\frac{dC}{dt} = -kC^n \tag{5.1}$$

$$\int_0^C \frac{1}{C^n} dC = \int_0^t -k dt$$

$$\frac{C^{1-n} - C_0^{1-n}}{1-n} = -k(t - t_0)$$

Multiply ${}^{1}/_{C_0^{1-n}}$ to both sides:

$$\frac{C^{1-n} - C_0^{1-n}}{C_0^{1-n}} = \frac{(n-1)k(t - t_0)}{C_0^{1-n}}$$

$$\left(\frac{C}{C_0}\right)^{1-n} - 1 = \frac{(n-1)k(t - t_0)}{C_0^{1-n}}$$

$$\frac{C}{C_0} = \left(\frac{(n-1)k(t-t_0)}{C_0^{1-n}} + 1\right)^{\frac{1}{1-n}} \quad (5.2)$$

Where t was total days of storage, t_0 was initial storage day, C_0, n, and k were estimated from predictive models proposed in *Chapter 4*, which were:

1. Anthocyanin predictive models:

1.1 Initial ACY concentration (Co_{ACY})

$$Co_{ACY} = \beta_1 + \beta_2(vitC) + \beta_3(gallic) + \beta_4(gallic - 140)^2$$

$\beta_1 = 28.24$, $\beta_2 = -8.71\times10^{-2}$, $\beta_3 = 1.06\times10^{-2}$, $\beta_4 = -8.08\times10^{-5}$ (5.3)

1.2 Order of degradation reaction (n_{ACY})

$$n_{ACY} = 5.31 - 0.0513(vitC) \quad (5.4)$$

1.3 Degradation rate constant of ACY (k_{ACY})

$$logk_{ACY} = \beta_1 + \beta_2(vitC) + \beta_3(gallic) + \beta_4((vitC - 60) \times (gallic - 140)) + \beta_5(vitC - 60)^2$$

$\beta_1 = -6.7882$, $\beta_2 = 7.2875\times10^{-2}$, $\beta_3 = -1.2934\times10^{-3}$, $\beta_4 = 1.8544\times10^{-5}$, $\beta_5 = -3.7809\times10^{-4}$ (5.5)

2. Red color intensity predictive models:

2.1 Initial color intensity (Co_{color})

$$Co_{color} = \beta_1 + \beta_2(vitC) + \beta_3(gallic) + \beta_4(gallic - 140)^2$$

$\beta_1 = 1.96$, $\beta_2 = -2.49\times10^{-3}$, $\beta_3 = 8.91\times10^{-4}$, $\beta_4 = -1.96\times10^{-6}$ (5.6)

2.2 Order of degradation reaction (n_{color})

$$n_{color} = 6.2 - 0.055(vitC) + 0.001(vitC - 60)^2 \quad (5.7)$$

2.3 Degradation rate constant of color (k_{color})

$$logk_{color} = \beta_1 + \beta_2(vitC) + \beta_3(gallic) + \beta_4((vitC - 60) \times (gallic - 140)) + \beta_5(vitC - 60)^2 + \beta_6(gallic - 140)^2$$

$\beta_1 = -2.6121$, $\beta_2 = 2.0902\times10^{-2}$, $\beta_3 = -1.6303\times10^{-3}$, $\beta_4 = 1.6511\times10^{-5}$, $\beta_5 = -3.9769\times10^{-4}$, $\beta_6 = 2.3897\times10^{-6}$ (5.8)

Referring to experimental treatments in this study, ranges of vitamin C and gallic acid concentration used in the models were suggested to be between 40–80, and 0–320 mg/100 mL, respectively. Meanwhile, product shelf-life in this study (t) was 16 days and t_0 was 1.

5.2 Example case study#1

A cranberry juice company would like to address consumers' complaint regarding browning color in cranberry juice product. This product was made from cranberry juice concentrate, while juice in each bottle of 8 oz (240 mL) was fortified with 132 mg vitamin C. How much gallic acid would be recommended to maintain at least 50% color retention by the end of shelf-life?

Solution procedures

Since units of vitamin C and gallic acid used in the models are "**mg/100 mL**", thus, vitamin C fortification at 132 mg/240 mL equals to 55 mg/100 mL.

*A. **Forward problem***

First of all, minimum and maximum color retention of the juice needed to be determined, to test whether 50% retention could be achieved by adding gallic acid. The gallic acid at 0 and 320 mg/100 mL are for minimum and maximum retention, respectively.

1A). At vitamin C = 55 mg/100 mL and gallic acid = 0 mg/100 mL, Co_{color} (Eq. (5.6)), n_{color} (Eq. (5.7)), and $\log k_{color}$ (Eq. (5.8)) are:

Co_{color} = 1.78 AU

n_{color} = 3.2

$\log k_{color}$ = –1.4140, hence k_{color} = 0.0385 $(AU)^{(1-n)}$ day^{-1}

2A). Minimum color retention (Eq. (5.2)) = 0.4589, or 45.89%

3A). At vitamin C = 55 mg/100 mL and gallic acid = 320 mg/100 mL, repeat calculation in 1A. and 2A.

Co_{color} = 2.04 AU

n_{color} = 3.2

$\log k_{color}$ = –1.9316, hence k_{color} = 0.0117 $(AU)^{(1-n)}$ day^{-1}

Maximum color retention = 0.6199, or 62.0%

Therefore, 45.89–62% is a range of color retention in cranberry juice with 55 mg/100 mL vitamin C fortification. According to the range, 50% color retention is within the limits.

The next step is to calculate what gallic acid concentration that would give 50% color retention. Equations (5.2), (5.6), (5.7), and (5.8) are needed in calculation. For Eq. (5.6) and (5.8), gallic acid is presented in many terms, which make it impossible to solve explicitly for gallic acid.

Therefore, this is a roots problem, and the solution to solve roots problem is giving an initial guess of gallic acid and iteratively solving until the guess value is close to actual value, such that function of value; f(x) ~ 0.

B. <u>Procedures for roots problem</u>

1B). Use function `fzero` in MATLAB to solve roots problem, which is:

```
[x, fx] = fzero(function, x0)
```

`X` is location of the root, which gives an answer of gallic acid concentration (mg/100 mL)

`fx` is function evaluated at that root, the function for roots problem was set as follows:

$$function = limit - retention$$

Where, *limit* is the required color retention, *retention* is the value from iterative guessing of gallic acid, and *f(x)* = 0, meaning the gallic acid that gives difference between retention and limit close to zero.

`function` is a function handle to the `fx` function, consists of Eq. (5.6), (5.7), and (5.8) for calculating retention (Eq. (5.2)). MATLAB syntax are shown in APPENDIX **C**.

`x0` is an initial guess of gallic acid concentration.

2B). The *retention* $\left({C_t}/{C_0}\right)$, Co_{color}, k_{color}, and n_{color} are simultaneously calculated by guessing `x0`= 70 mg/100 mL.

3B). The results are:

Gallic = 66.43

fx = 5.5511×10^{-17}

In order to maintain 50% color retention in cranberry juice, gallic acid at 66.43 mg/100 mL needs to be added in the juice, which is fortified with vitamin C at 55 mg/100 mL. Therefore, each juice bottle (240 mL), which contains 132 mg vitamin C, needs gallic acid = 66.43 x 2.4 = 159.43 mg.

The juice industry might be more interested in color retention than anthocyanin retention because color intensity has direct impact on consumers' perceptions. However, this study also proposed ACY predictive models, which help predict ACY retention and the information could be useful for advertising and/or educating consumers regarding the health benefit components. The ACY retention in the juice adding gallic acid at 66.43 mg/100 mL can be calculated using Eq. (5.2), while Co_{ACY}, n_{ACY}, and k_{ACY} were calculated using Eq. (5.3), Eq. (5.4), and Eq. (5.5), respectively.

Co_{ACY} = 23.72 mg/L

n_{ACY} = 2.49

$logk_{ACY}$ = –2.8686, hence k_{ACY} = 0.0014 $(mg/L)^{(1-n)}$ day^{-1}

Therefore, there is 37.16% ACY retention in the cranberry juice (240 mL), which contains 132 mg fortified vitamin C and 159.43 (66.43 x 2.4) mg gallic acid.

5.3 Example case study#2

Since anthocyanins in cranberry juice help lower risk of human-diseases, a cranberry juice company would like to know how much anthocyanins have been lost in the current product (refer to the same juice product in *Case study*#1), and wondering if anthocyanin retention in the juice

could be increased to 50%. If yes, how much gallic acid will need to be added? (Note that juice was fortified with 132 mg vitamin C in each 240-mL bottle.)

Solution procedures

A. Forward problem

Determine minimum and maximum ACY retention at gallic acid = 0 and 320 mg/100 mL, respectively.

1A). At vitamin C = 55 mg/100 mL, gallic acid = 0 mg/100 mL, Co_{ACY}, n_{ACY} and $\log k_{ACY}$ are calculated using Eq. (5.3), Eq. (5.4), and Eq. (5.5), respectively.

Co_{ACY} = 21.8658 mg/L

n_{ACY} = 2.49

$logk_{ACY}$ = –2.7765, hence k_{ACY} = 0.0017 $(mg/L)^{(1-n)}\,day^{-1}$

2A). Minimum ACY retention (Eq. 5.2)) = 35.43%

Without gallic acid addition, the cranberry juice has lost approximately 65% of anthocyanins.

3A). At vitamin C = 55 mg/100 mL, gallic acid = 320 mg/100 mL, maximum ACY retention is calculated by repeating procedure 1A. and 2A.

Co_{ACY} = 24.2236 mg/L

n_{ACY} = 2.49

$logk_{ACY}$ = –3.2201, hence k_{ACY} = 6.0241e-04 $(mg/L)^{(1-n)}\,day^{-1}$

Maximum ACY retention = 0.5338, or 53.38%

Thus, the target 50% ACY retention does not exceed the maximum limit.

*B. **Procedures for roots problem***

Similar to *Case study*#1, a roots problem is solved for gallic acid concentration that would give 50% ACY retention, whereas Eq (5.2), (5.3), (5.4), and (5.5) are used to solve for gallic acid.

1B). Use function `fzero` in MATLAB to solve roots problem, which is:

```
[x, fx] = fzero(function, x0)
```

`X` is location of the root, which gives an answer of gallic acid concentration (mg/100 mL)

`fx` is function evaluated at that root, the function for roots problem was set as follows:

$$function = limit - retention$$

Where, *limit* is the required ACY retention, *retention* is the value from iterative guessing of gallic acid, and *f(x)* = 0, meaning the gallic acid that gives difference between retention and limit close to zero.

`function` is a function handle to the `fx` function, consists of Eq. (5.3), (5.4), and (5.5) for calculating retention (Eq. (5.2)). MATLAB syntax are shown in APPENDIX C.

`x0` is an initial guess of gallic acid concentration.

2B). The *retention* $\left({C_t}/{C_0}\right)$, Co_{ACY}, k_{ACY}, and n_{ACY} are simultaneously calculated by guessing `x0`= 70 mg/100 mL.

3B). The results are:

Gallic = 282.3422

fx = -1.1102×10^{-16}

Therefore, in order to maintain 50% ACY after storage, gallic acid at 282.34 × 2.4 = 677.62 mg will need to be added in the juice, which is fortified with 132 mg vitamin C.

The above two case studies explained step-by-step calculation to address questions. However, overall trends of color and anthocyanin retention in cranberry juice were illustrated as 3D plots in **Figure 5.1**. Two main benefits of the 3D plots are:

- To estimate maximum retention of color and ACY at any fortified vitamin C level in the juice. For instance, at 80 mg fortified vitamin C, maximum color retention is 49.19% (**Figure 5.1A**), whereas maximum ACY retention is 40.57% (**Figure 5.1B**). Therefore, both color and ACY retention in the juice with the 80 mg vitamin C would never reach 50%, even maximum gallic acid (320 mg) is added.
- To identify vitamin C and gallic acid concentration that would give target retention of color and ACY.

5.4 Conclusions

In summary, the model application provides predictive information, which would be useful for process design. The guideline obtained from models could save resources on doing experiments, especially money and time, and hence decisions or conclusions could be made rapidly.

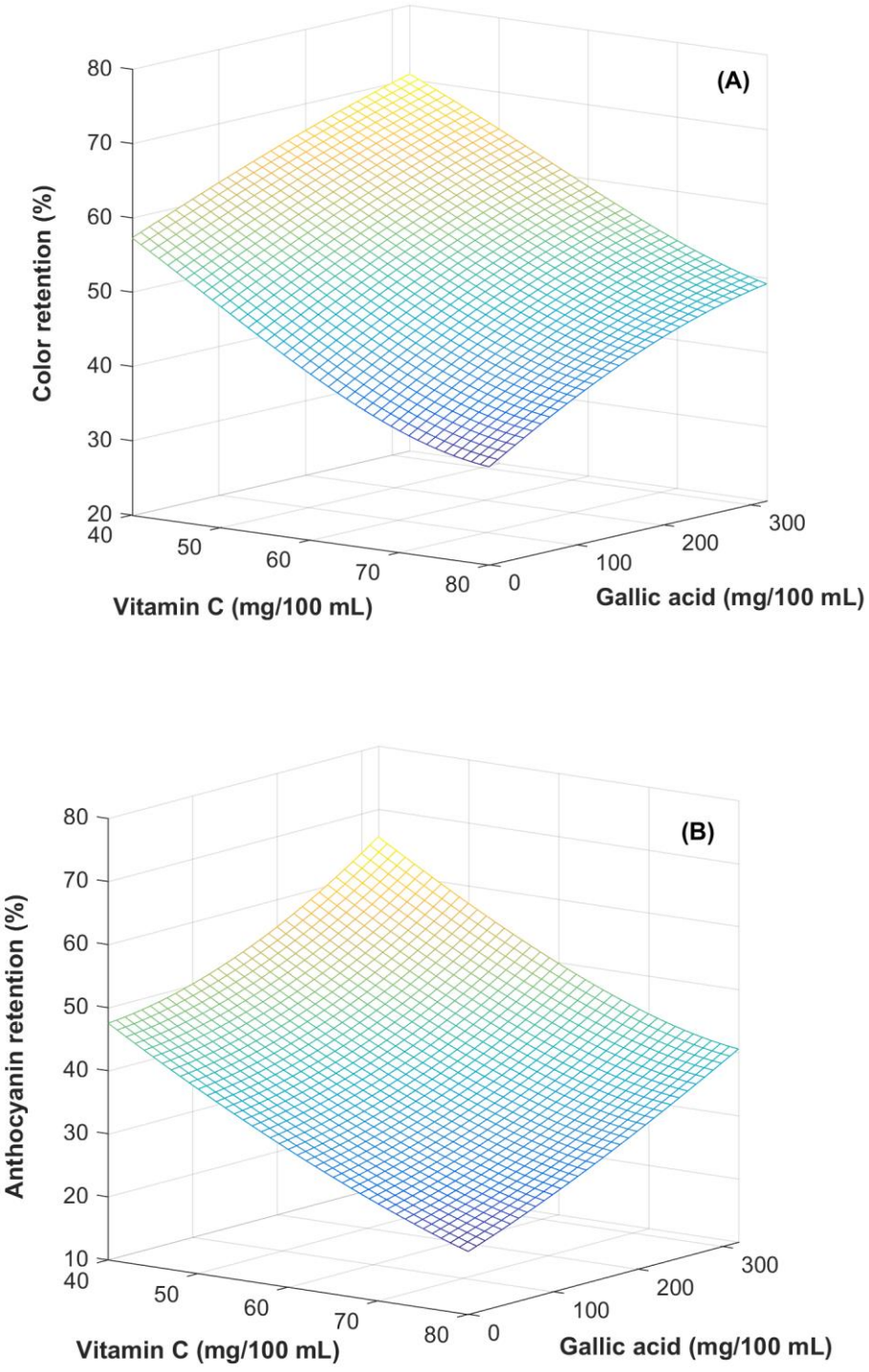


Figure 5.1 Retention of color (A) and anthocyanin (B) in pasteurized cranberry juice after storage at 23 °C, 16 days, with addition of vitamin C (40–80 mg/100 mL) and gallic acid (0–320 mg/100 mL).

CHAPTER 6

OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Overall conclusions

The novel contributions of this study were:

1. It showed potential protective effect of gallic acid against detrimental effect of fortified vitamin C on color and anthocyanins in cranberry juice. Therefore, gallic acid is a potent natural antioxidant in addressing consumer complaints regarding color change in the juice.

2. It showed the feasibility of applying gallic acid in the juice industry with regard to its high water solubility, and desired lack of effect on color upon dissolving.

3. It explained that the most likely protective mechanism of gallic acid is neutralization of vitamin C oxidation byproducts (i.e., •OH), rather than neutralizing of ascorbate radicals.

4. It showed that parameters *k* and *n* of the kinetic model, which are degradation rate constant and order of degradation reaction, respectively, were highly correlated and cannot be estimated simultaneously.

5. It showed an innovative statistical method to estimate *n*-values at the smallest root-mean-square-error.

6. It is the first study to report that *n*-order for both anthocyanins and color varied in a wide range from 1.2 to 4.4, and decreased with increasing fortified vitamin C level.

7. It reported that *n*-order was a function of only vitamin C, but not gallic acid. In contrast, *k* and C_0 were a function of both vitamin C and gallic acid.

8. It showed that proposed predictive models for *k*, *n*, and C_0 accurately predicted color and anthocyanin retention, as evaluated from residual structure in residual plots and histogram.

9. It provided examples in model application, using roots problem solution technique, to calculate concentration of gallic acid that meets the goal of maintaining a minimum required color and/or anthocyanin retention in the juice.

6.2 Future directions

The following topics are recommended for future study:

1. Sensory evaluation is necessary to obtain consumers' perception on the product regarding gallic acid addition.

2. Validation of secondary models with independent experiments on cranberry juice production.

3. This study was conducted with only cranberry juice, and thus all kinetic parameters (*k*, *n*, C_0) estimated in the present study might not apply to other kinds of juice. Therefore, estimating kinetic parameters for other pigment-rich juices is recommended for juices different from cranberry juice. The parameter estimation could be done by following procedures as used in this study, which are:

    2.1 Estimate parameters *k*, *n*, C_0 using ordinary least square (OLS) inverse method

        a. Check parameter correlation

        b. Check significance of parameters (CIs do not contain zero)

        c. Check error of parameters

2.2 Model predictive equations for k, n, C_0 using multiple linear regression method

a. Check error and AIC_c (corrected Akaike Information Criteria) of predictive models

b. Check goodness of fit of the models via residual plots and histogram

APPENDICES

APPENDIX A

Experimental measurements for red color intensity, anthocyanins, and L-ascorbic acid content

Table A1 Data of red color intensity, anthocyanins, and vitamin C in cranberry juice at day 0 after pasteurizing at 85 °C, the juice was not fortified with vitamin C and no antioxidant addition.

Replication no.	Color intensity (AU)	Anthocyanins (mg/L)	Vitamin C (mg/100 mL)
1	2.66	47.00	9.29
2	2.45	46.24	6.67
3	2.40	47.79	7.23
4	2.65	47.17	9.55
5	2.24	45.00	6.03
6	2.15	45.93	7.77
Avg ± SD	**2.42 ± 0.21**	**46.52 ± 1.00**	**7.76 ± 1.41**

Table A2 Data of red color intensity, anthocyanins, and vitamin C in cranberry juice pasteurizing at 85 °C, the juice was not fortified with vitamin C and no antioxidant addition.

Storage (days)	Color intensity (AU)	Anthocyanins (mg/L)	Vitamin C (mg/100 mL)
1	2.45	46.18	9.55
	2.34	45.73	9.29
2	2.23	43.60	8.71
	2.2	43.67	8.62
4	2.08	43.34	8.33
	2.19	42.65	7.77
6	2.02	40.04	7.23
	2.14	39.66	6.85
8	2.07	39.32	6.61
	2.02	38.59	6.31
10	2.10	38.66	6.03
	1.94	37.83	6.67
12	1.89	36.59	6.50
	1.79	35.71	5.90
14	1.71	35.87	5.96
	1.70	35.34	5.72
16	1.69	34.82	5.63
	1.60	34.45	5.55
Avg ± SD	**2.01 ± 0.24**	**39.6 ± 3.82**	**7.07 ± 1.22**

Table A3 Data of red color intensity in cranberry juice fortified with vitamin C (40–80 mg/100 mL) and gallic acid addition (0–320 mg/100 mL).

Vitamin C 40 mg/100 mL				
Storage (days)	**Color intensity (AU)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
1	1.829	1.896	2.005	2.104
	1.735	1.861	1.885	2.056
	1.845	1.963	2.005	2.101
	1.827	1.951	1.971	2.096
2	1.711	1.813	1.891	2.027
	1.585	1.73	1.776	1.977
	1.693	1.865	1.909	2.045
	1.687	1.842	1.829	2.027
4	1.504	1.617	1.739	1.891
	1.377	1.553	1.636	1.842
	1.5	1.671	1.706	1.898
	1.46	1.657	1.676	1.879
6	1.367	1.514	1.615	1.812
	1.291	1.435	1.51	1.761
	1.341	1.53	1.592	1.735
	1.331	1.552	1.545	1.764
8	1.274	1.366	1.539	1.718
	1.18	1.3014	1.426	1.663
	1.215	1.432	1.502	1.688
	1.21	1.433	1.446	1.665
10	1.231	1.34	1.468	1.681
	1.13	1.266	1.387	1.628
	1.172	1.333	1.414	1.604
	1.147	1.402	1.359	1.606
12	1.161	1.273	1.427	1.611
	1.083	1.246	1.327	1.552
	1.111	1.303	1.355	1.574
	1.136	1.363	1.328	1.571
14	1.109	1.251	1.378	1.571
	1.053	1.194	1.292	1.528
	1.073	1.269	1.296	1.5
	1.093	1.305	1.3	1.49
16	1.085	1.206	1.308	1.493
	1.007	1.162	1.245	1.466

Table A3 (cont'd)

Vitamin C 40 mg/100 mL				
Storage (days)	**Color intensity (AU)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
16	1.022	1.209	1.263	1.498
	1.041	1.251	1.21	1.446
Vitamin C 60 mg/100 mL				
1	1.735	1.915	1.869	2.044
	1.727	1.79	1.945	2.14
	1.737	1.873	1.962	2.136
	1.779	1.89	1.936	1.915
2	1.567	1.765	1.745	1.932
	1.582	1.623	1.796	2.002
	1.572	1.71	1.857	2.037
	1.593	1.755	1.835	1.798
4	1.311	1.541	1.516	1.769
	1.327	1.435	1.596	1.636
	1.291	1.439	1.634	1.821
	1.334	1.519	1.578	1.614
6	1.128	1.367	1.409	1.649
	1.171	1.218	1.462	1.705
	1.1	1.27	1.46	1.674
	1.13	1.302	1.389	1.463
8	1.031	1.23	1.264	1.507
	1.036	1.119	1.318	1.608
	0.977	1.118	1.325	1.547
	0.983	1.174	1.253	1.341
10	0.894	1.174	1.162	1.45
	0.913	1.026	1.209	1.51
	0.86	1.024	1.141	1.432
	0.86	1.074	1.187	1.252
12	0.852	1.098	1.122	1.354
	0.888	0.948	1.156	1.39
	0.782	0.944	1.133	1.32
	0.807	1.002	1.068	1.195
14	0.801	1.028	1.041	1.268
	0.831	0.893	1.086	1.371
	0.772	0.87	1.059	1.314
	0.742	0.974	1.006	1.156

Table A3 (cont'd)

Vitamin C 60 mg/100 mL				
Storage (days)	**Color intensity (AU)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
16	0.783	0.965	1.038	1.306
	0.818	0.889	1.057	1.322
	0.726	0.883	1.041	1.23
	0.687	0.91	0.97	1.082
Vitamin C 80 mg/100 mL				
1	1.681	1.746	1.896	1.975
	1.735	1.862	2.04	1.997
	1.747	1.761	1.799	1.886
	1.678	1.833	1.851	1.921
2	1.481	1.615	1.766	1.871
	1.555	1.718	1.891	1.878
	1.533	1.661	1.782	1.745
	1.43	1.655	1.755	1.779
4	1.183	1.325	1.504	1.68
	1.226	1.399	1.611	1.642
	1.222	1.362	1.452	1.511
	1.115	1.356	1.489	1.549
6	1.039	1.105	1.349	1.558
	1.026	1.227	1.418	1.49
	0.993	1.093	1.264	1.319
	0.926	1.168	1.31	1.359
8	0.911	1.007	1.18	1.401
	0.881	1.054	1.233	1.338
	0.867	0.92	1.162	1.178
	0.821	0.95	1.208	1.262
10	0.812	0.843	1.031	1.296
	0.751	0.955	1.142	1.242
	0.753	0.793	1.052	1.057
	0.705	0.888	1.049	1.143
12	0.748	0.809	0.99	1.152
	0.68	0.835	1.042	1.175
	0.667	0.749	0.979	0.996
	0.644	0.821	1.02	1.037

Table A3 (cont'd)

Vitamin C 80 mg/100 mL				
Storage (days)	**Color intensity (AU)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
14	0.668	0.714	0.898	1.132
	0.631	0.782	0.95	1.095
	0.639	0.694	0.874	0.936
	0.573	0.705	0.923	0.971
16	0.662	0.734	0.876	1.085
	0.586	0.726	0.918	1.037
	0.625	0.638	0.883	0.837
	0.552	0.695	0.886	0.834

Table A4 Data of anthocyanin content in cranberry juice fortified with vitamin C (40–80 mg/100 mL) and gallic acid addition (0–320 mg/100 mL).

Vitamin C 40 mg/100 mL				
Storage (days)	**Anthocyanin content (mg/L)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
1	22.94655	24.22136	29.76849	23.78493
	23.46336	28.08023	26.63315	24.11799
	26.70206	22.63646	32.14584	22.94655
	22.77428	24.15245	26.49534	24.29026
2	20.74147	24.80708	21.15492	22.602
	19.84566	22.25746	22.18855	21.67174
	26.28861	21.3961	23.91127	22.91209
	21.01711	21.98183	24.84153	26.08189
4	16.40023	18.74313	27.14997	22.73982
	15.26324	18.39858	30.62985	20.01793
	16.60696	17.7095	19.39776	19.94903
	17.88177	20.46584	18.05404	20.39693
6	14.22962	17.53723	18.05404	20.43139
	13.88507	15.36661	16.88259	21.36165
	16.81369	18.57085	20.19021	23.98018
	14.95316	17.77841	17.19268	20.29357
8	12.8859	15.6767	15.46997	16.98596
	13.16153	13.98844	15.64224	17.15823
	14.64307	16.1246	19.08767	22.1541
	14.16071	14.95316	15.78006	17.05487

Table A4 (cont'd)

Vitamin C 40 mg/100 mL				
Storage (days)	**Anthocyanin content (mg/L)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
10	13.54053	14.19516	15.81451	16.81369
	10.95646	13.05817	12.61026	16.71032
	12.67917	16.43469	15.33215	17.26159
	14.67752	13.57498	15.19434	17.53723
12	10.43965	13.05817	15.78006	15.53888
	10.64637	15.36661	13.09262	17.43386
	14.36743	14.81534	14.67752	18.08849
	14.95316	12.64472	15.09097	18.88094
14	10.4741	11.85227	12.43799	15.81451
	10.4741	12.61026	13.54053	18.01959
	11.64554	12.57581	13.91953	15.88342
	14.98761	14.4708	14.95316	18.26077
16	8.78584	10.95646	12.43799	13.78171
	10.26737	12.19681	15.40106	17.81286
	10.40519	11.92118	15.26324	16.88259
	12.40354	12.1279	13.60944	14.22962
Vitamin C 60 mg/100 mL				
1	23.36	22.87764	24.08354	23.49782
	22.18855	22.42973	23.15327	24.5659
	18.39858	20.1213	23.53227	25.73734
	18.36413	22.87764	26.32307	25.53062
2	19.39776	19.01876	20.53475	21.3961
	18.43304	19.05321	27.08106	18.12295
	22.63646	20.15575	22.56755	23.98018
	20.70702	19.01876	21.01711	20.98265
4	14.26407	13.91953	17.43386	18.70867
	14.81534	16.53805	17.3305	16.60696
	16.88259	17.50277	17.15823	19.98348
	14.12625	16.71032	17.84731	19.88012
6	13.05817	13.81616	14.50525	18.74313
	11.23209	14.60861	15.46997	16.15906
	12.81699	15.26324	15.46997	18.74313
	12.33463	15.05652	16.9515	22.22301

Table A4 (cont'd)

Vitamin C 60 mg/100 mL				
Storage (days)	**Anthocyanin content (mg/L)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
8	10.95646	11.85227	14.9187	14.5397
	9.716106	11.74891	13.60944	14.43634
	9.99174	13.12708	15.46997	20.22466
	11.05982	11.301	14.57416	16.81369
10	7.063126	8.78584	12.95481	14.22962
	11.57664	14.4708	12.26572	14.22962
	8.820294	12.71363	13.54053	15.09097
	9.612743	11.92118	14.71198	14.5397
12	6.477404	8.682477	12.36908	14.5397
	7.855575	7.993392	10.99091	10.74973
	4.789144	10.74973	13.95398	15.81451
	10.8531	12.36908	15.88342	16.77923
14	7.614395	6.787492	8.854749	12.36908
	6.959764	7.304306	8.613569	10.50855
	7.476578	11.57664	11.16319	14.36743
	6.718584	8.579114	11.02537	15.22879
16	7.338761	7.269852	8.820294	11.64554
	6.132861	7.338761	8.30348	9.50938
	7.924483	9.922831	9.647197	12.71363
	7.166489	7.269852	10.8531	13.85062
Vitamin C 80 mg/100 mL				
1	19.7423	17.26159	20.87929	21.46501
	16.29687	22.63646	24.35917	21.3272
	19.22549	24.22136	22.01628	22.36082
	22.25746	26.80543	27.32224	28.42478
2	13.57498	17.02041	20.25911	19.98348
	12.81699	16.91705	19.29439	18.50195
	18.43304	18.57085	19.63894	20.25911
	21.36165	23.77345	22.1541	23.98018
4	13.7128	13.50608	14.05734	18.26077
	11.88672	12.40354	14.43634	17.88177
	12.71363	18.26077	21.01711	19.81121
	14.81534	20.70702	19.60448	18.77758

Table A4 (cont'd)

Vitamin C 80 mg/100 mL				
Storage (days)	**Anthocyanin content (mg/L)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
6	10.40519	11.301	13.09262	21.43056
	8.613569	11.68	15.02206	15.22879
	11.74891	12.40354	16.88259	21.12047
	11.23209	15.7456	16.60696	17.02041
8	7.097581	7.614395	12.71363	16.91705
	8.992566	8.923657	12.43799	15.22879
	4.789144	10.74973	13.95398	15.81451
	10.8531	12.36908	15.88342	16.77923
10	5.891681	6.132861	8.648023	8.751386
	7.993392	8.682477	10.0951	11.95563
	7.132035	7.924483	12.1279	14.33298
	7.82112	9.406017	14.40189	15.7456
12	3.686607	11.92118	8.337935	6.718584
	3.824425	4.479056	9.785014	7.373215
	7.683303	7.442123	10.33628	12.71363
	6.615221	9.681651	10.30183	12.47245
14	3.996696	4.892507	8.0623	7.855575
	3.204248	5.374867	9.061474	7.476578
	5.030324	5.857227	8.78584	10.16401
	6.20177	8.648023	9.543834	11.43882
16	4.823599	4.168967	7.132035	4.926961
	3.54879	4.375693	8.0623	12.61026
	4.341239	5.926135	7.132035	10.92201
	5.443775	6.339587	9.233746	10.26737

Table A5 Data of L-ascorbic acid content in cranberry juice fortified with vitamin C (40–80 mg/100 mL) and gallic acid addition (0–320 mg/100 mL).

Vitamin C 40 mg/100 mL				
Storage (days)	**Ascorbic acid content (mg/100 mL)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
1	36.3229	41.13183	35.60204	42.47882
	36.92952	32.80256	36.32256	43.47239
	38.21195	33.11314	32.21028	33.45421
	36.11892	34.45634	31.83397	33.2663
2	33.36274	29.72246	28.18709	32.68222
	33.6729	22.03697	29.30677	28.75299
	31.94378	32.51333	29.72681	29.19796
	32.83359	31.77962	26.28412	28.85488
4	25.54045	25.27282	22.58175	24.10159
	24.01687	15.88206	22.08526	23.60849
	23.21415	22.70071	21.81643	21.54328
	23.68709	23.38758	20.6925	20.28221
6	18.61035	15.57333	15.01102	15.93928
	16.7043	10.16741	18.1156	14.69378
	17.23206	14.32243	9.688326	12.04274
	15.02702	14.23217	11.5714	11.70279
8	6.525108	6.295542	5.320056	4.666379
	10.97347	4.491095	6.744318	6.443753
	11.4693	11.61936	8.805205	9.745885
	10.43647	10.08143	8.891652	6.821836
10	11.83682	12.82278	12.59733	14.31421
	10.65812	12.50615	12.48349	12.25449
	10.77112	7.370475	9.783123	6.898716
	8.503306	6.812773	9.652785	7.053342
12	12.19921	12.01053	12.06532	12.1009
	11.91086	11.94662	11.95495	11.66625
	10.10084	8.699271	9.403349	8.565427
	9.412753	8.597139	9.315885	8.963072
14	12.20504	11.61474	11.47486	11.34447
	14.24295	12.3434	11.44224	12.7462
	11.08961	9.401822	9.132461	8.717103
	9.249405	8.162906	8.324431	8.715841

Table A5 (cont'd)

Vitamin C 40 mg/100 mL				
Storage (days)	**Ascorbic acid content (mg/100 mL)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
16	5.01376	4.222359	4.257198	4.266516
	4.391232	4.236779	3.872985	3.540066
	10.22933	9.380121	9.620822	9.814859
	10.16884	8.778902	9.284462	12.79641
Vitamin C 60 mg/100 mL				
1	53.26456	55.19244	51.05578	60.3403
	53.06407	59.16169	51.79537	48.74638
	54.15472	52.54554	49.92	49.05714
	52.32032	52.60621	50.03539	50.23886
2	48.49548	56.45525	48.07266	49.02292
	52.54339	52.87737	50.3761	45.68101
	48.98143	47.65177	44.11731	46.28749
	47.36521	50.58611	48.67238	44.53013
4	42.09105	38.93365	40.41004	37.02235
	45.35363	41.05715	40.66498	32.52461
	41.81605	42.72694	36.14204	35.10553
	40.71428	43.04518	37.94117	34.80431
6	32.32259	32.73227	29.01463	25.2124
	32.77727	29.1968	30.18211	22.51106
	31.91104	31.78803	30.22759	32.6144
	29.20099	31.76333	33.57818	32.06954
8	30.42813	23.15081	20.12191	24.36153
	26.2057	26.94568	20.44394	25.05665
	23.88631	22.44871	25.23229	17.66713
	23.49116	27.13346	25.76044	21.95771
10	17.75864	9.876488	14.16126	9.293497
	15.65142	13.08254	10.28377	7.57621
	19.93542	16.00908	14.56295	15.64509
	20.84028	18.39063	17.16811	16.93603
12	17.41633	14.91641	14.1669	14.3175
	14.6204	14.90733	18.25647	12.9838
	15.85584	10.04533	11.34136	12.26397
	12.39989	17.17749	11.8934	10.58831

Table A5 (cont'd)

Vitamin C 60 mg/100 mL				
Storage (days)	**Ascorbic acid content (mg/100 mL)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
14	12.19908	12.6203	12.10529	12.2634
	14.82886	12.17416	12.36419	12.59301
	13.32676	10.36762	9.617971	10.43298
	11.21497	10.79469	10.08815	9.326656
16	14.4353	12.41017	11.39981	10.69018
	12.91019	12.29309	11.16125	10.27953
	10.08432	9.064032	9.549488	13.0199
	9.411888	9.460272	10.37045	12.44799
Vitamin C 80 mg/100 mL				
1	63.74724	67.61069	87.24824	76.7644
	62.3397	66.3471	61.30447	74.28407
	68.21294	70.82319	66.41275	60.44126
	67.15324	70.67804	67.83211	61.30195
2	61.2389	65.77362	69.7705	56.1496
	61.06649	64.83855	64.93191	55.05276
	63.12511	70.48266	63.69341	58.04704
	65.1682	67.99291	63.92321	56.2545
4	54.61005	53.70349	31.75137	49.18951
	52.95725	56.46677	27.88105	48.68375
	55.63515	59.63047	56.40083	49.73936
	58.69792	56.47696	56.21045	51.67425
6	50.52604	44.92376	50.63154	40.62398
	49.42737	55.51184	43.00429	40.59164
	52.32296	51.79776	50.01584	44.20368
	49.4995	48.55869	48.03141	41.50566
8	46.56134	36.86035	34.62147	41.2536
	47.21523	37.44363	36.69912	43.61777
	41.69312	47.97312	37.63306	36.99927
	46.92883	44.66159	35.23364	32.67509
10	33.15531	28.6903	33.01338	31.57013
	33.2358	28.05638	27.30168	36.15037
	34.56136	36.35589	32.3095	28.02707
	41.24942	37.65857	25.78195	29.51492

Table A5 (cont'd)

Vitamin C 80 mg/100 mL				
Storage (days)	**Ascorbic acid content (mg/100 mL)**			
	GA-0mg	**GA-80mg**	**GA-160mg**	**GA-320mg**
12	21.87883	16.14706	20.32687	13.57536
	26.23138	23.31493	17.73734	13.74155
	22.62652	19.05891	21.1077	22.13557
	23.51296	22.13555	25.42336	17.84243
14	19.43074	22.04559	19.82995	16.10366
	19.81042	21.96311	13.47741	20.61134
	21.7281	20.71388	19.31578	18.11516
	16.95148	21.92914	17.26456	21.54035
16	13.45866	17.88653	12.99896	12.53311
	18.4545	16.82041	12.8103	14.01642
	16.13667	10.89922	9.441339	16.74058
	18.78586	12.9201	10.22739	15.8649

APPENDIX B

Example MATLAB syntax and additional results for Chapter 4

1. A syntax to estimate parameter *n* with smallest rmse

   Example syntax to estimate parameter *n* for color intensity at one treatment (vitamin C 80, Gallic acid 320)

   First function syntax: file name "forderdiff2Pnn":

```
function y = forderdiff2Pnn(beta,t)
%   called by inv_prob
global len
tspan=t(1:len,1);
n=t(1,2);%n comes from 2nd column
[t,y]=ode45(@ff,tspan,beta(1));
    function dy=ff(t,y) %function that computes the dydt
        dy(1)=-beta(2)*y(1).^n;
    end
t=[t;t;t;t]; y=[y;y;y;y];
end
```

   Following syntax are to paste in MATLAB as a script file, while the previous function file is open:

```
clear
format compact
%% Read in data
global len
data =xlsread('data_4rep.xlsx');
x1=data(:,1);
yobs1=data(:,2);
yobs2=data(:,3);
yobs3=data(:,4);
yobs4=data(:,5);
len=length(x1);
x=[x1;x1;x1;x1];
yobs=[yobs1;yobs2;yobs3;yobs4];

%% Initial parameter guesses

yo=2.0;
k=0.0000005;%make smaller to be able to reach lowest rmse

beta0(1)=yo; %initial guess yo
beta0(2)=k; %initial guess kr
beta=beta0;%set beta=to the initial guesses

%% nlinfit returns parameters, residuals, Jacobian (sensitivity
%coefficient matrix),
%covariance matrix, and mean square error.  ode45 is solved many times
iteratively
 xn=x; %copy x into xn
count =1;
for n= 0.05:.05:5
xn(:,2)=n;
% beta0(2)=beta0(2)*.8;
[beta,resids,J,COVB,mse] = nlinfit(xn,yobs,@forderdiff2Pnn,beta0);
beta
rmse(count)=sqrt(mse)
nx(count)=n;
count = count +1;
```

```
end
%% plot rmse versus n
figure
plot(nx,rmse,'-o')
xlabel('n')
ylabel('rmse')
title('VitC80Gallic320')
```

2. Two-parameters (k and C_0) estimation using Ordinary Least Square method, fixing n from the n with smallest rmse.

   Example syntax for estimating k and C_0 for color at treatment with vitamin C 60 and Gallic acid 320

   There are 3 function files:

   2.1 Function "forderdiff2P_for_SSC":

```
function y = forderdiff2P_for_SSC(beta,t)
%first-order model, differential form
%   called by inv_prob
%global len

%tspan=t(1:len);
tspan=t;
[t,y]=ode45(@ff,tspan,beta(1));

    function dy=ff(t,y) %function that computes the dydt
        dy(1)=-beta(2)*y(1).^(2.9); %n from estimate n with lowest
rmse
        % avg for ACY n=0.35, 3.3, 2.2, 1.2 for vit 0,40,60,80,
respectively
        % avg for COLOR n=0.65,4.4, 2.9,2.2  for vit 0,40,60,80,
respectively
    end
%t=[t;t;t;t]; y=[y;y;y;y];
end
```

   2.2 Function "forwarddiff2P":

```
function y = forderdiff2P( beta,t )
%first-order model, differential form
%   called by inv_prob
global len

tspan=t(1:len);

[t,y]=ode45(@ff,tspan,beta(1));

    function dy=ff(t,y) %function that computes the dydt

        dy(1)=-beta(2)*y(1).^(2.9); % n from estimate n with lowest
rmse
    % avg n for ACY=0.35, 3.3, 2.2, 1.2 for vit 0,40,60,80,
respectively
        % avg n for COLOR=0.65, 4.4, 2.9,2.2 for vit 0,40,60,80,
respectively
    end
t=[t;t;t;t]; y=[y;y;y;y];
```

```
end
```

2.3 Function "SSC_2P":

```
function Xp = SSC_2P( beta, x, func )
%computes scaled sensitivity coefficients
% uses forward-difference approximation
% beta are the parameters
% x is the independent variable
% func is the model
d=0.001;
ypred=func(beta,x);
figure
for i = 1:length(beta)  %scaled sens coeff for forward problem
            betain = beta; %reset beta
            betain(i) = beta(i)*(1+d);
            yhat{i} = func(betain,x);%function with only one
perturbed parameter
            Xp{i} = (yhat{i}-ypred)/d;%scaled sens coeff for ith
parameter
            ysensf=Xp{i}; hold on
            h2(i) = plot(x,ysensf,'-b','LineWidth',2);
end
ysensf1=Xp{1}; ysensf2=Xp{2}; %extract data from cell array into
vectors
hold on
YLine = [0 0];
XLine = [0 max(x)];
set(gca, 'fontsize',14,'fontweight','bold');
h2(1) = plot(x,ysensf1,'-b','LineWidth',2);
h2(2) = plot(x,ysensf2,'-r','LineWidth',2);
%h2(3) = plot(x,ysensf3,'-y','LineWidth',2);

legend(h2,'X''_{Co}','X''_k')
xlabel('time (days)','fontsize',16,'fontweight','bold')
ylabel('scaled sensitivity
coefficient','fontsize',16,'fontweight','bold' )
plot (XLine, YLine,'k'); %plot a straight black line at zero
grid on
end
```

Syntax in script file:

```
%% example of nlinfit using file name = inv_soln_first_2P.m
%This program can be used as a base for most nonlinear regression OLS
%using nlinfit
%% Housekeeping
%
clear all; % Clear the workspace.
close all; % Close all figures.
format compact
%% Read in data
global len

data =xlsread('data_4rep.xlsx');
x1=data(:,1);
yobs1=data(:,2);
yobs2=data(:,3);
yobs3=data(:,4);
yobs4=data(:,5);
len=length(x1);
```

```
x=[x1;x1;x1;x1];
yobs=[yobs1;yobs2;yobs3;yobs4];

%% Initial parameter guesses
yo=2;
k=0.00005; % if error happen, make initial k very small i.e. 0.00005
beta0(1)=yo; %initial guess yo
beta0(2)=k; %initial guess kr
%beta0(2)=n;
beta=beta0;%set beta=to the initial guesses

%% X' = scaled sensitivity coefficients using forward-difference
% This is a forward problem with known approximate parameters
xs=linspace(min(x1),max(x1),500);
Xp=SSC_2P(beta, xs, @forderdiff2P_for_SSC);
title('scaled sensitivity coefficients using initial guesses')

%% nlinfit returns parameters, residuals, Jacobian (sensitivity
%coefficient matrix),
%covariance matrix, and mean square error.  ode45 is solved many times
iteratively

[beta,resids,J,COVB,mse] = nlinfit(x,yobs,@forderdiff2P,beta0);
beta
rmse=sqrt(mse)
condX=cond(J)
detXTX=det(J'*J)
%type forderdiff2P.m
%uncomment if you wish to print the code of the function
%% confidence intervals for parameters
ci=nlparci(beta, resids,J)

%% R is the correlation matrix for the parameters, sigma is the standard
error vector
[R,sigma]=corrcov(COVB);
R
sigma
relerr=sigma./beta' %relative error for each parameter

%% Confidence and prediction intervals for the dependent variable

%nonlinear regression confidence intervals-- 'on' means simultaneous
%bounds; 'off' is for nonsimultaneous bounds; must use 'curve' for
%regression line, 'observation' for prediction interval
[ypred, delta] =
nlpredci('forderdiff2P',x,beta,resids,J,0.05,'on','curve'); %confidence
band for regression line
[ypred, deltaob]
=nlpredci('forderdiff2P',x,beta,resids,J,0.05,'on','observation');%predict
ion band for individual points

yspan=range(ypred)% total span of ypred
relrmse=rmse/yspan % ratio of rmse vs. yspan

%simultaneous confidence bands for regression line
CBu=ypred+delta;
CBl=ypred-delta;

%simultaneous prediction bands for regression line
PBu=ypred+deltaob;
PBl=ypred-deltaob;

%% Output--ypred and yobs vs. t
```

```
figure
hold on
h1(1)=plot(x(1:len),ypred(1:len),'-','linewidth',3); %predicted y values
h1(2)=plot(x,yobs,'square', 'Markerfacecolor', 'r');
xlabel('time (days)','fontsize',16,'fontweight','bold')
ylabel('Color (AU)','fontsize',16,'fontweight','bold')
grid on

%% Output --CIs and PIs

%plot Cobs, Cpred line, confidence band for regression line
h1(3) = plot(x(1:len),CBu(1:len),'--g','LineWidth',2);
plot(x(1:len),CBl(1:len),'--g','LineWidth',2);

%plot prediction band for regression line
h1(4) = plot(x(1:len),PBu(1:len),'-.','LineWidth',2);
plot(x(1:len),PBl(1:len),'-.','LineWidth',2);
legend(h1,'ypred','yobs','CB','PB')

%% residual scatter plot

figure
hold on
plot(x, resids, 'square','Markerfacecolor', 'b');
YLine = [0 0];
XLine = [0 max(x)];
plot (XLine, YLine,'R'); %plot a straight red line at zero
ylabel('Observed y - Predicted y','fontsize',16,'fontweight','bold')
xlabel('time (days)','fontsize',16,'fontweight','bold')
grid on

%% number of runs = number of times moving from one residual to the next
crosses zero
n=length(yobs)
rescross=resids(2:n).*resids(1:n-1);%multiply each pair of residuals
res_sign=sign(rescross);%get the sign of each multiplied pair
count=0;
for i=1:n-1
    if res_sign(i)<0 %if product of pair is < 0, that's a run
        count=count+1;
    end
end
fprintf('number of runs = %5.2f\n',count);
minrun=(n+1)/2; %count should be >=minrun
fprintf('Minimum required number of runs = %5.2f\n',minrun);
%% residuals histogram--same as dfittool, but no curve fit here

[n1, xout] = hist(resids,10); %10 is the number of bins
 figure
 hold on
 set(gca, 'fontsize',14,'fontweight','bold');
 bar(xout, n1) % plots the histogram
 xlabel('Y_{observed} - Y_{predicted}','fontsize',16,'fontweight','bold')
 ylabel('Frequency','fontsize',16,'fontweight','bold')

 Mean_of_error=mean(resids) % the closer to zero, the better-- prove
assumption2 (no measurement error)
%% scaled sensitivity coefficients using final estimated parameters
% This is a double-check to make sure X' has not changed much

Xp=SSC_2P(beta,xs,@forderdiff2P_for_SSC);
title('scaled sensitivity coefficients using beta estimates')
```

```
%% AIC analysis
N=length(x);
P=length(beta);
K=P+1;
SS=resids'*resids;
AIC=N*log(SS/N)+(2*K)
AIC_correct=AIC+(((2*K)*(K+1))/(N-K-1))
```

3. 3D plot for logk_{color} predictive model (Figure 4.5B)

   Syntax in script file:

```
%multiple linear regression
clear all
close all
data=xlsread('K_color.xlsx','logK_color_n');
vitC=data(:,1);
gallic=data(:,2);
logk_color=data(:,3);
vitCadj=data(:,4);
gallicAdj=data(:,5);
size(vitC);
%X=[ones(size(logvitC)) logvitC gallic];
%X=[ones(size(vitC)) vitC gallic vitCadj.*gallicAdj];
%X=[ones(size(vitC)) vitC gallic vitCadj.*gallicAdj gallicAdj.*gallicAdj];
%X=[ones(size(vitC)) vitC gallic vitCadj.*gallicAdj vitCadj.*vitCadj];
X=[ones(size(vitC)) vitC gallic vitCadj.*gallicAdj vitCadj.*vitCadj
gallicAdj.*gallicAdj];
[b, bint,r, rint, stats] = regress(logk_color,X);
format short e
format compact
b
bint
format short
mse=stats(4);
rmse=sqrt(mse)
yspan=max(logk_color)-min(logk_color)
Rsqr=stats(1)
%correlation of parameters
covb=mse.*((X.'*X))^(-1);
[R,sigma]=corrcov(covb)
%% AIC analysis
N=length(X);
P=length(b);
K=P+1;
resids=r;
SS=resids'*resids;
AIC=N*log(SS/N)+(2*K)
AIC_correct=AIC+(((2*K)*(K+1))/(N-K-1))
%Adjusted Rsqaure
SS_error=SS;
y=logk_color;
y_bar=mean(y);
SS_total=sum((y-y_bar).^2);
Rsq_adj = 1 - (N-1)./(N-P).*(SS_error./SS_total)
%% scatter 3D plot
figure
scatter3(vitC,gallic,logk_color,'filled')
hold on
x1fit = min(vitC):2:max(vitC);
x2fit = min(gallic):2:max(gallic);
```

```
[X1FIT,X2FIT] = meshgrid(x1fit,x2fit);
% y = b(1) + b(2)*x1 + b(3)*x2 + b(4)*x1*x2 standard statistical model
%YFIT = b(1) + b(2)*X1FIT + b(3)*X2FIT;
%YFIT = b(1) + b(2)*X1FIT + b(3)*X2FIT + b(4)*(X1FIT-60).*(X2FIT-140);
%YFIT = b(1) + b(2)*X1FIT + b(3)*X2FIT + b(4)*(X1FIT-60).*(X2FIT-140)+
b(5)*((X2FIT-140).^2);
%YFIT = b(1) + b(2)*X1FIT + b(3)*X2FIT + b(4)*(X1FIT-60).*(X2FIT-140)+
b(5)*((X1FIT-60).^2);
YFIT = b(1) + b(2)*X1FIT + b(3)*X2FIT + b(4)*(X1FIT-60).*(X2FIT-140)+
b(5)*((X1FIT-60).^2)+ b(6)*((X2FIT-140).^2);
mesh(X1FIT,X2FIT,YFIT);
xlabel('Vitamin C (mg/100mL)')
ylabel('Gallic acid (mg/100mL)')
zlabel('logk_{color} (AU^{(1-n)} day^{-1}) (n=4.4,2.9,2.2)')
%title('3D Plotting')
view(50,10)
hold off
```

4. Model fitting for color retention (Figure 4.6B)

   Syntax in script file:

```
clear
close all

%% Model fitting Vit C 40,Gallic 0 to 320
data=xlsread('colorData.xlsx','colorV40');
storage=data(:,1);
v40G0=data(:,2);
v40G80=data(:,3);
v40G160=data(:,4);
v40G320=data(:,5);
figure
hold on
plot(storage,v40G0,'mo');
plot(storage,v40G80,'r*');
plot(storage,v40G160,'x','MarkerSize',10);
plot(storage,v40G320,'^','Markerfacecolor','yellow');
legend('GA, 0 mg','GA, 80 mg','GA, 160 mg','GA, 320 mg','location','best')
xlabel('storage (days)','fontsize',12,'fontweight','bold')
ylabel('Red color intensity (AU)','fontsize',12,'fontweight','bold')
title('Vit C 40');
axis([0 22 0.8 2.2]);
text(17.5, 1.1,'Pred (GA, 0 mg)');
text(17.5, 1.3,'Pred (GA, 80 mg)');
text(17.5, 1.5,'Pred (GA, 160 mg)');
text(17.5, 1.6,'Pred (GA, 320 mg)');

% Predicted ACY vitC 40

C0color=xlsread('Co_color.xlsx');

for i=1:4
[t, c]=ode45(@color_pred, [1 17],[C0color(i)],[],i,1);
hold on
plot(t,c,'-','linewidth',1);
clear t c
end


%% Model fitting Vit C 60, Gallic 0 to 320
clear
data=xlsread('colorData.xlsx','colorV60');
storage=data(:,1);
```

```
v60G0=data(:,2);
v60G80=data(:,3);
v60G160=data(:,4);
v60G320=data(:,5);
figure
hold on
plot(storage,v60G0,'mo');
plot(storage,v60G80,'r*');
plot(storage,v60G160,'x','MarkerSize',10);
plot(storage,v60G320,'^','Markerfacecolor','yellow');
legend('GA, 0 mg','GA, 80 mg','GA, 160 mg','GA, 320 mg','location','best')
xlabel('storage (days)','fontsize',12,'fontweight','bold')
ylabel('Red color intensity (AU)','fontsize',12,'fontweight','bold')
title('Vit C 60');
axis([0 22 0.5 2.2]);
text(17.5, 0.8,'Pred (GA, 0 mg)');
text(17.5, 1,'Pred (GA, 80 mg)');
text(17.5, 1.2,'Pred (GA, 160 mg)');
text(17.5, 1.4,'Pred (GA, 320 mg)');

% Predicted ACY vitC 60

C0color=xlsread('Co_color.xlsx');

for i=5:8
[t, c]=ode45(@color_pred, [1 17],[C0color(i)],[],i,2);
hold on
plot(t,c,'-','linewidth',1);
clear t c
end

%% Model fitting Vit C 80, Gallic 0 to 320
clear
data=xlsread('colorData.xlsx','colorV80');
storage=data(:,1);
v80G0=data(:,2);
v80G80=data(:,3);
v80G160=data(:,4);
v80G320=data(:,5);
figure
hold on
plot(storage,v80G0,'mo');
plot(storage,v80G80,'r*');
plot(storage,v80G160,'x','MarkerSize',10);
plot(storage,v80G320,'^','Markerfacecolor','yellow');
legend('GA, 0 mg','GA, 80 mg','GA, 160 mg','GA, 320 mg','location','best')
xlabel('storage (days)','fontsize',12,'fontweight','bold')
ylabel('Red color intensity(AU)','fontsize',12,'fontweight','bold')
title('Vit C 80');
axis([0 22 0.3 2.2]);
text(17.5, 0.6,'Pred (GA, 0 mg)');
text(17.5, 0.8,'Pred (GA, 80 mg)');
text(17.5, 1,'Pred (GA, 160 mg)');
text(17.5, 1.2,'Pred (GA, 320 mg)');

% Predicted ACY vitC 80

C0color=xlsread('Co_color.xlsx');

for i=9:12
[t, c]=ode45(@color_pred, [1 17],[C0color(i)],[],i,3);
hold on
plot(t,c,'-','linewidth',1);
clear t c
```

```
end
```

5. Residual plot (Figure 4.7B)

   Syntax in script file:

```
%% vitc 60
clear all
data=xlsread('colorData.xlsx','colorV60');
storage=data(:,1);
v60G80=data(:,3);
%% plot residuals (predicted-observed)V60

t=[1 2 4 6 8 10 12 14 16 1 2 4 6 8 10 12 14 16 1 2 4 6 8 10 12 14 16 1 2 4
6 8 10 12 14 16];
logkcolorV60G80=b1+(b2.*vitc2)+(b3.*Gallic2)+(b4.*(vitc2-60)*(Gallic2-
140))+ (b5.*(vitc2-60)*(vitc2-60))+(b6.*(Gallic2-140)*(Gallic2-140));
kcolorV60G80 = 10.^logkcolorV60G80;
predictedV60G80=((n-1).*kcolorV60G80.*(t-to) + Co2.^(1-n)).^(1/(1-n));
obsV60G80=v60G80.';
residV60G80=predictedV60G80-obsV60G80;
figure;
hold on
plot(t,residV60G80,'*');
YLine= [0 0];
XLine= [0 max(t)];
plot(XLine, YLine,'R');
xlabel('storage (days)','fontsize',12,'fontweight','bold')
ylabel('Color_{observed} -
Color_{predicted}','fontsize',12,'fontweight','bold')
title('Residual plot Vit60Gallic80')
hold off
```

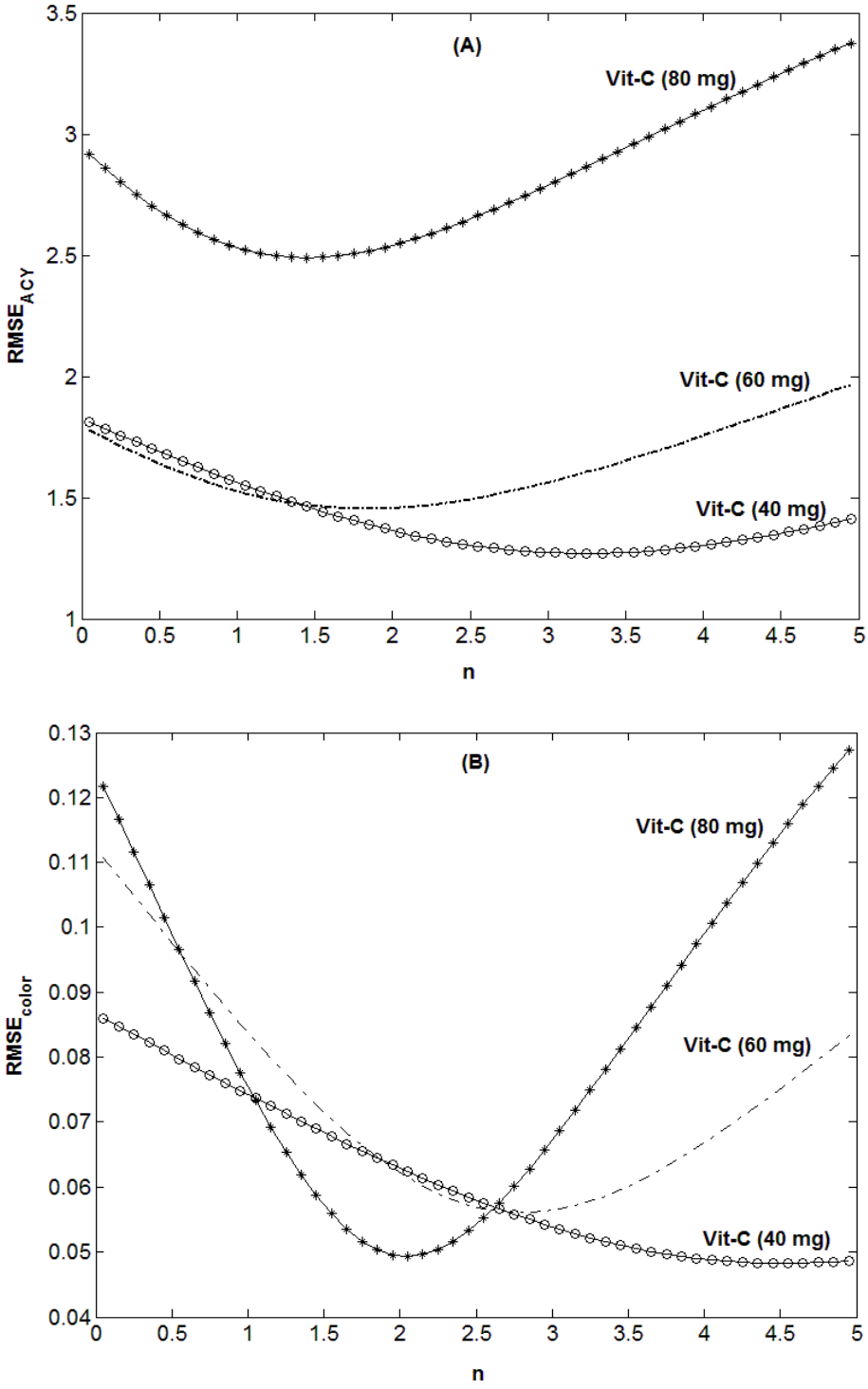


Figure B1 Plot of RMSE versus *n* (reaction order) of anthocyanins (A) and color (B) in cranberry juice with vitamin C (40–60 mg/100 mL) and gallic acid (80 mg/100 mL), during 16-day storage at 23 ºC.

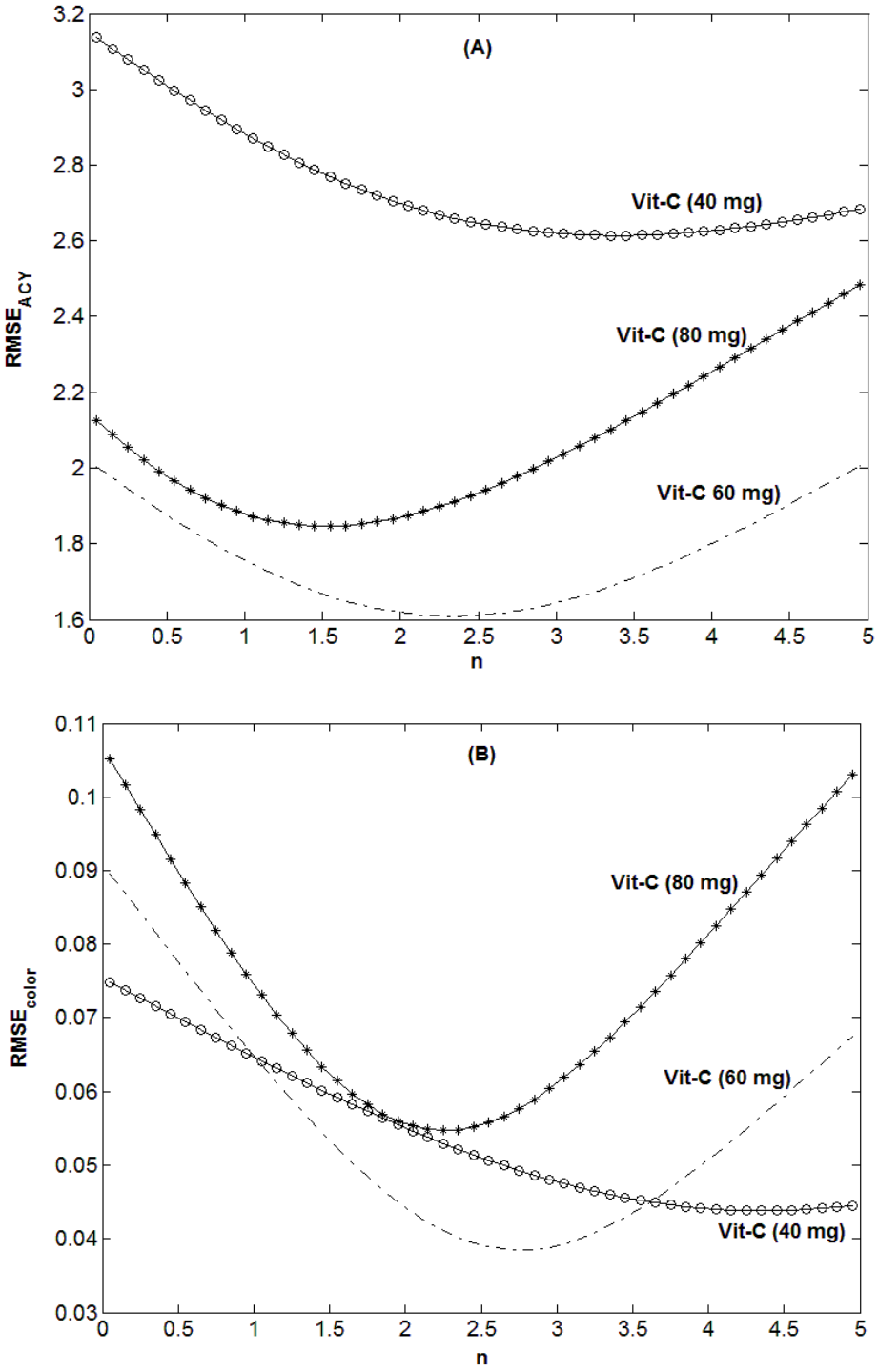


Figure B2 Plot of RMSE versus *n* (reaction order) of anthocyanins (A) and color (B) in cranberry juice with vitamin C (40–60 mg/100 mL) and gallic acid (160 mg/100 mL), during 16-day storage at 23 ºC.

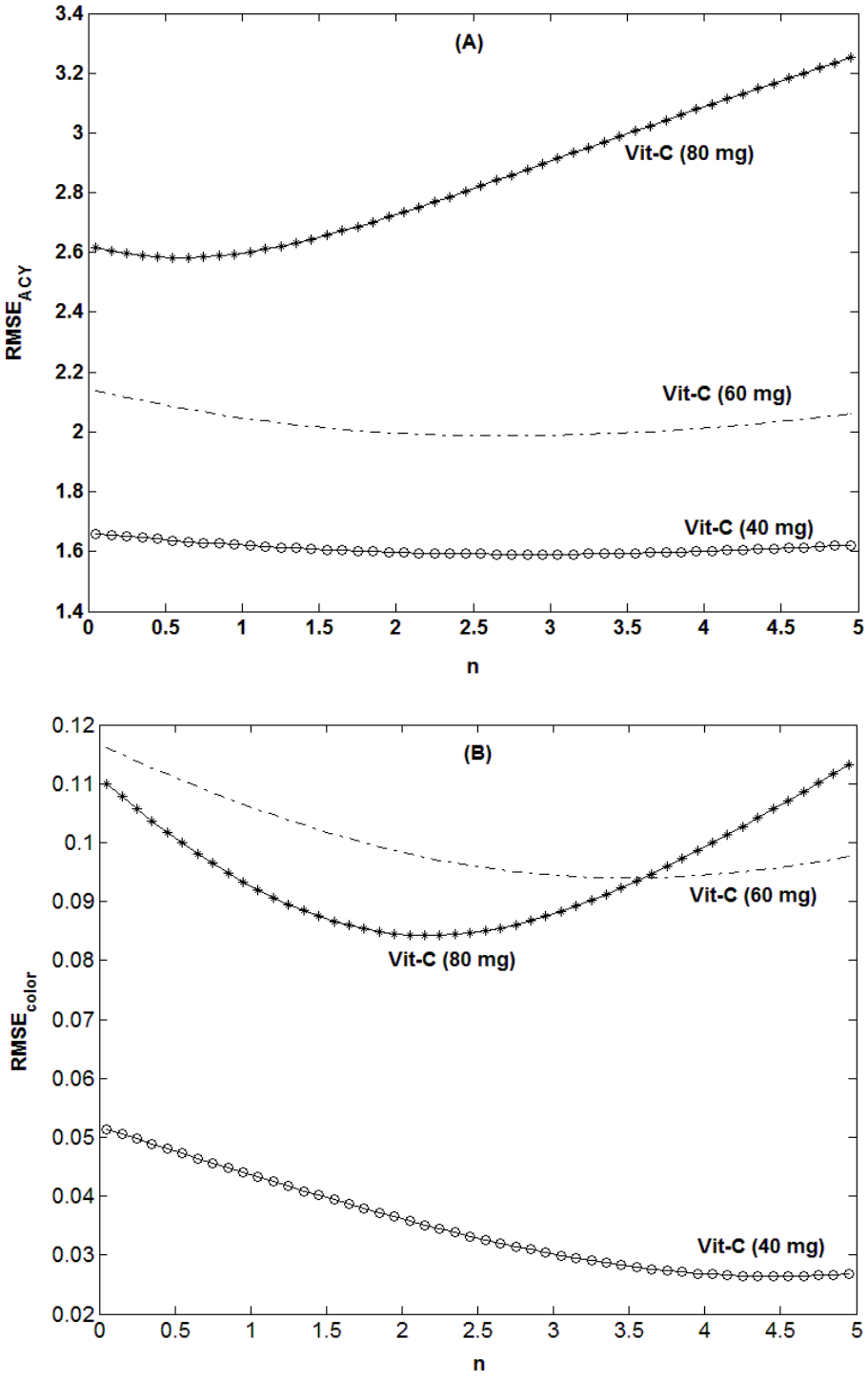


Figure B3 Plot of RMSE versus *n* (reaction order) of anthocyanins (A) and color (B) in cranberry juice with vitamin C (40–60 mg/100 mL) and gallic acid (320 mg/100 mL), during 16-day storage at 23 ºC.

Table B1 Summary of *n*-values with smallest RMSE of all 12 treatments for color and anthocyanins.

Vitamin C (mg/100mL)	**Gallic acid (mg/100mL)**	**Color**		**Anthocyanins**	
		n	rmse	n	rmse
40	0	4.25	0.03949	3.65	1.7281
	80	4.55	0.04826	3.25	1.2703
	160	4.35	0.04384	3.35	2.6143
	320	4.45	0.02641	2.95	1.6144
Average		**4.4**		**3.3**	
60	0	2.55	0.03466	1.75	1.5841
	80	2.85	0.05606	1.85	1.4558
	160	2.75	0.03475	2.35	1.6091
	320	3.45	0.09398	2.65	1.9869
Average		**2.9**		**2.15**	
80	0	2.35	0.042204	1.45	1.9023
	80	2.05	0.049306	1.45	2.4894
	160	2.25	0.054702	1.55	1.8463
	320	2.15	0.08425	0.55	2.5833
Average		**2.2**		**1.25**	

Calculate n_{ACY} and n_{color} at different vitamin C concentration using following equations;

$$n_{ACY} = 5.31 - 0.0513(vitC) \quad (4.5)$$

$$n_{color} = 6.2 - 0.055(vitC) + 0.001(vitC - 60)^2 \quad (4.6)$$

Table B2 The n_{ACY} and n_{color} calculated from Eq. (4.5) and Eq. (4.6), respectively.

Vitamin C	n_{ACY}	n_{color}
40	3.3	4.4
60	2.2	2.9
80	1.2	2.2

Table B3 Two parameter (k, C_0) estimation for anthocyanins in cranberry juice fortified with vitamin C (40-80 mg/100 mL) and gallic acid (0-320 mg/100 mL), during 16-day storage at 23 °C.

Vitamin C (mg/100 mL)	Gallic acid (mg/100 mL)	n (fixed)	Co_{ACY}; %Error	k_{ACY}; %Error
40	0	3.3	24.16; 3.10%	1.04×10^{-4}; 8.51%
	80	3.3	24.89; 2.12%	7.49×10^{-5}; 6.22%
	160	3.3	28.14; 3.93%	6.32×10^{-5}; 11.16%
	320	3.3	24.04; 2.40%	3.04×10^{-5}; 10.60%
60	0	2.2	21.70; 3.11%	4.10×10^{-3}; 7.21%
	80	2.2	21.97; 2.65%	2.90×10^{-3}; 6.61%
	160	2.2	24.37; 2.58%	2.30×10^{-3}; 6.66%
	320	2.2	23.77; 3.06%	1.50×10^{-3}; 9.53%
80	0	1.2	19.05; 3.76%	6.96×10^{-2}; 7.70%
	80	1.2	22.14; 4.17%	6.14×10^{-2}; 8.74%
	160	1.2	22.79; 2.87%	4.38×10^{-2}; 6.81%
	320	1.2	23.31; 3.85%	3.68×10^{-2}; 9.97%

Table B4 Two parameter (k, C_0) estimation for color in cranberry juice fortified with vitamin C (40-80 mg/100 mL) and gallic acid (0-320 mg/100 mL), during 16-day storage at 23 °C.

Vitamin C (mg/100 mL)	**Gallic acid (mg/100 mL)**	***n* (fixed)**	Co_{color} **; %Error**	k_{color} **; %Error**
40	0	4.4	1.82; 0.92%	1.47×10^{-2}; 3.17%
	80	4.4	1.93; 1.01%	8.40×10^{-3}; 3.91%
	160	4.4	1.97; 0.88%	6.80×10^{-3}; 3.59%
	320	4.4	2.09; 0.47%	3.60×10^{-3}; 2.34%
60	0	2.9	1.77; 0.88%	4.92×10^{-2}; 2.33%
	80	2.9	1.88; 1.20%	3.27×10^{-2}; 3.45%
	160	2.9	1.94; 0.78%	2.48×10^{-2}; 2.40%
	320	2.9	2.05; 1.71%	1.54×10^{-2}; 6.25%
80	0	2.2	1.71; 1.02%	7.85×10^{-2}; 2.40%
	80	2.2	1.83; 1.10%	6.14×10^{-2}; 2.68%
	160	2.2	1.92; 1.08%	4.10×10^{-2}; 2.99%
	320	2.2	1.95; 1.60%	3.38×10^{-2}; 4.75%

Developing secondary model for Co_{ACY} using multiple linear regression in JMP software

An empirical polynomial equation is;

$$Co_{ACY} = \beta_1 + \beta_2 vitC + \beta_3 Gallic + \beta_4(vitC \times Gallic) + \beta_5(vitC)^2 + \beta_6(Gallic$$

Table B5 Model comparison using *p-value* of parameters, and AICc as criteria.

Term	**Parameters**	***p-value*,** $\alpha = 0.05$	**AICc**
Model#1			
Intercept	$\beta_1 = 27.83$	< 0.0001	68.76
vitC	$\beta_2 = -8.71 \times 10^{-2}$	0.0033	
Gallic	$\beta_3 = 1.06 \times 10^{-2}$	0.0106	
(vitC-60) x (Gallic-140)	$\beta_4 = 2.866 \times 10^{-4}$	0.1157 (not sig diff)	
$(vitC-60)^2$	$\beta_5 = 1.53 \times 10^{-3}$	0.3750 (not sig diff)	
$(Gallic-140)^2$	$\beta_6 = -8.08 \times 10^{-5}$	0.0227	
Model#2			
Intercept	$\beta_1 = 28.24$	< 0.0001	53.24
vitC	$\beta_2 = -8.71 \times 10^{-2}$	0.0033	
Gallic	$\beta_3 = 1.06 \times 10^{-2}$	0.0121	
$(Gallic-140)^2$	$\beta_6 = -8.08 \times 10^{-5}$	0.0278	
Model#3			
Intercept	$\beta_1 = 27.69$	< 0.0001	54.66
vitC	$\beta_2 = -8.71 \times 10^{-2}$	0.0108	
Gallic	$\beta_3 = 6.41 \times 10^{-3}$	0.1217 (not sig diff)	

Model#2 has the lowest AIC_C among those three models, and all parameters in the mmodel#2 are significant different from zero. Therefore, model#2 is the best model for Co_{ACY}.

APPENDIX C

MATLAB syntax for Chapter 5

1. MATLAB function name "color_func" for roots problem in Case study#1:

```
function x = color_func( Gallic, limit )
limit = 0.5; % require 50% color retention
vitC = 55; % mg/100 mL fortified concentration in juice
t= 16; %end of storage (day)
to=1; %start storage at day 1
% Eq. (5.7) n-order as a function of vitamin C
n=6.2-0.055*(vitC)+0.001*(vitC-60).^2;


b1=  -2.6121e+00;
b2=   2.0902e-02;
b3=  -1.6303e-03;
b4=   1.6511e-05;
b5=  -3.9769e-04;
b6=   2.3897e-06;
% Eq. (5.8) logkcolor as a function of vitamin C and gallic acid
logkcolor=b1+(b2.*vitC)+(b3.*Gallic)+(b4.*(vitC-60)*(Gallic-140))+
(b5.*(vitC-60)*(vitC-60))+(b6.*(Gallic-140)*(Gallic-140));
kcolor = 10.^logkcolor;

c1=  1.96;
c2= -2.49e-03;
c3= 8.91e-04;
c4= -1.96e-06;
% Eq. (5.6) Co(color) as a function of vitamin C and gallic acid
Co= c1+(c2.*vitC)+(c3.*Gallic)+(c4.*(Gallic-140)*(Gallic-140));

%retention =C/Co;
retention = [((kcolor*(n-1)*(t-to))/(Co^(1-n)))+1]^(1/(1-n));
function = limit - retention;

end
```

The statement (script file) to solve the roots problem is:

```
Xo = 70; % initial guess of gallic acid that gives 50% retention
(C/Co=0.5)
[Gallic, fx] = fzero(@(Gallic)color_func(Gallic), Xo)
```

2. MATLAB function name "acy_func" for roots problem in Case study#2:

```
function x = acy_func( Gallic, limit )
limit = 0.5; % require 50% ACY retention
vitC = 55; % mg/100 mL fortified concentration in juice
t= 16; %end of storage (day)
to=1; %start storage at day 1
% Eq. (5.4) n-order as a function of vitamin C
n=5.31-0.0513*(vitC);
b1=  -6.7882e+00;
b2=   7.2875e-02;
b3=  -1.2934e-03;
```

```
b4=   1.8544e-05;
b5=  -3.7809e-04;

% Eq. (5.5) logkacy as a function of vitamin C and gallic acid
logkacy=b1+(b2.*vitC)+(b3.*Gallic)+(b4.*(vitC-60)*(Gallic-140))+
(b5.*(vitC-60)*(vitC-60));
kacy = 10.^logkacy;
c1=  28.24;
c2= -8.71e-02;
c3= 1.06e-02;
c4= -8.08e-05;
% Eq. (5.3) Co(acy) as a function of vitamin C and gallic acid
Co= c1+(c2.*vitC)+(c3.*Gallic)+(c4.*(Gallic-140)*(Gallic-140));
%retention =C/Co;
retention = [((kacy*(n-1)*(t-to))/(Co^(1-n)))+1]^(1/(1-n));
x = limit - retention;
end
```

The statement (script file) to solve roots problem is:

```
Xo = 70; % initial guess of gallic acid that gives 50% retention
(C/Co=0.5)
[Gallic, fx] = fzero(@(Gallic)acy_func(Gallic), Xo)
```

3. 3D plot color retention (Figure 5.1A)

Syntax in script file:

```
vitC = linspace(40,80,4)';
Gallic = linspace(0,320,4)';

t= 16; %end of storage (day)
to=1; %start storage at day 1
n=6.2-0.055.*(vitC)+0.001.*(vitC-60).^2; % n-order as a function of
vitamin C

b1=  -2.6121e+00;
b2=   2.0902e-02;
b3=  -1.6303e-03;
b4=   1.6511e-05;
b5=  -3.9769e-04;
b6=   2.3897e-06;

c1=  1.96;
c2= -2.49e-03;
c3=  8.91e-04;
c4= -1.96e-06;
Co= c1+(c2.*vitC)+(c3.*Gallic)+(c4.*(Gallic-140).*(Gallic-140)); %
Co_color regression

figure
x1fit = min(vitC):1:max(vitC);
x2fit = min(Gallic):10:max(Gallic);
[X1FIT,X2FIT] = meshgrid(x1fit,x2fit);

logkcolor=b1+(b2.*X1FIT)+(b3.*X2FIT)+(b4.*(X1FIT-60).*(X2FIT-140))+
(b5.*(X1FIT-60).*(X1FIT-60))+(b6.*(X2FIT-140).*(X2FIT-140));
kcolor = 10.^logkcolor;
n=6.2-0.055.*(X1FIT)+0.001.*(X1FIT-60).^2;
```

```
Co= c1+(c2.*X1FIT)+(c3.*X2FIT)+(c4.*(X2FIT-140).*(X2FIT-140));
YFIT = 100.*[((kcolor.*(n-1).*(t-to))./(Co.^(1-n)))+1].^(1./(1-n)); % YFIT
= retention
mesh(X1FIT,X2FIT,YFIT);
axis([40 80 0 320 20 80])
xlabel('Vitamin C (mg/100 mL)')
ylabel('Gallic acid (mg/100 mL)')
zlabel('Color retention (%)')
%title('3D Plotting')
view(50,10)
```

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REFERENCES

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