ANALYZING THE EFFECTS OF STORAGE TEMPERATURE ABUSE ON CABBAGE QUALITY

by

SABRINA BUCK

(Under the Direction of Laurel L. Dunn)

ABSTRACT

There has been growing demand for healthy and convenient fresh cut produce, but shredded cabbage can be exposed to loss of refrigeration conditions at many points during storage and distribution. This study analyzed the effect of mild temperature abuse conditions (25 °C) on the quality characteristics of cabbages stored over a three-week period. The cabbages were exposed to temperature abuse intervals of 8, 16, and 24 hours prior to refrigerated storage and compared to cabbages kept under refrigeration. Cabbages exposed to temperature abuse conditions for 8 hours did not significantly differ from those kept under refrigeration conditions for any of the quality parameters assessed in this study. However, the L-ascorbic acid and glucosinolate contents of the shredded cabbages that were temperature abused for 16 and 24 hours were lower in comparison to the refrigerated cabbages. Anthocyanins were not significantly impacted by temperature abuse but were degraded during shredding.

INDEX WORDS: anthocyanins, polyphenol oxidase activity, glucosinolates, color parameters

ANALYZING THE EFFECTS OF STORAGE TEMPERATURE ABUSE ON CABBAGE QUALITY

by

SABRINA BUCK

B.S.A., University of Georgia, 2021

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2023

© 2023

Sabrina Buck

All Rights Reserved

ANALYZING THE EFFECTS OF STORAGE TEMPERATURE ABUSE ON CABBAGE QUALITY

by

SABRINA BUCK

Major Professor:	Laurel L. Dunn
Committee:	Ronald B. Pegg
	Govindaraj Dev Kumar

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
May 2023

DEDICATION

This thesis is dedicated to my beautiful grandmother, Patricia Niezgoda, who always supported my higher education and goals.

ACKNOWLEDGEMENTS

I want to express my sincere gratitude to all the professors on my advisory committee for their time and patience throughout this project. I cannot express my gratitude for Dr. Ronald Pegg enough. Without his guidance and facilities this project would not have been possible, and I am forever appreciative of all he taught me during my time in his lab. I would also like to thank the Georgia Department of Agriculture for funding this research. I want to thank Dr. Tyl for teaching me the foundations for this project during my time here as an undergraduate student and Dr. Kerr for allowing me to use his equipment and lab space. Additionally, I owe a sincere thank-you to my lab mates, Patrick Connolly, Jennifer Dorick, Amelia Payne, Anna Townsend, and Andrew Widmer, for their help processing the cabbages. This project also would not have been possible without J.R. Hyatt teaching me how to use the analytical equipment, and Samuel Ogundipe providing lab support and encouragement.

I am also extremely grateful for my parents and brothers for helping me pursue this degree and always being just a phone call away. I love you all deer-ly, family Bucks. I also want to thank my boyfriend, Justin Berry, for all his encouragement and keeping me grounded throughout graduate school. I am so lucky to have such an amazing support system, and I have thoroughly enjoyed my time in the University of Georgia Food Science program.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

In Georgia, cabbages and other leafy greens account for about 20% of the state's vegetable acreage (University of Georgia Extension, 2017). However, temperature fluctuations during storage and transport can negatively impact the quality of this important crop. There may be loss of refrigeration conditions while the cabbage sits on loading docks, due to faulty cooling units during shipping, or during retail display. While previous research has been conducted on the effects of thermal processing on cabbage quality characteristics (Volden et al., 2008; Lafarga et al., 2018), little research exists on the effects of the milder temperature abuse conditions that cabbage is likely to encounter during shipping and retail. Additionally, there has been increased demand for shredded cabbage in recent years due to its desirable health properties and convenience (Francis et al., 2012), but shredded cabbage is also susceptible to faster deterioration, especially in the absence of proper refrigeration. Thus, the purpose of this project was to determine the onset of quality changes in whole and shredded cabbages caused by storage temperature abuse.

This research has important applications for consumer safety, product quality, and limiting food waste. Farmers and processors with cabbages that have been exposed to temperature abuse need to know what conditions are the most harmful to their product quality and whether the cabbage poses a safety risk. The information obtained from this study can also help limit food waste, as cabbage that has been exposed to temperature abuse conditions but is not susceptible to quality or safety concerns may be salvaged. Additionally, components of the cabbage, such as the anthocyanin pigments, may still be able to be isolated and purified for further use after partial or

full spoilage. Applications of this research are also important for growers who field package with limited access to cooling equipment.

Temperature abuse conditions may cause deterioration of quality that negatively impacts the economic value of the crop as well as the potential safety. From the consumer standpoint, cabbage that lacks desirable sensory characteristics or that is beginning to spoil is undesirable to purchase. Of particular concern is shredded cabbage, which has additional exposed surfaces for undesirable microbial growth and chemical reactions. Thus, it is important to understand what durations of temperature abuse negatively affect the quality parameters of cabbage to better educate the industry on best handling practices and provide guidance on when to salvage or discard cabbage.

The quality characteristics studied include those related to the flavor, health, and color of the cabbages. Compounds with beneficial health characteristics including glucosinolates, anthocyanins, and L-ascorbic acid were all quantified. Glucosinolates also are important as they contribute to the sensory profile of cabbage by imparting a bitter taste (Nor et al., 2020). Anthocyanins play an important role in cabbage color and are responsible for the red and purple hues of red cabbage. High performance liquid chromatography (HPLC) techniques were used to assess both the concentration of individual anthocyanin pigments as well as the overall anthocyanin content of the cabbage samples. Lastly, polyphenol oxidase (PPO) activity was measured. PPOs play an important role in the browning and thus degradation of shredded produce when in the presence of molecular oxygen (Singh et al., 2018). The objective of this study was to determine what durations of storage temperature abuse significantly affect these cabbage quality characteristics.

References

Francis, G. A., Gallone, A., Nychas, G. J., Sofos, J. N., Colelli, G., Amodio, M. L., & Spano, G. (2012). Factors affecting quality and safety of fresh-cut produce. *Critical Reviews in Food Science and Nutrition, 52*(7), 595–610.

Lafarga, T., Bobo, G., Vinas, I., Collazo, C., Aguilo-Aguayo, I. (2018). Effects of thermal and non-thermal processing of cruciferous vegetables on glucosinolates and its derived forms. *Journal of Food Science Technology, 55,* 1973-1981. https://doi.org/10.1007/s13197-018-3153-7.

Nor, N.D.M., Lignou, S., Bell, L., Houston-Price, C., Harvey, K., & Methven, L. (2020). The relationship between glucosinolates and the sensory characteristics of steamed-pureed turnip (Brassica Rapa subsp. Rapa L.). *Foods*, *9*(11), 1719. https://doi.org/10.3390/foods9111719.

Singh, B., Suri, K., Shevkani, K., Kaur, A., Kaur, A., Singh, N. (2018). Enzymatic browning of fruit and vegetables: a review. In: Kuddus, M. (eds) *Enzymes in Food Technology*. Springer, Singapore. https://doi.org/10.1007/978-981-13-1933-4_4.

University of Georgia Extension. (2017, January 30). *Commercial production and management of cabbage and leafy greens.* Retrieved April 30, 2022, from https://extension.uga.edu/publications/detail.html?number=B1181&title=Commercial%20Production%20and%20Management%20of%20Cabbage%20and%20Leafy%20Greens.

Volden, J., Borge, G.I.A., Bengtsson, G.B., Hansen, M., Thygesen, I.E., & Wicklund, T. (2008). Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (Brassica oleracea L. ssp. capitata f. rubra*). Food Chemistry, 109*, 595–605. http://dx.doi.org/10.1016/j.foodchem.2008.01.010.

CHAPTER 2

LITERATURE REVIEW

2.1 Cabbage growth and production

Cabbage (*Brassica oleracea* subsp. *capitata L.*) is a cool weather crop that is commonly eaten as a fresh item, cooked, or processed into fermented products like sauerkraut (University of Georgia Extension, 2017). It is mainly grown in the spring, fall, and winter months in Georgia (University of Georgia Extension, 2017). In 2021, Georgia farms harvested 8,600 acres of cabbage, and the United States harvested a total of 57,000 acres of cabbage (United States Department of Agriculture, 2022). The economic value of the U.S. cabbage crop in 2021 totaled $457 million (United States Department of Agriculture, 2022). Out of the 21.1 million hundredweight (cwt) cabbages in utilized production, 18.1 million cwt went to the fresh market and 147,760 tons were used for further processing (United States Department of Agriculture, 2022).

Quality degradation during cabbage production can be attributed to many factors including disease, physical damage sustained during harvest, handling, and loss of temperature control during storage and distribution. Common diseases in cabbage include black-rot caused by *Xanthomonas campestris* pv. *campestris* and black leg caused by the fungus *Phoma lingam* (University of Georgia Extension, 2017). Diseases can be transmitted via an array of pathways including from contaminated seed, the environment (wind/ water/ soil), small animals, or from human activity (e.g., transplanting and transporting the cabbage) (Sharma et al., 2018).

Cabbage is usually harvested by hand and field packed. During field packing, cabbage is often thrown into large harvest bins, which may result in damage including breaking of outer leaves

or the breaking open of the cabbage head (University of Georgia Extension, 2017). Physical damage is a threat to cabbage quality as the injury serves as an entry point for pathogens, and the cut tissue can be more susceptible to accelerated chemical degradation reactions. Furthermore, consumers will refuse to purchase whole heads of cabbage that have obvious signs of physical damage, so further processing such as shredding is required.

In terms of temperature, quality is best maintained when cabbage is precooled before shipping; then the refrigeration chain will continue throughout storage and transport. Under refrigeration conditions (32 °F) and 90-95% relative humidity, cabbage can be stored for 3 to 6 weeks (University of Georgia Extension, 2017). If proper storage temperatures are not maintained, cabbages may be more susceptible to microbial spoilage and pathogens may grow, making the cabbages unsafe to eat. Additionally, cabbages not stored under proper refrigeration conditions may undergo loss of nutrients, water, and desirable color characteristics (Zhang et al., 2013).

2.2 *Listeria monocytogenes* in cabbage

Listeria monocytogenes contamination in fresh produce has been attributed to the growing, post-harvest processing, and retail environments (Zhu, Gooneratne, & Hussain, 2017). The pathogen causes listeriosis, which is characterized by symptoms including fever, diarrhea, meningitis, myalgia, abortion in pregnant women, stillbirth, and death (World Health Organization, 2018). The resulting disease is serious, with ~94% hospitalization rates and high fatality rates of 20-30% (Haskell & DeAngelo, 2022). Each year, the costs associated with *L. monocytogenes* infections in the US are estimated to range from $228 million-$7.6 billion (Hoffman, Maculloch, & Batz, 2015).

L. monocytogenes was first recognized as a foodborne pathogen in 1981 after an outbreak in coleslaw from Canada resulted in 41 cases with 18 deaths (Garner & Kathariou, 2016). There was speculation that the cabbage used for the coleslaw became contaminated from sheep manure; however, strains from the implicated flock were not preserved so a definite conclusion was never reached. In 2019, Mann Packing Company voluntarily recalled multiple fresh cut vegetable products, including green cabbage, due to potential *L. monocytogenes* contamination (U.S. Food & Drug Administration, 2019). In 2021, there were two additional recalls associated with cabbage and *L. monocytogenes* growth. In July 2021, 5000 Years Food Inc. recalled their kimchi after *L. monocytogenes* was found within their production facility (U.S. Food & Drug Administration, 2021a). Later in December of 2021, Fresh Express initiated a recall after the Garden Salad, which contained red cabbage, tested positive for *L. monocytogenes* (U.S. Food & Drug Administration, 2021b). Furthermore, the worst foodborne disease outbreak in U.S. history in terms of fatalities was due to whole cantaloupes contaminated by *L. monocytogenes* due to improper post-harvest handling (McCollum et al., 2013). Of the 147 reported cases, 99% of patients were hospitalized and 33 died. While cabbage was not the implicated commodity in this outbreak, the severe morbidity and high mortality indicate that fresh produce can pose a significant health hazard if contaminated.

Previous studies have been conducted on *L. monocytogenes* growth in heat-treated cabbage. In 2010, Ells and Hansen held shredded and intact cabbage inoculated with *L. monocytogenes* and *Listeria* spp. at 50 °C for 3 min, then returned the samples to refrigerated storage at 5 °C. Growth in the heat-treated cabbage was up to 2.36 log CFU/g greater than in the non-heat treated cabbage. On the intact cabbage, all *Listeria* strains declined in population (up to 1.0 log CFU/cm^2) over the 28-day storage period. On the intact cabbage coupons, decreases in

Listeria populations of up to 1.0 log CFU/cm^2 were observed. However, the cut tissue coupons supported growth (up to 1.2 log CFU/cm^2), especially during the first 7-14 days of incubation.

One potential theory as to why cruciferous plants exhibit higher levels of microbial growth following a brief heat treatment is that myrosinase is inactivated. Myrosinase is the enzyme that cleaves glucosinolates into toxic compounds which are inhibitory to microbial plant pathogens and insects (Mithen et al., 2000). Another study analyzed the effects of modified atmosphere packaging (MAP) on *L. monocytogenes* growth in shredded cabbage over a 17-day storage period (Kallander et al., 1991). Shredded cabbage was inoculated with *L. monocytogenes*, then stored in either ambient air or under a modified atmosphere (70% CO_2 and 30% N_2) at 5 °C and 25 °C. Under both the ambient and modified atmosphere at 25 °C, *L. monocytogenes* colony counts decreased below detectable levels (<20 CFU/g) within 6 days. The cabbages stored under a modified atmosphere, however, had a lower initial increase in *L. monocytogenes* growth and a more rapid decline in growth overall. In the refrigerated cabbages stored at 5 °C, *L. monocytogenes* increased by about 1 log CFU/g under both atmospheres. In the refrigerated cabbage samples, the cabbages stored under normal atmosphere decreased in *L. monocytogenes* counts between days 13 and 17; however, no decrease in growth under refrigeration conditions was reported in the cabbages stored using MAP.

2.3 Lactic acid bacteria spoilage in cabbage

Lactic acid bacteria (LAB) are Gram-positive, non-spore-forming, facultative-anaerobes that produce lactic acid via their metabolic processes. In cabbage, LAB are responsible for both desirable food fermentation processes that produce kimchi and sauerkraut as well as undesirable food spoilage. A 2007 study analyzed the bacterial composition of LAB present in sauerkraut

during fermentation by using DNA fingerprinting (Plengvidhya et al., 2007). LAB present in cabbage include *Leuconostoc mesenteroides, Lactobacillus brevis, Pediococcus pentosaceus*, and *Lactobacillus plantarum* (Plengvidhya et al., 2007). The researchers found that during sauerkraut fermentation, heterolactic LAB species will dominate the first 2-3 days of fermentation, then be replaced by acid-tolerant homofermentative LAB species (Plengvidhya et al., 2007). In this study, initial LAB populations in the shredded cabbage ranged from 10^4 CFU/g to 10^6 CFU/g, and the authors speculated that this could be contributed to LAB growth during transport prior to analysis.

Another study analyzed the growth of *E. coli* O157:H7 and *L. monocytogenes* during sauerkraut fermentation (Niksic et al., 2005). There was a higher survival of both *L. monocytogenes* and lactic acid bacteria in the whole heads of cabbage as compared to the shredded cabbage. The authors proposed this was likely because the shredding process rapidly released many of the nutrients, allowing for rapid consumption by the bacteria and an initial population spike. However, after this population increase, the high acidity generated by metabolic activity resulted in a decrease in the bacterial populations. In the whole heads of cabbage, nutrients diffused out of the cabbage slowly. Thus, there was a slower production of acid, and the bacterial populations survived for longer periods of time.

2.4 Colorimetric parameters of cabbage

The CIELAB color scale provides a standard color scale that allows color values to be compared (HunterLab, 1996). The color space in the CIELAB scale is organized in a geometric cube form (see Figure 2.1). The L* axis runs from top to bottom and measures the lightness of the sample, with L* = 100 representing white and L* = 0 representing black. The a* axis measures red/green colors, with the positive a* representing red and the negative a* representing green. The

b* axis measures yellow/blue colors, with positive b* values representing yellow and negative b* values representing blue.

A previous study analyzed the color parameters within 3 different layers of red cabbage, from the outside to the inside leaves (Leahu et al., 2018). The L* values ranged from 23.96-25.87, with the outside leaves being darker in color (Leahu et al., 2018). The a* values were 3.59, 6.84, and 8.84 for the outer, mid, and inner layers respectively, indicating a red color with increasing intensity in the inner leaves (Leahu et al., 2018). The b* values for the outer, mid, and inner layers were -1.04, -3.43, and -3.91, respectively. (Leahu et al., 2018). A previous study conducted on green, shredded cabbage found that the L* values dropped from 75.9 to 71.21, a* values increased from -2.15 to -1.05, and b* values decreased from 14.18 to 12.99 over a four-week storage period (Ibrahim et al., 2004).

2.5 PPO activity of cabbage

A major quality concern in cabbage is polyphenol oxidase (PPO) activity. PPOs are enzymes that are present in the cytoplasm of the cell while their chemical substrates, mainly polyphenols, are contained in cell plastids (Singh et al., 2018). When plant tissue is damaged during processing or handling, the plastids are ruptured and PPOs come into contact with the phenolic substrates (Singh et al., 2018). The first reaction that PPOs catalyze is the hydroxylation of monophenols to diphenols, and then PPO catalyzes the oxidation of *o*-diphenols to *o*-quinones (see Figure 2.2) (Singh et al., 2018). Both reactions require the presence of molecular oxygen. After these PPO-catalyzed reactions, the non-enzymatic polymerization of quinones then results in the formation of dark-colored melanin pigments (see Figure 2.2) (Singh et al., 2018).

The rate of enzymatic browning is affected by both temperature and pH. At temperatures below 7 °C, PPO activity is inhibited, while temperatures above 70 °C can inactivate PPOs (Singh et al., 2018). During extraction, chilled temperatures (usually near 4 °C) are ideal to limit enzyme degradation (Sabarre Jr & Yagonia-Lobarbio, 2021). If using equipment that can generate heat during extraction, such as a blender, the equipment should be used in short bursts so as not to overheat the enzymes (Sabarre Jr & Yagonia-Lobarbio, 2021). The optimum pH for PPO activity varies between 5.0-7.0, and low pH values below 3.0 inhibit PPO activity (Singh et al., 2018). During extraction, pH buffers between 6.8-7.2 are recommended (Sabarre Jr & Yagonia-Lobarbio, 2021).

Methods of PPO control in produce include heating and cooling, high-pressure processing, and adding chemical anti-browning agents. Blanching can be used to prevent enzymatic browning but may also result in undesirable tissue softening (Singh et al., 2018). Freezing decreases the availability of water for enzymatic reactions and is often used in combination with blanching to slow enzymatic browning (Singh et al., 2018). High pressure processing deactivates enzymes without changing the nutrient or flavor profile of foods. However, the high pressure of 3000-8000 bars is unsuitable for fresh produce as the integrity of the porous vegetable structure is disturbed in the process (Singh et al., 2018). Chemical anti-browning agents include reducing agents, acidifying agents, chelating agents, and antioxidant agents (Singh et al., 2018). Reducing agents, such as ascorbic acid, control PPO activity by reducing *o*-quinones back to polyphenolic substrates (Singh et al., 2018). Chelating agents, such as citric acid, form complexes with PPOs, preventing PPOs from binding with other substrates, and acidifying agents reduce the pH below the PPO optimum to decrease enzyme activity (Singh et al., 2018).

A previous study on cabbage PPO activity reported high thermal stability, with about 40% of the PPO activity remaining after heat treatment at 100 °C for 10 minutes (Fujita et al., 1995). The study also found the optimum temperature for PPO activity in cabbage was 40 °C (Fujita et al., 1995). Another study conducted by Li et al. (2020) studied the PPO activity in purple cabbage stored under a modified atmosphere for 20 days. In the early stages of storage, PPO activity ranged from 4.56 to 11.59 U g^{-1} min^{-1} (Li et al., 2020); U is an arbitrary enzyme activity unit representing an increase in absorbance of 0.001. The PPO activities in the purple cabbage reached a minimum on day 16 of storage before increasing again by 20 days of storage (Li et al., 2020). By the end of the storage cycle (day 20), the PPO activity ranged from 11.47 U g^{-1} min^{-1} to 24.1 U g^{-1} min^{-1} (Li et al., 2020).

2.6 Anthocyanins in red cabbage

Anthocyanins are the pigments responsible for the red, purple, and blue color in many fruits and vegetables. In addition to being color pigments, anthocyanins are antioxidants and have been linked to health benefits. Studies suggest that consumption of anthocyanins may help reverse age-related neurodegenerative declines, protect against cancers, improve cardiovascular health, stimulate insulin secretion, and ameliorate oxidative stress (Charron et al., 2007).

Monomeric anthocyanins undergo a structural change as a function of pH, resulting in a color change that can be quantified using spectrophotometry (see Figure 2.3). At a pH of 1.0, the colored oxonium form predominates, and at a pH of 4.5, the colorless hemiketal form predominates (Lee, Durst, & Wolstrad, 2005). The difference in the absorbance of the pigments at a wavelength of 520 nm is then proportional to the total anthocyanin concentration (Lee, Durst, & Wolstrad, 2005).

Previous studies have reported large variations in the anthocyanin content of red cabbage. Mazza & Miniati (1993) reported the total monomeric anthocyanin content in red cabbage ranged from 25-495 mg/100 g fresh weight cabbage. Podsedek et al. (2006) reported the anthocyanin levels in red cabbage were 40.53 mg cyanidin-3-glucoside/ 100 g fresh weight for the Koda cultivar and 76.16 mg cyanidin-3-glucoside / 100 g fresh weight for the Kissendrup cultivar. Ahmadiani et al. (2014) reported the average total anthocyanin content in red cabbage to be about 145-150 mg cyanidin-3-glucoside equivalents/100 g fresh cabbage, and Kannan (2011) found a total anthocyanin content of 12.89 mg cyanidin-3-glucoside/ 100 g fresh weight cabbage. Potential reasons that help account for this large variation include the utilization of different solvent systems and extraction techniques as well as potential differences between cabbage cultivars (Kannan, 2011).

To determine the individual anthocyanin profile, additional methods, such as high-performance liquid chromatography (HPLC) are required. For anthocyanins, reversed-phase analytical HPLC is common, which employs a polar mobile phase and non-polar stationary phase (Peña et al., 1997). Thus, the more polar anthocyanin pigments elute first. After separation using HPLC, the isolated peaks can be analyzed using mass spectroscopy to identify individual anthocyanins. Anthocyanins in red cabbage are based on cyanidin-3-*O*-diglucoside-5-*O*-glucoside, which can be non-acylated, mono-acylated, or di-acylated with *p*-coumaric, cafferic, ferulic, and sinapic acids (McDougall et al., 2007). Previous studies on the anthocyanin profile of red cabbage have found between 8 to 23 anthocyanin pigments, depending on the cultivar of the cabbage (Ahmadiani et al., 2014). Additionally, a previous study reported that red cabbage anthocyanins have higher thermal stability than anthocyanins in other foods (Dyrby, Westergaard, & Stapelfeldt, 2001). Anthocyanin colorants from red cabbage were stored at 25 °C, 40 °C, 60 °C and 80 °C for

up to six hours, and only the samples held at 80 °C underwent determinable thermal degradation (Dyrby, Westergaard, & Stapelfeldt, 2001). This study proposed that the increased stability of red cabbage anthocyanins is due to their complex sugar residues (Dyrby, Westergaard, & Stapelfeldt, 2001). Another study compared the anthocyanin profile of black carrot at 4 °C, 25 °C, and 40 °C for 90 days and found that the acylated anthocyanins were more stable than the nonacylated anthocyanins at all storage temperatures (Turker, Aksay, & Ekiz, 2004).

2.7 Vitamin C in cabbage

An important health component in cabbage is the vitamin C content. It has been linked to increased iron absorption, lowered cholesterol levels, and is an antioxidant that protects the body from free radicals and toxins (Chambial et al., 2013). Vitamin C is used as an index of the nutrient quality for produce products because it is very sensitive to degradation during food processing and storage (Pathy, 2018). Degradation may be due to anaerobic or aerobic pathways, with aerobic oxidative destruction mainly occurring during food processing and anaerobic degradation primarily occurring during food storage (Pathy, 2018).

A study conducted in 2013 by Park et al. analyzed the vitamin C content of red and green cabbages using HPLC. Key findings include that the vitamin C content of red and green cabbages ranged from 22.72-129.90 mg/100 g fresh weight cabbage, and red cabbages had higher vitamin C contents than green cabbages (Park et al., 2013). Another study analyzing phytochemicals in cabbage, including vitamin C, found that the vitamin C content ranged from 5.66 to 23.50 mg/100 g fresh weight (Singh et al., 2006).

Li et al. (2020) also analyzed the vitamin C content of shredded purple cabbage over a 21-day storage period. At the beginning of storage, vitamin C contents ranged from 24.8-26.3 mg/

100 g fresh weight cabbage. By the end of storage, vitamin C content ranged from 8.70 mg/100 g fresh weight cabbage to 21.6 mg/100 g fresh weight cabbage, depending on the modified atmosphere packaging gas composition and packaging material.

2.8 Glucosinolates in cabbage

Glucosinolates are sulfur-containing compounds responsible for the bitter taste of cabbage (Nor et al., 2020). When plant tissue is damaged, glucosinolate hydrolysis leads to the formation of degradation products such as isothiocyanates, nitriles, oxazolidinethiones, indoles, and thiocyanates (Verkerk, Dekker, & Jongen, 2001). Isothiocyanates have important anti-cancer properties and have been linked to multiple pathways including apoptosis, oxidative stress, and cell cycle progression (Wu, Zhou, & Xu, 2009).

A previous study conducted by Song & Thornalley (2007) analyzed the effects of storage and processing on the glucosinolate contents of broccoli, Brussel sprouts, cauliflower, and green cabbage. The study found that storage at ambient temperature and refrigeration temperature over the course of seven days resulted in minor losses (9-26%) of glucosinolates, but there were no significant differences in the glucosinolate levels between the two temperatures (Song & Thornalley, 2007). Additionally, storing the vegetables at -85 °C caused significant loss of glucosinolates because the freezing and subsequent thawing of the plant tissue resulted in the release of myrosinase, which broke down the glucosinolates (Song & Thornalley, 2007). The study also found that green cabbage lost about 60% of total glucosinolates after being shredded (Song & Thornalley, 2007).

A 2014 study used HPLC to analyze glucosinolates in red and green cabbage lines (Park et al., 2014). In green cabbages, an average of 78% of the glucosinolates were glucoraphanin,

sinigrin, and glucobrassicin. 65.6% of the aliphatic glucosinolate content was attributed to sinigrin in the green cabbages. The average total glucosinolate contents in green cabbages were 1.7 μmol/g dry weight of glucoraphanin and 4.6 μmol/g dry weight of sinigrin. The study also concluded that there were large differences in the total glucosinolate content and composition between the green and red cabbage lines.

2.9 Impact on the cabbage industry

Determining the effects of storage temperature abuse on cabbage is important to the cabbage industry to help ensure safe, quality produce. Loss of refrigeration conditions is frequently encountered along the produce production chain. From a safety perspective, it is vital to understand at what point cabbage that has undergone temperature abuse poses a threat to public health. It is also important to understand how bioactive compounds with potential health benefits, such as vitamin C, anthocyanins, and glucosinolates, are impacted by temperature abuse. From an industry perspective, cabbage that has undergone loss of refrigeration conditions may still be salvageable. Thus, better understanding the effects of temperature abuse can help limit food waste and increase cabbage profitability. On the other hand, cabbage that has not been maintained under refrigeration conditions may also reach the end of shelf life sooner, and differences in degradation reactions between whole and shredded cabbages that have been temperature abused need to be further understood to distinguish when temperature abuse results in significant losses in cabbage quality.

Additionally, this study can help guide farmers on proper cabbage handling practices postharvest. Farmers that field pack their cabbages need to know how long whole cabbages can sit on trucks in the field before going to refrigerated storage. There has also been increased demand for shredded produce, as it is minimally processed and convenient for consumers to store and

handle (Francis et al., 2012). Shredded produce is more expensive than whole heads because there are additional processing steps. This makes it have enhanced economic value in the cabbage industry, but it also needs to be handled carefully because shredded tissues are more susceptible to pathogenic contamination as well as degradation reactions.

Furthermore, cabbage that has spoiled may still be able to be used for other purposes. An emerging topic in the food industry is food-waste valorization. Up to 45% of food is wasted in the food supply chain, so there is interest in extracting natural compounds from spent food to use for other purposes (Cecilia et al., 2019). For example, red cabbage is rich in anthocyanins, which could potentially be extracted and used as natural food coloring agents even after cabbage spoilage (Patras, 2019).

Therefore, cabbage storage temperature abuse needs to be further studied because of the potential negative impacts loss of refrigeration has on the flavor, health, and color characteristics of cabbage. The cabbage industry needs guidance on best handling practices for cabbages after harvest and further processing. The results from this study will help cover a literature gap on storage temperature abuse in shredded produce as well as aid in determining what durations of temperature abuse pose a significant threat to cabbage quality.

References

Ahmadiani, N., Robbins, R. J., Collins, T. M., & Giusti, M. M. (2014). Anthocyanins contents, profiles, and color characteristics of red cabbage extracts from different cultivars and maturity stages. *Journal of Agricultural and Food Chemistry*, *62*(30), 7524–7531. https://doi.org/10.1021/jf501991q.

Barth, M., Hankinson, T.R., Zhuang, H., & Breidt, F. (2009). Microbiological spoilage of fruits and vegetables. In: Sperber, W.H. & Doyle, M.P. (eds) *Compendium of the microbiological spoilage of foods and beverages*. Food Microbiology and Food Safety. https://doi.org/10.1007/978-1-4419-0826-1_6.

Cecilia, J. A., García-Sancho, C., Maireles-Torres, P. J., & Luque, R. (2019). Industrial food waste valorization: a general overview. *Biorefinery: Integrated Sustainable Processes for Biomass Conversion to Biomaterials, Biofuels, and Fertilizers*, 253-277.

Červenski, J., Vlajić, S., Ignjatov, M., Tamindžić, G., & Zec, S. (2022). Agroclimatic conditions for cabbage production. *Field & Vegetable Crops Research / Ratarstvo i Povrtarstvo*, *59*(2), 43–50. https://doi.org/10.5937/ratpov59-36772.

Chambial, S., Dwivedi, S., Shukla, K. K., John, P. J., & Sharma, P. (2013). Vitamin C in disease prevention and cure: an overview. *Indian Journal of Clinical Biochemistry*, *28*(4), 314–328. https://doi.org/10.1007/s12291-013-0375-3.

Charron, C. S., Clevidence, B. A., Britz, S. J., & Novotny, J. A. (2007). Effect of dose size on bioavailability of acylated and nonacylated anthocyanins from red cabbage (Brassica oleracea L. Var. capitata). *Journal of agricultural and food chemistry*, *55*(13), 5354–5362.

Dyrby, M., Westergaard, N., & Stapelfeldt, H. (2001). Light and heat sensitivity of red cabbage

extract in soft drink model systems. *Food Chemistry, 72*(4), 431–437. https://doi.org/10.1016/S0308-8146(00)00251-X.

Ells, T., Hansen, L.T. (2010). Growth of *Listeria* spp. in shredded cabbage is enhanced by a mild heat treatment. *Journal of Food Protection, 73*(3), 425–433. https://doi.org/10.4315/0362-028X-73.3.425.

Francis, G. A., Gallone, A., Nychas, G. J., Sofos, J. N., Colelli, G., Amodio, M. L., & Spano, G. (2012). Factors affecting quality and safety of fresh-cut produce. *Critical Reviews in Food Science and Nutrition, 52*(7), 595–610.

Fujita, S., Saari, N., Maegawa, M., Tetsuka, T., Hayashi, N., & Tono, T. (1995). Purification and properties of polyphenol oxidase from cabbage (Brassica oleracea L.). *Journal of Agricultural and Food Chemistry, 43*, 1138–1142. https://doi.org/10.1021/jf00053a005.

Garner, D., & Kathariou, S. (2016). Fresh produce–associated listeriosis outbreaks, sources of concern, teachable moments, and insights. *Journal of Food Protection*, *79*(2), 337–344.

Haskell, S., DeAngelo, K. (2022, January 15*). What's on the menu for 2022? Covid-19? Listeria? Salmonella? E. coli?* Institute for Food Laws and Regulations. Retrieved from https://www.canr.msu.edu/news/what-s-on-the-menu-for-2022-covid-19-listeria-salmonella-e-coli-1.

Hoffman, S., Maculloch, B., Batz, M. (2015) *Economic Burden of Major Foodborne Illnesses Acquired in the United States* (Bulletin Number 140). United States Department of Agriculture, Economic Research Service.

HunterLab. (1996). *CIE L*a*b* color scale.* Hunter Associates Laboratory, Inc. Retrieved from http://lib3.dss.go.th/fulltext/glass/GlassTheories/CIE_Lab_info.pdf.

Ibrahim, R., Osman, A., Saari, N., & Rahman, R. A. (2004). Effects of anti-browning treatments

on the storage quality of minimally processed shredded cabbage. *Journal of Food Agriculture and Environment, 2*, 54–58.

Kallander, K. D., Hitchins, A. D., Lancette, G. A., Schmieg, J. A., Garcia, G. R., Solomon, H. M., & Sofos, J. N. (1991). Fate of *Listeria monocytogenes* in shredded cabbage stored at 5 and 25°C under a modified atmosphere. *Journal of Food Protection, 54*(4), 302–304. https://doi.org/10.4315/0362-028X-54.4.302.

Kannan, V. (2011). *Extraction of bioactive compounds from whole red cabbage and beetroot using pulsed electric fields and evaluation of their functionality.*

Leahu, A., Ghinea, C., Oroian, M. & Damian, C. (2018). Determination of essential and toxic elements, ascorbic acid content and color of different leaves in two cabbage varieties. *Ovidius University Annals of Chemistry, 29*(2), 110–116. https://doi.org/10.2478/auoc-2018-0016.

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *Journal of AOAC International, 88*(5), 1269–1268. https://doi.org/10.1093/jaoac/88.5.1269.

Li, H., Li, X., Wang, R.., Xing, Y., Xu, Q., Shui, Y., Guo, X., Li W., Yang, H., Bi., X., & Che, Z. (2020). Quality of fresh-cut purple cabbage stored at modified atmosphere packaging and cold-chain transportation. *International Journal of Food Properties, 23(1*), 138–153. https://doi.org/10.1080/10942912.2020.1716795.

Manwell, P. M., & Ackland, M. R. (1986). Rapid detection of microbial growth in vegetable salads at chill and abuse temperatures. *Food Microbiology*, *3*(1), 59–65.

Mazza, G. & Miniati, E. (1993). *Anthocyanins in fruits, vegetables, and grains.* Boca Raton: CRC

Press.

McCollum, J. T., Cronquist, A. B., Silk, B. J., Jackson, K. A., O'Connor, K. A., Cosgrove, S., ... & Mahon, B. E. (2013). Multistate outbreak of listeriosis associated with cantaloupe. *New England Journal of Medicine, 369*(10), 944-953.

McDougall, G. J., Fyffe, S., Dobson, P., & Stewart, D. (2007). Anthocyanins from red cabbage–stability to simulated gastrointestinal digestion. *Phytochemistry, 68*(9), 1285–1294.

Mithen, R.F., Dekker, M., Verkerk, R., Rabot, S., & Johnson, I.T. (2000). The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *Journal of the Science of Food and Agriculture, 80*(7), 967–984. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<967::AID-JSFA597>3.0.CO;2-V.

Niksic, M., Niebuhr, S. E., Dickson, J. S., Mendonca, A. F., Koziczkowski, J. J., & Ellingson, J. L. (2005). Survival of *Listeria monocytogenes* and *Escherichia coli* O157: H7 during sauerkraut fermentation. *Journal of Food Protection, 68*(7), 1367–1374.

Nor, N.D.M., Lignou, S., Bell, L., Houston-Price, C., Harvey, K., & Methven, L. (2020). The relationship between glucosinolates and the sensory characteristics of steamed-pureed turnip (*Brassica Rapa subsp. Rapa* L.). *Foods*, *9*(11), 1719. https://doi.org/10.3390/foods9111719.

Park, S., Valan Arasu, M., Lee, M.K., Chun, J.-H., Seo, J. M., Lee, S.W., Al-Dhabi, N. A., & Kim, S.-J. (2014). Quantification of glucosinolates, anthocyanins, free amino acids, and vitamin C in inbred lines of cabbage (Brassica oleracea L.). *Food Chemistry*, *145*, 77–85. https://doi.org/10.1016/j.foodchem.2013.08.010.

Pathy, K. (2018). Process for preparation of vitamin C and method for determination of vitamin c in tablets. *Journal of Chemical Research*, *2*(1), 2.

Patras, A. (2019). Stability and colour evaluation of red cabbage waste hydroethanolic extract in presence of different food additives or ingredients. *Food Chemistry, 275*, 539–548.

Peña, A., Garcia, V., de la Luz Romero, M., & Capella, S. (1997). Reversed-phase ion-pair chromatography of anthocyanins in red wines. *Journal of Chromatographic Science*, *35*(4), 161–164.

Plengvidhya, V., Breidt, F., Jr, Lu, Z., & Fleming, H. P. (2007). DNA fingerprinting of lactic acid bacteria in sauerkraut fermentations. *Applied and Environmental Microbiology*, *73*(23), 7697–7702. https://doi.org/10.1128/AEM.01342-07.

Podsedek, A., Sosnowska, D., Redzynia, M., & Anders, B. (2006). Antioxidant capacity and content of Brassica oleracea dietary antioxidants. *International Journal of Food Science & Technology*, *41*, 49–58.

Reyes, F.L., Villarreal, E.J., & Cisneros-Zevallos, L. (2007). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, *101*(3), 1254–1262. https://doi.org/10.1016/j.foodchem.2006.03.032.

Sabarre Jr, D. C., & Yagonia-Lobarbio, C. F. (2021). Extraction and characterization of polyphenol oxidase from plant materials: a review. *Journal of Applied Biotechnology Reports, 8*(2), 83–95.

Sharma, A., Rathore, J. P., Ali, A., Qadri, I., Hussain, S. M., & Angmo, T. (2018). Major diseases and pathogen ecology of cabbage. *The Pharma Innovation Journal*, *7*(7), 574–578.

Singh, J., Upadhyay, A. K., Bahadur, A., Singh, B., Singh, K. P., & Rai, M. (2006). Antioxidant phytochemicals in cabbage (*Brassica oleracea L. var*. capitata). *Scientia Horticulturae*, *108*(3), 233–237.

Singh, B., Suri, K., Shevkani, K., Kaur, A., Kaur, A., Singh, N. (2018). Enzymatic browning of

fruit and vegetables: a review. In: Kuddus, M. (eds) *Enzymes in Food Technology* (pp.63–78). Springer, Singapore. https://doi.org/10.1007/978-981-13-1933-4_4.

Song, L., & Thornalley, P. J. (2007). Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food and Chemical Toxicology, 45*(2), 216–224. https://doi.org/10.1016/j.fct.2006.07.021.

Tsuda T. (2016). Recent progress in anti-obesity and anti-diabetes effect of berries. *Antioxidants, 5*(2), 13. https://doi.org/10.3390/antiox5020013.

Turker, N., Aksay, S., & Ekiz, H. I. (2004). Effect of storage temperature on the stability of anthocyanins of a fermented black carrot (*Daucus carota* var. L.) *Journal of Agricultural and Food Chemistry, 52*(12), 3807–3813. https://doi.org/10.1021/jf049863s.

United States Department of Agriculture. (2022). *Vegetables 2021 Summary.* National Agricultural Statistics Service. https://downloads.usda.library.cornell.edu/usda-esmis/files/02870v86p/zs25zc490/9593vz15q/vegean22.pdf.

U.S. Food & Drug Administration. (2019, November 3). *Mann Packing Co., Inc. voluntarily recalls vegetable produce sold in the United States and Canada due to potential health risks.* https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/mann-packing-co-inc-voluntarily-recalls-vegetable-products-sold-united-states-and-canada-due.

U.S. Food & Drug Administration. (2021a, July 13). *5000 Years Foods, Inc. recall cabbage kimchi because of possible of health risk.* https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/5000-years-foods-inc-recall-cabbage-kimchi-because-possible-health-risk.

U.S. Food & Drug Administration. (2021b, December 27). Fresh Express Announces Recall of Fresh Salad Products Due to Potential Health Risk.

https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/fresh-express-announces-recall-fresh-salad-products-due-potential-health-risk.

University of Georgia Extension. (2017, January 30). *Commercial production and management of cabbage and leafy greens.* https://extension.uga.edu/publications/detail.html.

Verkerk, R., Dekker, M., & Jongen, W. M. (2001). Post-harvest increase of indolyl glucosinolates in response to chopping and storage of Brassica vegetables. *Journal of the Science of Food and Agriculture, 81*(9), 953–958. https://doi.org/10.1002/jsfa.854.

World Health Organization. (2018, February 20). *Listeriosis.* Retrieved from https://www.who.int/news-room/fact-sheets/detail/listeriosis.

Wu, X., Zhou, Q. H., & Xu, K. (2009). Are isothiocyanates potential anti-cancer drugs? *Acta Pharmacologica Sinica*, *30*(5), 501–512. https://doi.org/10.1038/aps.2009.50.

Zhang, L. P., Xie, J., Wang, T., & Xiong, Q. (2013). Study of physicochemical properties of Chinese small cabbage (Brassica chinensis L.) stored at four temperatures. In *Advanced Materials Research* (Vol. 690, pp. 1275–1281). Trans Tech Publications Ltd.

Zhu, Q., Gooneratne, R., & Hussain, M. A. (2017). Listeria monocytogenes in fresh produce: outbreaks, prevalence and contamination levels. *Foods*, *6*(3), 21. https://doi.org/10.3390/foods6030021.

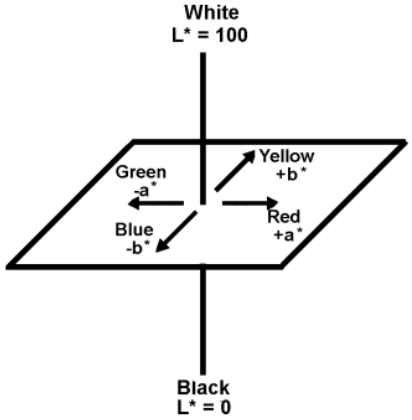


Figure 2.1: CEILAB color scale diagram (HunterLab, 1996)

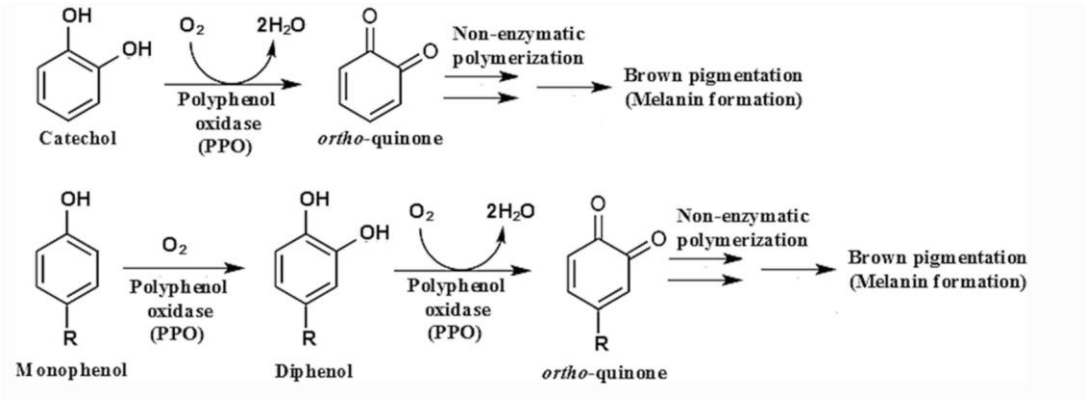


Figure 2.2: Polyphenol oxidase reaction mechanism (Singh et al., 2018)

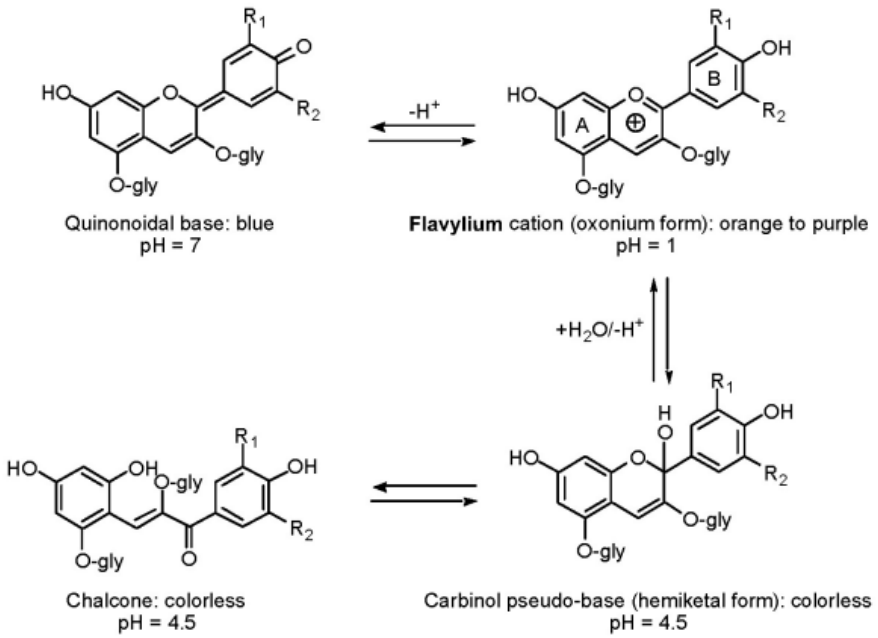


Figure 2.3: Anthocyanin structural change at different pH levels (Lee, Durst, & Wolstrad, 2005)

CHAPTER 3

CHANGES IN THE COLOR, FLAVOR, AND HEALTH CHARACTERISTICS OF STORAGE TEMPERATURE ABUSED CABBAGE [1]

[1] Buck, S. P., Dunn, L., Kerr, W. L., & Pegg, R.B.

To be submitted to *Food Chemistry*

ABSTRACT

Modified atmosphere packaging and the convenience of shredded produce has resulted in a large increase in demand for fresh cut cabbages. This study analyzed the effects of storage temperature abuse on the quality characteristics of cabbage. The cabbages were exposed to temperature abuse conditions of 25 °C for intervals of 8, 16, and 24 hours, then sampled once a week over a 3-week storage study. Whole heads of cabbage did not undergo quality degradation for any of the storage abuse conditions analyzed in this study. The shredded green cabbages that were temperature abused for 24 hours prior to refrigerated storage underwent loss of desirable colors as well as decreased vitamin C and glucosinolate contents. Anthocyanins were resistant to temperature abuse but susceptible to degradation caused by shredding. There is potential to extract anthocyanins from spent cabbages to use as food colorants.

3.1 Introduction

Cabbages may be exposed to temperature abuse conditions at many points from farm to retail. Lack of refrigeration conditions may be encountered during field-packing, from sitting on loading docks for prolonged periods of time, or due to faulty refrigeration units during storage and transport. While several studies have been conducted on the effects of higher temperature cooking and processing treatments on cabbage characteristics (Fujita et al., 1995; Phungamngoen, Chiewchan, & Devhastin, 2013; Wiczkowski, Szaawara-Nowak, & Topolska, 2015), there is a lack of literature covering storage temperature abuse conditions cabbages could potentially encounter. Thus, the purpose of this study was to analyze the effects of moderate storage temperature abuse on the quality characteristics of cabbage.

Another emerging trend in the produce industry is the increased consumption of shredded, ready-to-eat produce. Shredded cabbage is more convenient to handle and easier to portion but requires additional processing steps to retain its quality and safety attributes. In recent years, the fresh-cut produce industry has grown into a multi-billion-dollar sector as demand for healthy, minimally processed, and convenient produce has grown (Francis et al., 2012). Fresh cut products also cost more for consumers as they have additional processing steps, so the economic value of fresh cut cabbage within the cabbage industry has also grown with the increase in consumer demand.

Modified atmosphere packaging (MAP) is essential for retaining the quality of shredded produce and has enabled the exponential growth of the fresh cut produce industry. Once shredded, enzymes responsible for undesirable browning activity, such as polyphenol oxidases, become active in the presence of oxygen, resulting in undesirable browning as well as the loss of important bioactives, such as vitamin C. MAP changes the gas composition within the packaged food from that of air (21% O_2, 0.01% CO_2, 78% N_2) to a modified gas mixture with higher levels of CO_2 and decreased levels of O_2 (Sandhya, 2010). The higher concentration of CO_2 used in MAP packaging helps to inhibit microbial growth and preserve produce quality. This study compares whole heads of cabbage to shredded cabbages packaged under MAP to determine the effects of the physical form of the cabbage over the duration of temperature abuse and storage.

Additionally, most US cabbages are green cabbages. Forty-five percent of cabbages are used for processing coleslaw, 35% of cabbages are sold as fresh heads, 12% are used to make sauerkraut, and 5-10% are sold as fresh-cut products (Agricultural Marketing Resource Center, 2021). Thus, due to the increased demand of shredded produce and the dominant consumption of green cabbages, this project focused primarily on shredded green cabbages. Red cabbages were

also analyzed, but most of the research was focused on color characteristics and anthocyanin content while green cabbage was examined for its color, flavor, and health attributes.

Previous studies have found that visual, aroma, and health related attributes are among the most important factors consumers consider when purchasing produce (Moser, Raffaelli, & Thilmany-McFadden, 2011). Color can be analyzed using a scanning flatbed colorimeter to obtain L*a*b* values, which reflect the brightness and color hues of cabbage samples. The enzyme responsible for browning in cut produce tissues, polyphenol oxidase, was also analyzed as enzymatic browning decreases product quality and potential shelf life.

Glucosinolates are secondary metabolites found in plants from the Brassicaceae family. Upon digestion, the myrosinase enzyme hydrolyzes glucosinolates into various metabolites such as isothiocyanates, nitriles, oxazolidine-2-thiones, and indole-3-carbinols (Prieto, Lopez, & Simal-Gandara, 2019). Glucosinolates and their metabolic products have been cited as cancer-prevention agents, have anti-inflammatory effects, and exhibit biocidal activity against plant pathogens (Prieto, Lopez, & Simal-Gandara, 2019). The glucosinolate metabolites, such as isothiocyanates, are also important as they contribute to the pungent and bitter taste as well as sulfurous aroma of food products (Prieto, Lopez, & Simal-Gandara, 2019).

Vitamin C is an important micronutrient linked to healthy skin, gums, and blood vessels as well as increased iron absorption (Lee & Kader, 2000). Its consumption is necessary for the prevention of scurvy, and vitamin C exhibits antioxidant activity as well as reduces the risk of cardiovascular disease, arteriosclerosis, and some cancers. However, the vitamin C content of cabbage is also greatly affected by exposure to heat, light, and physical damage (Pathy, 2018).

Anthocyanins are phenolic compounds responsible for the red, purple, and blue color hues in many plants. Anthocyanins possess numerous health benefits such as reducing the risk of

cardiovascular disease and cancer as well as protecting DNA against damage from oxidative agents (Charron et al., 2007). An emerging topic in the food industry is food-waste valorization. Up to 45% of food is wasted in the food supply chain, so there is interest in extracting natural compounds from spent food to use for other purposes, such as food colorants (Cecilia et al., 2019). Anthocyanins, particularly in the acylated form, are not as susceptible to degradation as other cabbage compounds (Wiczkowski et al., 2013), so there is potential that anthocyanins may remain high even after cabbage spoilage and can be extracted and utilized.

The objective of this study was to determine how varying durations of mild storage temperature abuse that cabbages could be exposed to along the logistics chain impact the glucosinolate, anthocyanin, and L-ascorbic acid content as well as the color characteristics and PPO activity of cabbages. Additionally, both whole cabbages and shredded cabbages packed under MAP were analyzed to determine the role the physical form of the cabbage may have on quality degradation due to temperature abuse. Red cabbage anthocyanins were analyzed in fresh and spoiled samples to evaluate whether anthocyanins could potentially be extracted from wasted cabbages and used as food colorants.

3.2 Materials and Methods

3.2.1 Cabbage acquisition and processing

Fresh cabbages were purchased from Publix Supermarket in Watkinsville, Georgia. Four different groups of cabbage were tested: whole green cabbage, shredded green cabbage, whole red cabbage, and shredded red cabbage. For shredded cabbage samples, whole cabbages were obtained the day prior to processing and temperature abuse. For the whole heads of cabbage, the cabbages were obtained the morning of the start of the temperature abuse cycle.

Shredded cabbages were shredded using a commercial food chopper (HOBART, Troy, OH) and rinsed with 80 ppm PAA (Pace International, Wapato, WA) for 1 min. The PAA was drained off the cabbage using a salad spinner (Farberware Professional Salad Spinner, Lifetime Brands Inc) and 750 g of shredded cabbage was packed in CN545 polymeric packaging material (Sealed Air, Charlotte, NC). The bags were flushed with an 80% N_2, 15% CO_2, and 5% O_2 gas mixture (Airgas, Radnor, PA) using a vacuum packaging machine (Original Henkelman Vacuum Systems, Type 600, No 60009042, The Netherlands); the bags were under a vacuum for 15 s, backflushed for 17 s, then sealed.

3.2.2 Storage cycle

For each storge cycle, cabbages were held at the temperature abuse conditions outlined in Table 3.1. For both the whole and shredded cabbages, three cabbages from each temperature abuse condition were sampled on days 0, 7, 14, and 21 following temperature abuse. Three individual cabbages held at the same time and temperature abuse conditions were sampled on each day; the same cabbage was not sampled across all time points. Thus, at each time point, 3 of the control cabbages, 3 of the T8 cabbages, 3 of the T16, and 3 of the T24 cabbages were sampled for a total of 12 cabbages per day. The colorimeter and PPO data were obtained the day the cabbages were taken out of refrigerated storage (on days 0, 7, 14 and 21). For the vitamin C analysis, the cabbages were frozen overnight (-80 °C) then sampled the next day. The samples used for glucosinolate analysis were frozen the day cabbages came out of refrigerated storage, then subsequently freeze dried within the next week using a FreeZone 2.5-L Freeze Dryer (Labconco Corporation, Kansas City, MO). The samples used for the HPLC analysis were frozen (-80 °C) until analysis. On the day of analysis, samples being analyzed were kept on ice to limit degradation. For each shredded

bag of cabbage, the contents were mixed then samples were weighed out from the mixed cabbage. For whole cabbage samples, each head was cut into wedge shapes to ensure material from the inside, middle, and outside of the head were included in each sample. Only green cabbages were examined for L-ascorbic acid and glucosinolate content because this study was focused on the more widely consumed green cabbage as opposed to red cabbage. The color parameters and PPO activity of red cabbage were analyzed for comparison and to determine the potential of extracting anthocyanins to use as colorants after cabbage spoilage.

3.2.3 Materials

Monobasic sodium phosphate, sodium hydroxide pellets, 2,6-dichloroindophenol sodium salt hydrate (98%+ purity), HPLC grade water, acetonitrile, acetone, and sodium acetate trihydrate were obtained from Fisher Scientific (Fair Lawn, NJ). Dibasic sodium phosphate was obtained from Honeywell Fluka (Seelze, Germany). Catechol and sinigrin were purchased from TCl America (Portland, OR). Sodium bicarbonate, chloroform, and potassium ferricyanide were purchased from Sigma Aldrich (St. Louis, MO). The USP grade ascorbic acid, methanol, formic acid (88%), hydrochloric acid, meta-phosphoric acid pellets, glacial acetic acid and sodium chloride pellets were obtained from VWR International (Radnor, PA).

3.2.4 Colorimetric Parameters

A calibrated Aeros Benchtop Colorimeter (Model A60-1018-195 ver 1.0, HunterLab, Reston, VA) was used to obtain L*a*b* values for the cabbage samples. Each cabbage sample was individually loaded onto the sample tray until the bottom of the tray was uniformly covered. Whole cabbages were first cut into small pieces to fit in the tray while shredded cabbages were loaded

directly into the tray. The hue angle, which represents color tint, was then calculated as $\tan^{-1}(b/a)$ when a > 0 or $180 + \tan^{-1}(b/a)$ when a < 0. Chroma was measured as $\sqrt{a^2 + b^2}$ and reflects the color saturation, with higher chroma values indicating more saturated colors.

3.2.5 Vitamin C (L-ascorbic acid) quantification

Vitamin C in the form of L-ascorbic acid was analyzed using the Association of Analytical Chemistry Method 967.21 (Ascorbic acid in vitamin preparations and juices: 2,6-dichloroindophenol titrimetric method) as outlined in Li (2013). Only green cabbage samples were analyzed because green cabbage is more widely consumed than red cabbage and was the focus of health parameters in this study. The 2,6-dichloroindophenol reagent (98%+ purity) was standardized by pipetting 5 mL of a *meta*-phosphoric acid (3% w/v)- acetic acid (8% v/v) reagent into a 50 mL Erlenmeyer flask followed by 1 mL of ascorbic acid standard solution. The solution was titrated with 2,6-dichloroindopenol solution until a light-pink color was produced that lasted at least 5 s. The standardization was conducted in triplicate. Three blanks were also prepared containing 5 mL of *meta*-phosphoric acid-acetic acid and 1 mL distilled water. For each cabbage sample, 10 g of cabbage were placed in a mortar along with approximately 5 mL of *meta*-phosphoric acid-acetic acid reagent. The cabbage was ground until it had a consistency similar to apple sauce. Then the solution was filtered through a glass funnel containing glass wool into a Pyrex 100 mL Class A low-actinic red volumetric flask. The flask was filled to volume with *meta*-phosphoric acid-acetic acid and inverted three times. 30 mL aliquots were then transferred to a 125 mL Erlenmeyer flask and titrated with 2,6-dichloroindophenol solution until a light-pink color was produced that lasted at least 5 s. The quantity of added ascorbic acid was then calculated using the following equation:

$$\textbf{mg ascorbic acid/g} = (X-B) \times (F/E) \times (V/Y)$$

where X = average mL for cabbage solution titration, B = average mL for blank titration, F = mg ascorbic acid equivalent to 1.0 mL indophenol standard solution, E = g of cabbage assayed, V = volume initial test solution, and Y = volume test solution titrated.

3.2.6 PPO activity

PPO activity was determined using the method outlined by Li et al. (2020) with minor modifications. PPO activity was recorded for both green and red cabbages in the whole and shredded form. Ten grams of cabbage were homogenized in cold (4 °C) sodium phosphate buffer (0.1 M, pH 6.8) in a 1.5-cup mini food chopper (Mainstays, Las Vegas, USA). The cabbage solution was blended for 15 s, the sides of the chopper were scraped to remove adhered cabbage, then the chopper was run again for 30 s; short bursts reduced overheating of the enzyme extract. The puree was then filtered through a Büchner funnel lined with Whatman No. 4 filter paper (Whatman International Ltd, Maidstone, England) and brought to 25 mL in a volumetric flask using cold sodium phosphate buffer; flasks were kept on ice until analysis (< 30 min).

A 120 µL aliquot of the extracted filtrate was combined in a quartz cuvette with 2.88 mL of catechol solution (0.2 M catechol in 0.1 M sodium phosphate buffer). A spectrophotometer (Agilent 8453 diode array spectrophotometer, Agilent Technologies, Wilmington, DE) equipped with a TC 1 Temperature Controller (Quantum Northwest, USA) was set to stir at 1200 rpm. The spectrophotometer was blanked with sodium phosphate buffer and the absorbance at 412 nm was measured for 1 min in 15 s intervals. The activity unit (U) was defined as an increase of 0.001 in absorbance per one min.

3.2.7 HPLC of anthocyanins

Cabbage anthocyanin composition was determined using the HPLC method outlined in Ahmadiani et al. (2014). A 30 g sample of cabbage was frozen at -80 °C until analysis. Whole heads of cabbage were cut into fine shreds using scissors while the shredded cabbage was analyzed as is. The frozen cabbage was combined with 30 mL of acetone and put on a wrist action shaker (Burrell Scientific, Model 75, Pittsburgh, PA) for 15 min. The solution was then vacuum filtered through Whatman No. 1 filter paper. The extraction was repeated two more times for a total of 90 mL of filtrate collected. The remaining cabbage was then washed with 100 mL of 70% aqueous acidified acetone (0.1% formic acid) until the cabbage was white in color. The filtrates were then combined, transferred to a 500 mL separatory funnel, and mixed with 190 mL of chloroform. The separatory funnel was inverted and swirled three times, then the organic and aqueous phases were separated overnight.

The next day, the aqueous phase containing the anthocyanins was collected and evaporated at 38 °C using a Büchi Rotovapor R-210 equipped with a V-700 vacuum pump connected to a V-850 vacuum controller (Büchi Corporation, New Castle, DE). After evaporation, the aqueous extract was passed through an AccuBond II ODS-C18 cartridge (Agilent Technologies, United Kingdom). The cartridge was activated with 5 mL of methanol and washed with 5 mL of acidified water (0.01% HCl) before loading 2.5 mL of the sample. The sample was then washed with 3 mL of distilled water and eluted with 3 mL of acidified methanol (0.01% HCl). One milliliter of the sample was then filtered through a 3 mL syringe (Monoject, Covidien, Mansfield, MA) with a 0.2 µm regenerated cellulose syringe filter attached (Phenomenex, Torrance, CA) into a 2 mL amber HPLC vial (Agilent Technologies, USA).

An Agilent 1260 Infinity II UPLC system consisting of an Agilent 1260 Infinity II quaternary pump with internal degasser, autosampler, and diode array detector was used. The column was a Kinetex 2.6 µm F5 100 Å LC Column size 150 x 4.6 mm (Phenomenex, Torrance, CA). Phase A was 5% formic acid in HPLC grade water and Phase B was HPLC Grade acetonitrile. The following gradient was employed: 0-3 min 97% A and 3% B, 3-8 min 92% A and 8% B, 8-15 min 92-91% A and 8-9% B, 15-20 min 91-80% A and 9-20% B, 20-32 min 80-65% A and 20-35% B, 32-45 min 65-97% A and 35-3% B, 45-60 min 97% A and 3% B. The column oven was kept at 45 °C, the injection volume was 40 µL, and the flow rate was 0.8 mL/min. Elution of anthocyanins was monitored at 520 nm, which was confirmed to be the absorbance maxima in preliminary studies that recorded the wavelengths of the anthocyanin extracts using a spectrophotometer. An external calibration curve was prepared using cyanidin-3-*O*-glucoside chloride (Indofine Chemical Company, Hillsborough, NJ) at concentrations of 5, 25, 50, 100, 150, 200, 250, 300, 350, and 400 µg/mL.

3.2.8 Glucosinolate content

Glucosinolate content was only recorded for green cabbages. The method outlined in Gallaher, Gallaher, and Peterson (2012) was followed. A 0.2 g portion of freeze-dried cabbage was added to a PYREX 15 mL screw cap culture tube (Corning Incorporated, Corning, NY) and heated in an Aqua Pro Shaking Water Bath (Grant Instruments Ltd, Cambridge, UK) at 80 °C and 50 rpm. 4.5 mL of methanol were added to the tubes, which were then capped and heated for 25 min in the shaking water bath. At the same time, water was brought to a boil on a hot plate. After 25 min, the tubes were uncapped, 1 mL of boiling water was added, the tubes were vortexed, re-capped, and then the tubes were returned to the shaking water bath for 30 min.

The samples were then removed from the water bath, cooled to room temperature, and homogenized using a 150 Handheld Homogenizer (Fisher Scientific, Fair Lawn, NJ) equipped with a plastic disposable homogenizer probe (Fisher Scientific, Fair Lawn, NJ). The samples were transferred to 15 mL centrifuge tubes (VWR International, Radnor, PA) and centrifuged for 20 min at 1800g in an accuSpin 1R Centrifuge (Fisher Scientific, Germany). The supernatant was transferred to a small beaker, 2.5 mL of 90% (v/v) methanol were added back to the cabbage extract in the centrifuge tube, and the samples were centrifuged again for 20 min at 1800g. This was repeated until the supernatants had been pooled three times. The supernatants were then transferred to 11 mL glass test tubes through a glass funnel lined with Whatman No. 1 filter paper. The samples were dried under nitrogen gas using a N-EVAP 111 (Organomation Associates Inc, Berlin, MA) equipped with nitrogen gas (Airgas, Radnor, PA). Once evaporation was complete, the glass tubes were covered with film, wrapped in aluminum foil, and transferred to the freezer.

Within the next three days, the sample was resuspended in 3 mL of distilled water and vortexed. The samples were then run through a Supelco Supleclean LC-SAX SPE Tube (Sigma Aldrich, St. Louis, MO) to isolate the glucosinolate extract. The column was activated using 3 mL of methanol, followed by 3 mL of distilled water, 2.5 mL of sodium acetate (0.5 M, pH = 4.6), then 2.5 mL of distilled water. The 3 mL samples were then filtered using a Monject 3 mL syringe (Covidien, Mansfield, MA) plugged with glass wool. After the sample was added, the glass tube containing the sample was rinsed with 2.5 mL of distilled water and the rinse was applied to the syringe containing glass wool. The column was rinsed with another 2.5 mL of distilled water, and the glucosinolates were then eluted with 3 mL of 0.5 M sodium chloride. Once the glucosinolates were eluted, 2 mL of 2 M NaOH were added to each sample to hydrolyze the glucosinolates. After 30 min, samples were neutralized by adding 310 µL of HCl.

The spectrophotometer was equipped with a TC 1 Temperature Controller set to stir at 1200 rpm. The absorbance was set at 420 nm and blanked using sodium phosphate buffer (0.4 M, pH =7.0). For each sample, 1.5 mL of the neutralized glucosinolate extract was combined with 1.5 mL of 2 mM potassium ferricyanide in sodium phosphate buffer (0.4 M, pH =7.0) in a quartz cuvette. The absorbance was recorded 2 min after the addition of the potassium ferricyanide solution.

A standard curve was prepared as follows: 5 mg of sinigrin were diluted in 1 mL of distilled water. A 0.5 mL aliquot of the sinigrin solution was then combined with 0.5 mL of 2 M NaOH, incubated for 30 min, and then 77 µL of HCl were added. The sinigrin stock solution was then added in volumes of 0, 25, 50, 75, 100, and 125 µL and brought to 1.5 mL with 1 M NaOH. The standard solution was then combined with 1.5 mL of 2 mM potassium ferricyanide in sodium phosphate buffer (0.4 M, pH =7.0) and the absorbance at 420 nm was recorded 2 min later.

3.2.9 Statistical analysis

The effects of shredding, temperature abuse, and duration of refrigerated storage for each quality parameter were analyzed using a main effects model 3-Way ANOVA. For each type of cabbage (green whole/ green shredded/ red whole/ red shredded) the effects of duration of temperature abuse and refrigerated storage were analyzed using a 2-Way ANOVA including the interaction term. Tukey's post hoc analysis at a confidence level of 95% was employed to determine which specific temperature abuse and storage time groups were significantly different from one another.

3.3 Results and Discussion

3.3.1 Visual spoilage characteristics

For the whole heads of cabbage, there were no obvious visible signs of spoilage throughout the study regardless of storage temperature abuse or duration of storage. For the shredded red cabbages, the first T24 sample exhibited spoilage characteristics by day 7, with more samples spoiling on day 14 and day 21 (see Figure 3.1). On day 14, one of the T16 samples was spoiled and two of the T24 samples were spoiled. On day 21, two of both the T16 and T24 shredded red cabbage samples had spoiled. Spoilage characteristics included loss of structural integrity, collection of liquid in the bag, color changes from dark purple to hot pink, and a pungent aroma. The shredded green cabbages underwent less drastic spoilage (see Figure 3.2). The green shredded cabbage spoilage was characterized by loss of structural firmness, the formation of a slimy texture on the shredded cabbage surface, and a putrid aroma when the bags were opened. The green shredded cabbages also spoiled at later storage times. For the green shredded cabbages, one of the T24 cabbages was spoiled on day 14, and both the T16 and T24 cabbages had spoiled samples by day 21.

3.3.2 Colorimetric parameters

L* values measure the lightness of a sample on a scale of 0-100, with higher L* values being lighter in color. The L* values of the whole green cabbages (Figure 3.3) were significantly higher than those of the shredded green cabbages ($P < 0.001$). For the whole green cabbages, the average L* values ranged from 75.95 to 84.12. The control cabbages for the whole green group had higher L* values on days 0 and 7, but the difference was not significant. Additionally, there was no clear relationship between temperature abuse and L* values for the green whole cabbages.

For the green shredded cabbages, the average L* values ranged from 60.60-74.76, indicating the shredded green cabbages were darker in color than the whole heads. The duration of refrigerated storage as well as temperature abuse conditions prior to storage both had a significant effect on the green shredded cabbage. The green, shredded cabbages that were temperature abused for 16 and 24 h had significantly lower L* values than the control cabbages that were kept under refrigeration conditions ($P < 0.05$). Additionally, cabbages sampled on days 0 and 7 had significantly higher L* values than the cabbages sampled on days 14 and 21 ($P < 0.05$). When comparing both temperature abuse and storage time, the T24 sample on day 21 was significantly darker in color in comparison to the other samples ($P < 0.05$). Thus, temperature abuse prior to storage as well as longer durations of storage both resulted in darkening color of the shredded green cabbage samples. These findings correspond to previous studies that also found that L* values of green, shredded cabbage decreased over a 4-week storage period (Ibrahim et al., 2004).

For the red cabbages, the shredded cabbages had higher L* values than the whole cabbages ($P < 0.001$) and thus were brighter in comparison to the whole cabbages (see Figure 3.4). Neither the whole nor the shredded red cabbages varied significantly due to storage time or temperature abuse conditions. The average L* values ranged from 20.23 to 25.72 for the whole red cabbages, and the shredded red cabbages displayed average L* values ranging from 23.38 to 28.65. The T16 and T24 cabbages on days 14 and 21 had lower L* values, but the difference was not significant. This indicates that the temperature abused samples darkened slightly after temperature abuse, but the change was not significant. A previous study (Li et al., 2020) found that L* values of fresh cut purple cabbage decreased significantly over a 20-day storage period. Thus, it was expected that the color of the red cabbages would darken over the duration of storage based on previous literature. A potential reason for this not occurring is that different cabbages were sampled in each

group, so there may have been more variation between individual cabbages than because of temperature abuse or storage duration.

The a* values of green cabbages are shown in Figure 3.5. The a* axis measures how green or red a sample is, with red colors indicated by positive a* values and green colors indicated by negative a* values. The shredded cabbages had lower a* values than the whole cabbages ($P < 0.001$), and all the values were negative for both the whole and shredded green cabbages. This indicates that the shredded cabbages were greener in color than the whole cabbages. There was little variation in the a* values of the whole heads of green cabbage, with all the average a* values ranging from -1.23 to -3.64. There were no significant effects due to storage time or temperature abuse duration on the a* values of the whole heads of green cabbage. On the other hand, a* values of shredded green cabbages were both significantly affected by both temperature abuse duration and storage time. The a* values of the day 0 shredded green cabbages were significantly lower than the day 21 shredded green cabbages ($P < 0.01$). In comparison to the T8 and control cabbages, the T16 cabbages and T24 cabbages had significantly less negative a* values ($P < 0.05$). Thus, the green colors of the whole green cabbages were not affected by temperature abuse or storage, but the shredded green cabbages that were temperature abused and stored for longer durations underwent a loss in green color.

The a* values of red cabbages are shown in Figure 3.6. The shredded cabbages had significantly lower a* values than the whole cabbages ($P < 0.001$), indicating the whole cabbages had more red color in comparison to the shredded cabbages. For both the whole and shredded red cabbages, temperature abuse conditions and duration of storage had no significant effect on the a* values. However, for the shredded cabbages that were temperature abused for 24 h prior to storage, there were large standard deviations on days 14 and 21. This was likely due to variability in

cabbages spoiling. On these days, at least 1 of the 3 cabbages were spoiled while the others were still fresh in quality, resulting in variations in their a* values. The spoiled cabbages changed from purple and white to hot pink in color and had soft tissues (see Figure 3.1). The color change to hot pink in the spoiled cabbages resulted in higher a* values and increased standard deviations. Further research is required to confirm the significance of these findings.

The b* values of green cabbage are shown in Figure 3.7. The b* values represent color on the yellow/blue axis, with positive b* values representing yellow and negative b* values representing blue. The b* values for the green cabbages were lower for the shredded cabbages as compared to the whole heads at the same storage temperature and time conditions ($P < 0.001$). For the whole heads of green cabbages, neither temperature abuse duration nor duration of refrigerated storage had significant effects on the b* values of the cabbages. For the shredded green cabbages, both temperature abuse prior to storage as well as duration of refrigerated storage significantly affected the b* values of the cabbage. The day 0 cabbages had significantly higher b* values than the than the cabbages that were temperature abused for 16 and 24 h on day 21 ($P < 0.05$). The shredded cabbages most likely had decreased b* values on day 21 because the cabbages began to spoil and became duller in color. There was also less variation in the average b* values of the green shredded cabbages than in the whole cabbages. The average b* values of the green shredded cabbages ranged from 13.17 to 18.20, while the average b* values of the whole green cabbages ranged from 15.64 to 28.5.

Figure 3.8 depicts the b* values of the red cabbages. The b* values are all negative, indicating more blue than yellow colors in the red cabbage samples. The whole red cabbages had lower, more negative b* values as compared to the shredded red cabbages ($P < 0.001$), indicating the whole heads of red cabbage had more blue colors than the shredded heads. The average b*

values for the whole red cabbages ranged from -5.46 to -8.52, while the average b* values for the shredded red cabbages ranged from -2.35 to -5.97. There were no significant interactions between day of refrigerated storage or temperature abuse and the b* values of the whole heads of red cabbages. However, the b* values of the shredded red cabbages were significantly affected by duration of storage but not temperature abuse conditions. The day 0 shredded red cabbages had significantly lower b* values than the day 7, 14, and 21 cabbages ($P < 0.05$).

The hue angle and chroma of each group of cabbages were also recorded (see Table 3.2). Hue angle measures the tint of a color while chroma measures the saturation or vividness of the color (Lancaster et al., 1997). Neither hue angle nor chroma showed any clear trends with storage temperature abuse nor duration of refrigerated storage, so the results were divided by type of cabbage rather than by storage day or temperature abuse group. For the green cabbages, the hue angle was about 178.5°, which represents a green color tint. Red cabbage hue angles for all groups were 359.5°, indicating purple-violet color tint. There were no significant differences in chroma based on temperature abuse or storage time. However, whole cabbages had higher chroma values and thus more vibrant colors in comparison to shredded cabbages for both the green and red cabbages. The chroma was highest for the green whole cabbages and lowest for the red shredded cabbages, indicating the whole green cabbages had the most vibrant colors while the red shredded cabbages had the least vibrant colors of the cabbages analyzed in this study.

Overall, both the red and green whole cabbages did not exhibit fluctuations in L*, a*, or b* values throughout the duration of the study regardless of temperature abuse or subsequent refrigerated storage duration. The L* and b* values of the shredded green cabbages were significantly affected by temperature abuse for 24 h prior to refrigerated storage ($P < 0.05$). For the shredded red cabbages, the L*a*b* values did not exhibit any significant relationships with

storage temperature abuse or storage duration. However, the T24 samples on day 21 did exhibit a decrease in brightness and a spike in a* value. Thus, the green shredded cabbages were the most susceptible to colorimetric degradation from temperature abuse while both the green and red whole cabbages changed little in their color parameters throughout this study.

A previous study conducted by Phungamngoen, Chiewchan, & Devhastin (2013) found that fresh cabbage had an L* value of 62.01, a* value of -3.8, and b* value of 9.8. Steam blanching resulted in a reduction of the L*, a*, and b* values to 59, -2.8, and 6.0 respectively. Thus, it was expected that heat treatment would reduce the L*a*b* values of the green cabbage. This trend was also observed in the T24 green shredded cabbages on day 21 of this study.

3.3.3 L-ascorbic acid content

The L-ascorbic acid contents of the green cabbages are shown in Figure 3.9. The shredded cabbages had significantly lower amounts of L-ascorbic acid than the whole cabbages ($P < 0.01$). For whole heads of green cabbages, the duration of temperature abuse and refrigerated storage did not significantly affect the L-ascorbic acid content of the cabbages. However, the shredded cabbages did exhibit significant interactions for all the temperature abuse conditions when compared to the T24 temperature abuse condition on day 14 ($P < 0.001$).

An unexpected finding was that the shredded day 14 cabbages that were temperature abused for 24 h had lower L-ascorbic acid contents on day 14 than on day 21. This is likely due to individual cabbages being analyzed on each day of the study. Had the same cabbage samples been analyzed at both consecutive storage points, the L-ascorbic acid content likely would have decreased on day 21 for the T24 group. The increase is likely due to variations between the individual cabbages and not the temperature abuse or storage conditions. However, the T24

cabbages still showed notable decreases in L-ascorbic acid content by day 21; the losses just varied slightly based on the cabbages sampled. Therefore, the results still indicate that the T24 samples underwent larger losses of L-ascorbic acid, and that storage temperature abuse negatively impacts the vitamin C content of shredded cabbages.

The L-ascorbic acid content of cabbages found in this study corroborates findings from previous studies. In 2020, Li et al. analyzed the vitamin C content of shredded purple cabbages over a 21 day storage period. The initial vitamin C contents ranged from 24.8-26.3 mg/ 100 g fresh weight cabbage. By the end of storage, vitamin C content ranged from 8.70 mg/100 g fresh weight cabbage to 21.6 mg/100 g fresh weight cabbage. The initial vitamin C contents of the cabbages used in this study were slightly lower than the Li et al. study but still in the ballpark of other studies (Singh et al., 2006; Li et al., 2020). However, the same decrease in vitamin C seen in Li et al. (2020) was also observed within the 21-day storage period of this study. Additionally, the whole cabbages retaining their L-ascorbic acid content throughout the study was expected. Cruciferous vegetables typically undergo lower losses of ascorbic acid during storage as compared to other produce products due to high levels of glutathione and sulfur molecules (Dominguez-Perles et al., 2014).

A weakness of the method used was that the 2,6-dichloroindophenol titrimetric method quantifies L-ascorbic acid, not total vitamin C. L-ascorbic acid is oxidized to dehydroascorbic acid in the presence of high temperatures, light, metals (Fe^{3+}, Ag^{+}, Cu^{2+}), or oxygen (Spinola, Llorent-Martinez, & Castilho, 2014). Dehydroascorbic acid represents less than 10% of total ascorbic acid content in fresh produce (Spinola, Llorent-Martinez, & Castilho, 2014). Thus, the L-ascorbic acid content of the cabbages calculated in this paper is less than the total vitamin C content. Alternative methods to measure total vitamin C include the microfluorometric method (AOAC Method

967.22) or HPLC. AOAC Method 967.22 uses a microfluorometric method where all the L-ascorbic acid in the sample is oxidized to L-dehydroascorbic acid then combined with *o*-phenylenediamine to produce a fluorescing compound, allowing for both dehydroascorbic acid and L-ascorbic acid to be quantified. Additionally, HPLC can be used to detect both the reduced and oxidized forms of ascorbic acid and obtain total vitamin C contents. The titrimetric method was selected because it was less expensive and faster to run in comparison to the other methods, and the cabbage samples could not be stored as storage also degrades vitamin C. However, future studies may want to consider HPLC or the microfluorometric method as an alternative to the one utilized in this research.

3.3.4 PPO activity

PPO activity was defined in arbitrary enzyme activity units (U) that correspond to an increase in absorbance of 0.001. For the whole green cabbages, the control and T8 had about the same PPO activity (Figure 3.10) throughout the duration of the study. Additionally, the T16 and T24 cabbages had higher PPO values in comparison to the control and T8 cabbages throughout the duration of storage for the whole green cabbages ($P < 0.05$). The largest differences between the control and T8 whole cabbages as compared to the T16 and T24 whole cabbages were observed on days 0 and 7.

For the shredded green cabbages, the PPO activity increased slightly over time for the T8, T16, and T24 cabbages. The largest increase was observed in the shredded cabbages that were kept at refrigeration conditions ($P < 0.001$). The cabbages that were temperature abused for 16 and 24 h were expected to undergo a larger increase in PPO activity. One possible explanation for the lower than expected PPO values in the shredded cabbage is that respiration increased with

increasing temperatures (University of California Postharvest Center, 2001), consuming the O_2 in the modified atmosphere environment. Thus, there was less oxygen available to react with polyphenol oxidase, slowing the reaction.

The PPO activities of whole and shredded red cabbages are displayed in Figure 3.11. There was a trend for the shredded cabbages to have higher PPO values than whole cabbages at the same time and temperature abuse conditions ($P < 0.05$). Additionally, for both the whole red cabbages and the shredded red cabbages, temperature abuse conditions significantly affected relative PPO activity, but day of storage did not. For the shredded red cabbages, the T24 cabbages had significantly higher relative PPO activities than the other temperature abuse groups ($P < 0.001$). For the whole red cabbages, the T16 and T24 cabbages had significantly higher PPO values than the T8 and control (refrigerated) cabbages ($P < 0.05$).

For the duration of the study, the PPO values of all the cabbages were lower than previously reported (Li et al., 2020). The increase in PPO activity in the T24 cabbages seen in the red cabbages and in the green, whole cabbages was expected as the 25 °C storage abuse temperature was higher than room temperature but not high enough to inactivate the enzyme. Previous studies have indicated PPO has high thermal stability in cabbage (Fujita et al., 1995), and the optimum temperature for PPO activity in cabbages is 40 °C (Fujita et al., 1995; Nagai & Suzuki, 2001). Therefore, the 25 °C storage abuse temperature in this study did not inactivate the PPO enzyme. Instead, the enzyme activity likely increased during temperature abuse as the temperature was closer to the enzyme's optimum temperature than the refrigeration conditions. Thus, it was expected that the temperature abused samples would have higher PPO values than the control cabbages, especially for the shredded cabbages where the tissues were ruptured, releasing the PPO enzyme. In this study, the whole heads of cabbage both had higher PPO values for the T16 and

T24 cabbages, and the red shredded cabbage samples temperature abused for 24 hours prior to refrigerated storage also exhibited an increase in PPO activity as expected. However, the green shredded cabbages did not show an increase due to storage temperature abuse, with the control group having the highest PPO activity instead.

3.3.5 Anthocyanin content

Anthocyanins were quantified using HPLC (Table 3.3). An external calibration curve was run using cyanidin-3-*O*-glucoside chloride (see Figure 3.12), and anthocyanins were quantified in cyanidin-3-*O*-glucoside equivalents/ 100 g fresh weight cabbage. A chromatogram for the HPLC profile of the whole head is displayed in Figure 3.13, and a chromatogram for the anthocyanin profile of a shredded head is displayed in Figure 3.14. The anthocyanins were tentatively identified using previous research (Ahmadiani et al., 2014). Peaks 1-7 were tentatively identified as cyanidin-3-diglucoside-5-glucoside (Cy-3diG-5G), Cy-3diG-5G + p-coumaric, Cy-3diG-5G + ferulic, Cy-3diG-5G + sinapic, Cy-3diG-5G + ferulic and ferulic, Cy-3diG-5G + sinapic and ferulic, and Cy-3diG-5G + sinapic and sinapic. To confirm these tentative identities, mass spectrometry would need to be run.

For the whole heads of cabbage, the main pigment was tentatively identified as Cy-3diG-5G + p-coumaric. For the shredded cabbages, the main pigment was tentatively identified as Cy-3diG-5G + sinapic. Previous studies also found 7 major anthocyanins with Cy-3diG-5G + p-coumaric and Cy-3diG-5G + sinapic having the highest quantities relative to the other peaks (Zhang, Wang, & Liu, 2016). The results of a 3-way ANOVA evaluating the effects of temperature abuse duration, storage time, and physical form of the cabbage indicated that only the physical form of the cabbage significantly affected the total anthocyanin content ($P < 0.001$). The decline

in anthocyanin content with shredding is clearly displayed by both the decrease in total anthocyanin content before and after shredding as well as by the decrease in total number of anthocyanin peaks between the whole and shredded cabbages. For the whole heads of cabbage, the total number of anthocyanin peaks ranged from 25-33 peaks with an average of 28 anthocyanin pigments per sample, while shredded red cabbages had between 17-22 peaks with an average of 19 anthocyanin pigments per sample. The whole heads of cabbage had larger variations in their anthocyanin content than the shredded cabbages exhibited, as can be seen in the large standard deviations seen for the whole heads of cabbage in Table 3.3.

Previous studies have also indicated that acylated anthocyanins have higher stability to light, heat, and temperature abuse as compared to non-acylated anthocyanins (Giusti & Wrolstrad, 2003). The results from the current study confer with these findings. The acylated anthocyanins in this study did not significantly differ whether the cabbages were temperature abused for 24 h prior to storage or not. Similar findings were also found in previous studies that compared anthocyanin colorants from red cabbage during storage at 25 °C, 40 °C, 60 °C and 80 °C for up to six hours (Dyrby, Westergaard, & Stapelfeldt, 2001). These researchers found only the sample held at 80 °C underwent determinable thermal degradation.

This is important because anthocyanin content remained high even in samples that had physically spoiled. On day 21, the shredded control cabbages remained fresh in appearance while the shredded T24 samples had spoiled, yet the anthocyanin content was comparable regardless of spoilage state. Thus, anthocyanin pigments could potentially be recovered from cabbage waste to use as food colorants, as has been seen in previous studies (Patras, 2019). This could help repurpose cabbages and reduce food waste. The advantages of using red cabbage waste as food colorants include stability over larger pH intervals than other natural dye sources such as

blackcurrant, good stability during refrigerated storage, and better heat stability than colorants extracted from other foods such as grapes or cranberries (Patras, 2019). The increased stability of red cabbage anthocyanins as opposed to other food sources is due to the complex acylated cyanidin derivatives that exist within red cabbage (Patras, 2019).

3.3.6 Glucosinolate content

Glucosinolate content was recorded in Table 3.4. The glucosinolate contents were lower for the shredded green cabbages as compared to the whole green cabbages at each temperature abuse condition and day of storage ($P < 0.001$). Additionally, the T24 shredded cabbage samples has significantly lower glucosinolate contents than the other temperature abuse conditions the shredded cabbages encountered ($P < 0.05$). The percent decreases in glucosinolate contents in shredded cabbages were also much higher (72-83%) in the cabbages that were temperature abused for 24 h when compared to the cabbages that underwent shorter durations of temperature abuse prior to storage. Thus, shredding resulted in losses of glucosinolates that were then further reduced in shredded cabbages that were temperature abused for 24 h prior to refrigerated storage.

This study covered a research gap in the glucosinolate content of cabbage. Previous studies have found that mild heat treatments such as blanching did not have a significant effect on glucosinolate content in cabbage while cooking and canning processes caused severe degradation of glucosinolates (Oerlemans et al., 2006). However, there was a lack of literature on lower temperature abuse conditions for longer durations of time. Additionally, the optimum temperature for myrosinase, the enzyme that cleaves glucosinolates, was reported as 60 °C in cabbages from previous studies (Yen & Wei, 1993). It is likely there is increased activity of myrosinase in the

shredded cabbages during the storage abuse at room temperature, which accounts for the loss of glucosinolates in the shredded cabbages that were temperature abused for longer durations.

Another study analyzed the effects of ambient air conditions versus refrigeration conditions on cabbage glucosinolates over the course of 7 days and reported losses of 9-26% in total glucosinolate content, but there was no significant difference in glucosinolate contents between the two temperatures (Song & Thornalley, 2007). Furthermore, the study found that shredding resulted in a loss of about 60% of the total glucosinolate content of the cabbages (Song & Thornalley, 2007). The findings from the current study contradict the Song & Thornalley study in that room temperature abuse conditions did cause loss of glucosinolates in the shredded cabbage samples as compared to the refrigerated samples, but the studies corroborate in that both determined glucosinolates were significantly lost due to shredding processes.

3.3.7 Limitations and potential for future research

There are several limitations in this study that should be addressed in future research. First, the study could be improved by using larger sample sizes. Each temperature/time condition had three replicates, but running more samples at each condition could help identify potential outliers and reduce the standard deviations found in this study. Additionally, individual cabbages were sampled over the duration of the 21-day storage period. It would be better to analyze the same cabbage over the duration of the study, so variations could be contributed to the conditions being studied and not due to variations among cabbages. For the shredded cabbages, the T16 and T24 cabbages were spoiled by day 14, so including more time points between day 0 and day 14 could help better account for changes the cabbages underwent before spoilage. The cabbages were also analyzed at different times of the year, which could potentially contribute to some of the variation

in quality characteristics. The vitamin C content and anthocyanin content of the cabbages may also have decreased when the cabbages were put in frozen storage. Previous research has confirmed that cabbage quality characteristics vary based on growing conditions and harvest time (Ahmadiani et al., 2014), so analyzing cabbages from the same growing season would be ideal. Furthermore, obtaining cabbages from the same farm so the soil and growing climate was identical would also strengthen the study.

Lastly, it is imperative that future research be conducted on the microbiological characteristics of cabbage. Previous studies have indicated that pathogens like *Listeria monocytogenes* proliferate in temperature abused cabbage (Ells & Hansen, 2010). Thus, it is possible the cabbages may not undergo losses in quality but will pose a threat to consumer health after temperature abuse.

3.4 Summary and Conclusions

The results of this study emphasized the importance of maintaining cold chain conditions throughout storage and transport, particularly for shredded cabbages. Cabbage is desired for its color, health, and flavor attributes. Shredded green cabbage was the focus of this study as green cabbage is more widely consumed than red cabbage, and there has been a growing market for shredded cabbage due to its convenience. Additionally, shredded cabbage has additional economic value as the further processing steps increase the price of shredded cabbages in comparison to whole cabbages. However, cabbages can be exposed to loss of refrigeration conditions at many points along the logistics chain.

In this study, shredded cabbages that were temperature abused for 24 h prior to storage underwent darkening as well as losses in glucosinolate and L-ascorbic acid contents. Thus,

temperature abuse resulted in loss of color, flavor, and nutritive compounds in the shredded cabbages. These results indicate that the cabbage industry should take additional steps to ensure short holding times on loading docks, proper operation of refrigeration units on transportation trucks, and that retail displays are maintaining refrigerated conditions to preserve the quality of shredded cabbage.

The anthocyanin content of red cabbage was not impacted by storage temperature abuse but did significantly decrease due to shredding ($P < 0.001$). Anthocyanin content remained high even after cabbage spoilage, indicating the potential to collect anthocyanins from spoiled cabbages to use as colorants and help reduce food waste. Future studies could study the effects of storage temperature abuse on the concentrations of individual anthocyanin pigments, as the identities of the pigments were never confirmed in this study.

The whole cabbages were not susceptible to loss of quality attributes following storage temperature abuse at 25 °C for up to 24 h. Thus, field packaging cabbages should not pose a threat to food quality as long as the cabbage heads are not damaged and are not held on unrefrigerated trucks for extended durations longer than 24 h. However, additional steps still need to be taken once the cabbages are shredded to prevent contamination and loss of refrigeration conditions. Further research needs to be done to evaluate the effects of the temperature abuse intervals encountered in this study on the microbiological safety of cabbages. There is potential that temperature abused cabbages may look safe to eat and not undergo determinable loss of quality but may still pose a food safety risk.

References

Agricultural Marketing Resource Center. (2021, October). *Cabbage*. https://www.agmrc.org/commodities-products/vegetables/cabbage.

Ahmadiani, N., Robbins, R. J., Collins, T. M., & Giusti, M. M. (2014). Anthocyanins contents, profiles, and color characteristics of red cabbage extracts from different cultivars and maturity stages. *Journal of Agricultural and Food Chemistry*, *62*(30), 7524–7531. https://doi.org/10.1021/jf501991q.

AOAC International. (2006). AOAC Official Method 967.21, Ascorbic acid in vitamin preparations and juices 2,6-dichloroindophenol titrimetric method, 45.1.14.

AOAC International (2006). AOAC Official Method 967.22, Vitamin C (total) in vitamin preparations microfluorometric method, 45.1.15.

Cecilia, J. A., García-Sancho, C., Maireles-Torres, P. J., & Luque, R. (2019). Industrial food waste valorization: a general overview. *Biorefinery: Integrated Sustainable Processes for Biomass Conversion to Biomaterials, Biofuels, and Fertilizers*, 253-277.

Charron, C. S., Clevidence, B. A., Britz, S. J., & Novotny, J. A. (2007). Effect of dose size on bioavailability of acylated and nonacylated anthocyanins from red cabbage (Brassica oleracea L. Var. capitata). *Journal of Agricultural and Food Chemistry*, *55*(13), 5354–5362.

Domínguez-Perles*, R., Mena*, P., Garcia-Viguera, C., & Moreno, D. A. (2014). Brassica foods as a dietary source of vitamin C: A review. *Critical Reviews in Food Science and Nutrition*, *54*(8), 1076–1091.

Dyrby, M., Westergaard, N., & Stapelfeldt, H. (2001). Light and heat sensitivity of red cabbage

extract in soft drink model systems. *Food Chemistry*, *72*(4), 431–437. https://doi.org/10.1016/S0308-8146(00)00251-X.

Ells, T., Hansen, L.T. (2010). Growth of *Listeria* spp. in shredded cabbage is enhanced by a mild heat treatment. *Journal of Food Protection*, *73*(3), 425–433. https://doi.org/10.4315/0362-028X-73.3.425.

Francis, G. A., Gallone, A., Nychas, G. J., Sofos, J. N., Colelli, G., Amodio, M. L., & Spano, G. (2012). Factors affecting quality and safety of fresh-cut produce. *Critical Reviews in Food Science and Nutrition*, *52*(7), 595–610.

Fujita, S., Saari, N., Maegawa, M., Tetsuka, T., Hayashi, N., & Tono, T. (1995). Purification and properties of polyphenol oxidase from cabbage (Brassica oleracea L.). *Journal of Agricultural and Food Chemistry, 43,* 1138–1142. https://doi.org/10.1021/jf00053a005.

Gallaher, C. M., Gallaher, D. D., & Peterson, S. (2012). Development and validation of a spectrophotometric method for quantification of total glucosinolates in cruciferous vegetables. *Journal of Agricultural and Food Chemistry*, *60*(6), 1358–1362.

Giusti, M. M., & Wrolstad, R. E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*, *14*(3), 217–225.

Ibrahim, R., Osman, A., Saari, N., & Rahman, R. A. (2004). Effects of anti-browning treatments on the storage quality of minimally processed shredded cabbage. *Journal of Food Agriculture and Environment*, *2*, 54–58.

Lancaster, J. E., Lister, C. E., Reay, P. F., & Triggs, C. M. (1997). Influence of pigment composition on skin color in a wide range of fruit and vegetables. *Journal of the American Society for Horticultural Science*, *122*(4), 594–598.

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin

pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *Journal of AOAC International, 88*(5), 1269–1268. https://doi.org/10.1093/jaoac/88.5.1269.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, *20*(3), 207–220.

Li, L. (2013). *Selected nutrient analyses of fresh, fresh-stored, and frozen fruits and vegetables.* [Master's thesis, University of Georgia]. University of Georgia Libraries.

Li, H., Li, X., Wang, R.., Xing, Y., Xu, Q., Shui, Y., Guo, X., Li W., Yang, H., Bi., X., & Che, Z. (2020). Quality of fresh-cut purple cabbage stored at modified atmosphere packaging and cold-chain transportation. *International Journal of Food Properties, 23(1*), 138–153. https://doi.org/10.1080/10942912.2020.1716795.

Moser, R., Raffaelli, R., & Thilmany, D. D. (2011). Consumer preferences for fruit and vegetables with credence-based attributes: A review. *International Food and Agribusiness Management Review*, *14*(1030-2016-82774), 121–142.

Nagai, T., & Suzuki, N. (2001). Partial purification of polyphenol oxidase from Chinese cabbage Brassica rapa L. *Journal of Agricultural and Food Chemistry*, *49*(8), 3922–3926.

Oerlemans, K., Barrett, D. M., Suades, C. B., Verkerk, R., & Dekker, M. (2006). Thermal degradation of glucosinolates in red cabbage. *Food Chemistry, 95*(1), 19–29.

Pathy, K. (2018). Process for preparation of vitamin C and method for determination of vitamin c in tablets. *Journal of Chemical Research*, *2*(1), 2.

Patras, A. (2019). Stability and colour evaluation of red cabbage waste hydroethanolic extract in presence of different food additives or ingredients. *Food Chemistry*, *275*, 539–548.

Phungamngoen, C., Chiewchan, N., & Devahastin, S. (2013). Effects of various pretreatments

and drying methods on Salmonella resistance and physical properties of cabbage. *Journal of Food Engineering*, *115*(2), 237–244.

Prieto, M. A., López, C. J., & Simal-Gandara, J. (2019). Glucosinolates: Molecular structure, breakdown, genetic, bioavailability, properties and healthy and adverse effects. *Advances in Food and Nutrition Research*, *90*, 305–350.

Sandhya. (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. *LWT-Food Science and Technology*, *43*(3), 381–392.

Singh, J., Upadhyay, A. K., Bahadur, A., Singh, B., Singh, K. P., & Rai, M. (2006). Antioxidant phytochemicals in cabbage (Brassica oleracea L. var. capitata). *Scientia Horticulturae*, *108*(3), 233–237.

Song, L., & Thornalley, P. J. (2007). Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food and Chemical Toxicology*, *45*(2), 216–224. https://doi.org/10.1016/j.fct.2006.07.021.

Spínola, V., Llorent-Martínez, E. J., & Castilho, P. C. (2014). Determination of vitamin C in foods: Current state of method validation. *Journal of Chromatography A*, *1369*, 2–17.

University of California Postharvest Center. (2001). *Vegetables produce facts English.* https://postharvest.ucdavis.edu/Commodity_Resources/Fact_Sheets/Datastores/Vegetables_English.

Wiczkowski, W., Szawara-Nowak, D., & Topolska, J. (2015). Changes in the content and composition of anthocyanins in red cabbage and its antioxidant capacity during fermentation, storage and stewing. *Food Chemistry*, *167*, 115–123.

Yen, G. C., & Wei, Q. K. (1993). Myrosinase activity and total glucosinolate content of

cruciferous vegetables, and some properties of cabbage myrosinase in Taiwan. *Journal of the Science of Food and Agriculture*, *61*(4), 471–475.

Zhang, J., Wang, Z., & Liu, X. (2017). Characterization of acylated anthocyanins in red cabbage via comprehensive two-dimensional high performance liquid chromatography and HPLC-MS. *Journal of Food Processing and Preservation*, *41*(2).

Table 3.1: Cabbage temperature abuse conditions

Cabbage Group	Total # Cabbages	Temperature Abuse Conditions
Control (C)	12	Refrigerated at 4°C
T8	12	Temperature abused for 8 h at 25°C then moved to refrigeration conditions (4°C)
T16	12	Temperature abused for 16 h at 25°C then moved to refrigeration conditions (4°C)
T24	12	Temperature abused for 24 h at 25°C then moved to refrigeration conditions (4°C)

Table 3.2: Hue angle and chroma of cabbages

Type of Cabbage	Hue Angle (°)	Chroma
Green Whole	178.53 ± 0.03	21.60 ± 3.75
Green Shredded	178.60 ± 0.10	16.85 ± 1.82
Red Whole	359.57 ± 0.04	15.57 ± 2.03
Red Shredded	359.64 ± 0.10	12.34 ± 2.24

Table 3.3: Anthocyanin content of red cabbages exposed to temperature abuse conditions

Day	Temp Abuse [a]	Type	Peak 1[b,c]	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Total # Peaks	Total Content
0	Control	Whole	2.84 ± 0.63 A	29.50 ± 6.18 A	16.38 ± 3.70 A	24.06 ± 3.14 AB	3.66 ± 0.44 A	2.01 ± 0.28 ABC	3.96 ± 0.12 A	26.33 ± 1.25 A	97.93 ± 19.47 A
0	T24	Whole	3.76 ± 0.47 A	26.62 ± 4.97 A	13.10 ± 1.19 A	20.53 ± 2.19 ABC	3.97 ± 1.40 A	1.74 ± 0.32 ABC	3.52 ± 0.90 A	27.00 ± 1.63 A	88.46 ± 15.45 A
0	Control	Shred	1.27 ± 0.46 A	5.54 ± 0.66 B	8.20 ± 0.72 BC	18.51 ± 1.38 ABC	1.08 ± 0.20 B	1.83 ± 0.17 ABC	5.30 ± 1.01 A	19.67 ± 0.94 B	49.15 ± 2.16 B
0	T24	Shred	2.08 ± 0.53 A	4.66 ± 0.51 B	6.67 ± 0.61 C	15.18 ± 0.88 ABC	0.79 ± 0.09 B	1.27 ± 0.17 BC	3.77 ± 0.56 A	18.67 ±1.25 B	47.32 ± 1.96 B
21	Control	Whole	2.55 ± 1.44 A	29.20 ± 3.57 A	14.27 ± 2.53 A	25.51 ± 4.87 A	5.50 ± 1.62 A	2.55 ± 0.73 A	5.51 ± 1.45 A	28.67 ± 2.49 A	105.93 ± 18.92 A
21	T24	Whole	2.54 ± 0.68 A	27.72 ± 8.30 A	13.33 ± 3.90 AB	21.71 ± 5.43 ABC	5.29 ± 1.59 A	2.49 ± 0.52 AB	5.34 ± 0.97 A	30.67 ± 2.62 A	98.82 ± 2.29 A
21	Control	Shred	1.28 ± 0.75 A	3.95 ± 0.26 B	5.70 ± 0.29 C	12.73 ± 1.35 C	0.85 ± 0.13 B	1.65 ± 0.12 ABC	5.14 ± 1.06 A	18.00 ± 0.82 B	43.65 ± 4.33 B
21	T24	Shred	1.64 ± 0.48 A	4.97 ± 0.92 B	6.98 ± 1.14 C	14.18 ± 1.71 BC	0.65 ± 0.02 B	1.21 ± 0.10 C	3.91 ± 0.50 A	19.00 ± 2.16 B	40.92 ± 4.71 B

[a] Cabbages were exposed to temperature abuse conditions at 25°C for 24 h (T24) or kept under refrigeration conditions of 4°C (control) prior to refrigerated storage.

[b] Peaks were quantified using a cyanidin-3-*O*-glucoside (Cy3G) external standard and are expressed in mg Cy3G equivalents/ 100 grams fresh weight cabbage.

[c] Samples within each column were analyzed using a 3-way ANOVA followed by Tukey's test for post hoc comparison. Significantly different groups ($P < 0.05$) are identified by different letters.

Table 3.4: Glucosinolate contents of whole and shredded green cabbages exposed to temperature abuse conditions then stored for 21 days

Day	Storage Condition	Whole Cabbage Glucosinolates (mg sinigrin/ g dry weight)[b]	Shredded Cabbage Glucosinolates (mg sinigrin/ g dry weight)	% Decrease
0	Control[a]	10.23 ± 1.29 AB	7.04 ± 1.45 ABC	31.18
0	T8	11.74 ± 4.16 AB	8.36 ± 5.03 ABC	28.79
0	T16	11.34 ± 4.65 AB	8.66 ± 1.30 ABC	23.63
0	T24	12.88 ± 2.07 AB	3.60 ± 2.88 BC	72.05
7	Control	18.51 ± 1.10 A	8.66 ± 1.39 ABC	53.21
7	T8	17.63 ± 0.58 AB	10.94 ± 4.13 AB	37.95
7	T16	17.96 ± 1.98 AB	5.67 ± 4.2 ABC	68.43
7	T24	18.73 ± 4.24 A	3.31 ± 1.81 C	82.33
14	Control	14.27 ± 2.36 AB	7.87 ± 0.49 ABC	44.85
14	T8	9.05 ± 2.95 B	5.35 ± 2.35 ABC	40.88
14	T16	8.94 ± 0.24 B	5.39 ± 1.16 ABC	39.71
14	T24	16.84 ± 4.12 AB	2.84 ± 1.12 C	83.14
21	Control	16.84 ± 3.55 AB	12.4 ± 1.15 A	26.37
21	T8	15.36 ± 0.84 AB	6.71 ± 3.12 ABC	56.32
21	T16	17.59 ± 4.09 AB	3.35 ± 0.49 C	80.96
21	T24	18.44 ± 4.79 A	2.61 ± 1.36 C	85.85

[a] Control cabbages were kept at 4°C throughout the study while T8, T16, and T24 cabbages were temperature abused at 25°C for 8, 16, and 24 h respectively then returned to refrigerated storage at the start of the study.

[b] Samples were measured in triplicate and expressed as mg sinigrin per g of dry weight cabbage. Different letters in the same column indicate significant differences ($P < 0.05$).

A)

B)

Figure 3.1: (A) One of the red shredded cabbage samples exposed to 25°C for 24 h began showing signs of spoilage on D7. On day 14, the shredded red cabbages that were exposed to 25°C for 16 h also began to spoil, as seen by the hot pink cabbage on the far right in (B).

Figure 3.2: Shredded green cabbages on day 21 of the storage cycle. The image on the left displays cabbage that did not undergo any temperature abuse prior to storage and was kept in refrigeration conditions (4 °C) throughout the duration of the study. The cabbage on the left was exposed to 25 °C temperature abuse conditions prior to the 21-day refrigerated storage cycle and underwent increased browning and tissue softening.

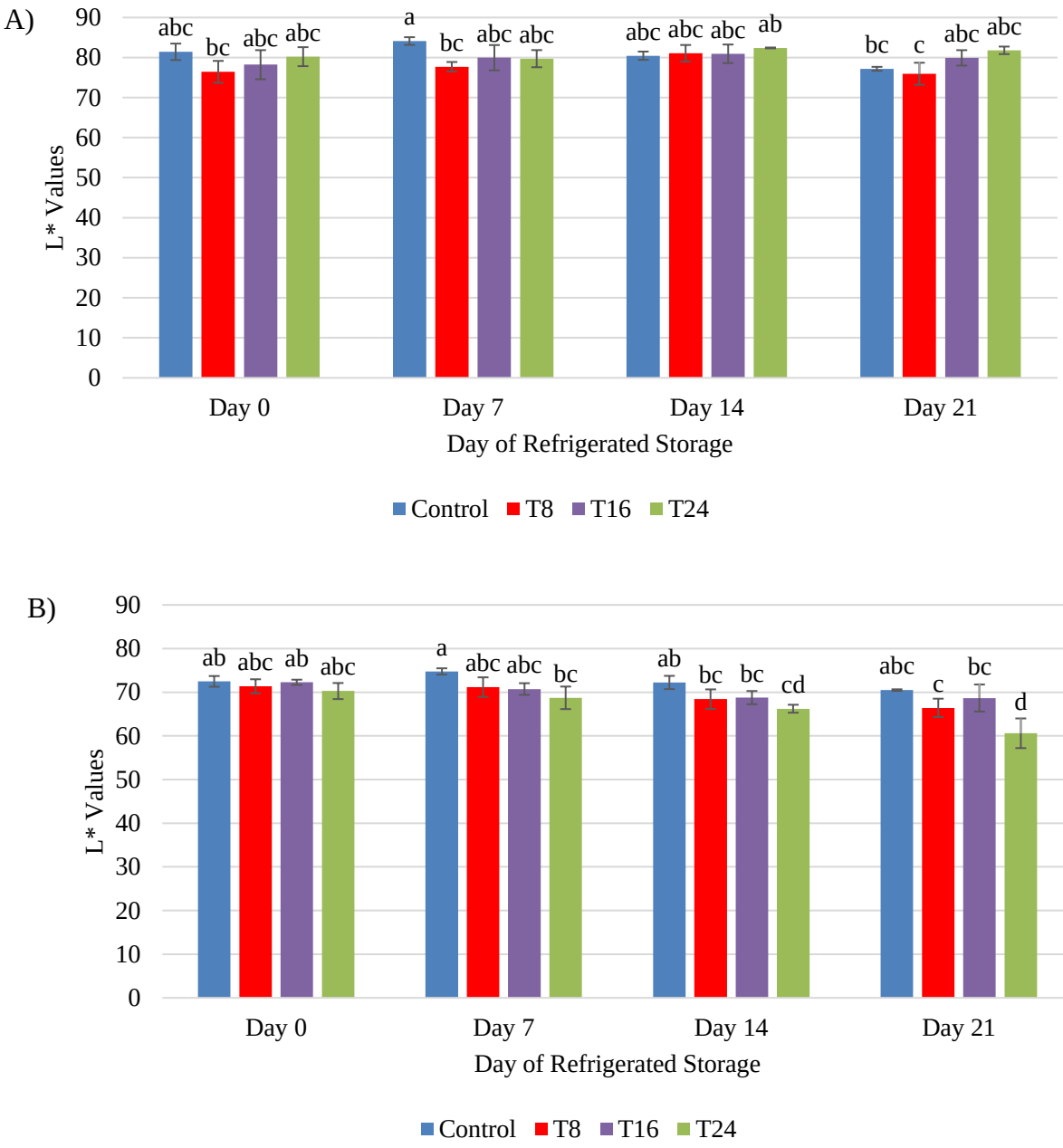


Figure 3.3: L* Values of whole (A) and shredded (B) green cabbages exposed to temperature abuse conditions of 25 °C for 0 (control), 8 (T8), 16 (T16), and 24 (T24) h then stored for 21 days under refrigeration conditions at 4 °C. All measurements were taken in triplicate, with one reading from three individual cabbages held at the same storage conditions. Different letters indicate significant differences ($P < 0.05$).

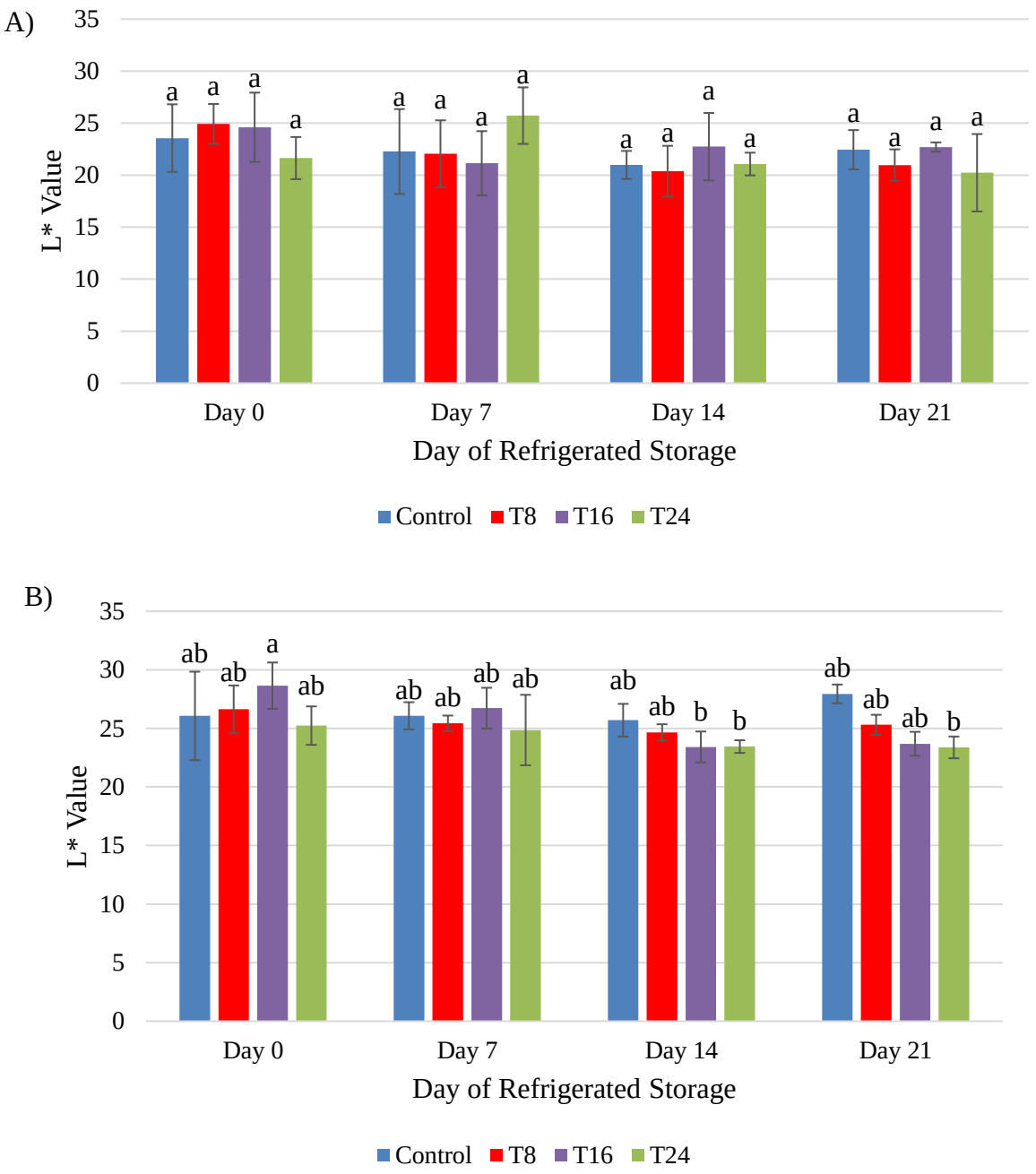


Figure 3.4: L* values of whole (A) and shredded (B) red cabbages exposed to mild temperature abuse conditions then stored for 21 days. The cabbages were exposed to temperature abuse conditions of 25 °C for 0 (control), 8 (T8), 16 (T16), and 24 (T24) h then stored for 21 days under refrigeration conditions at 4 °C. Different letters indicate significant differences ($P < 0.05$).

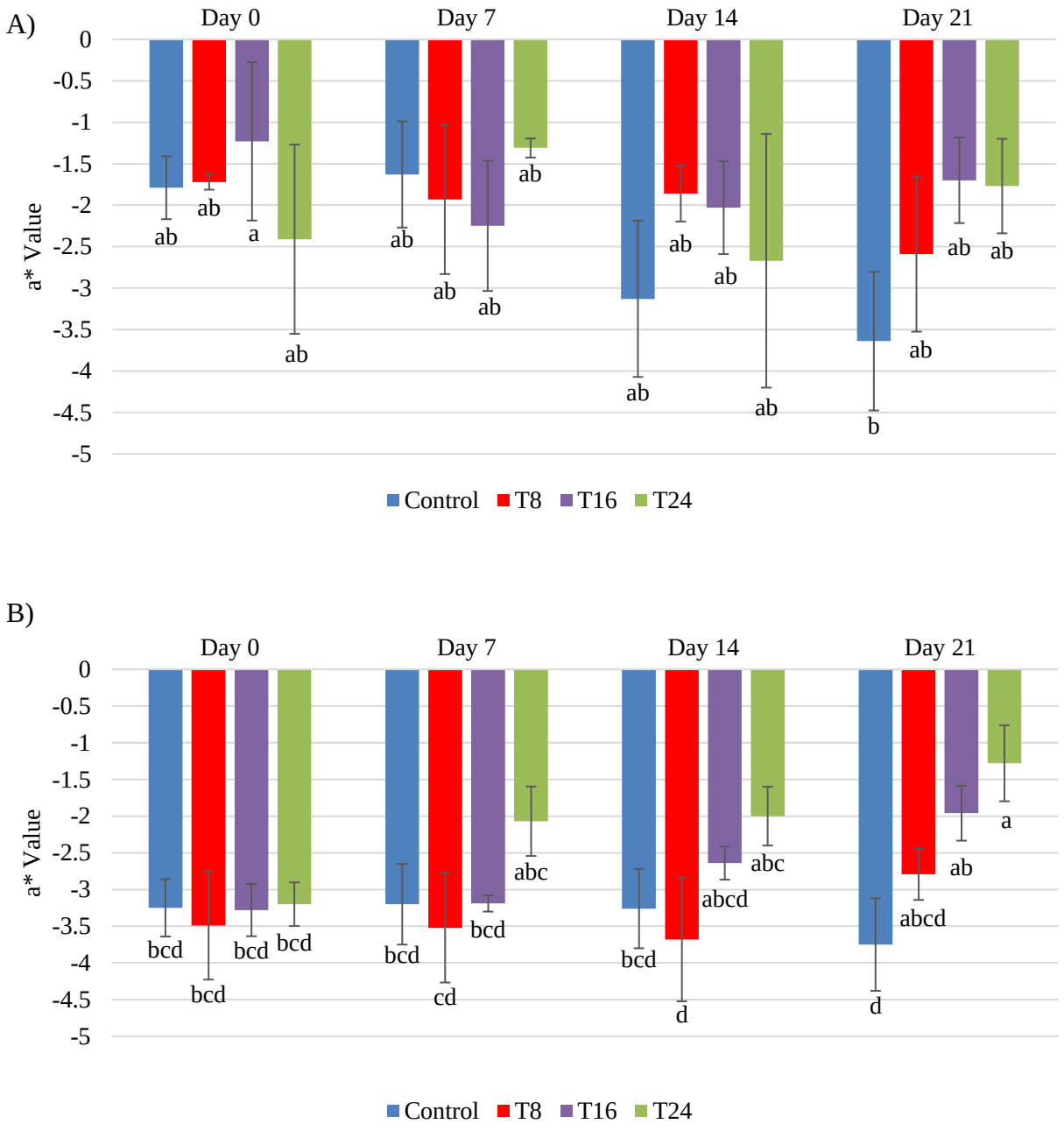


Figure 3.5: The a* values of whole (A) and shredded (B) green cabbages exposed to temperature abuse conditions of 25 °C for 0 (control), 8 (T8), 16 (T16), and 24 (T24) h then stored for 21 days under refrigeration conditions at 4 °C. All measurements were taken in triplicate, with one reading from three individual cabbages held at the same storage conditions. Different letters indicate significant differences ($P < 0.05$).

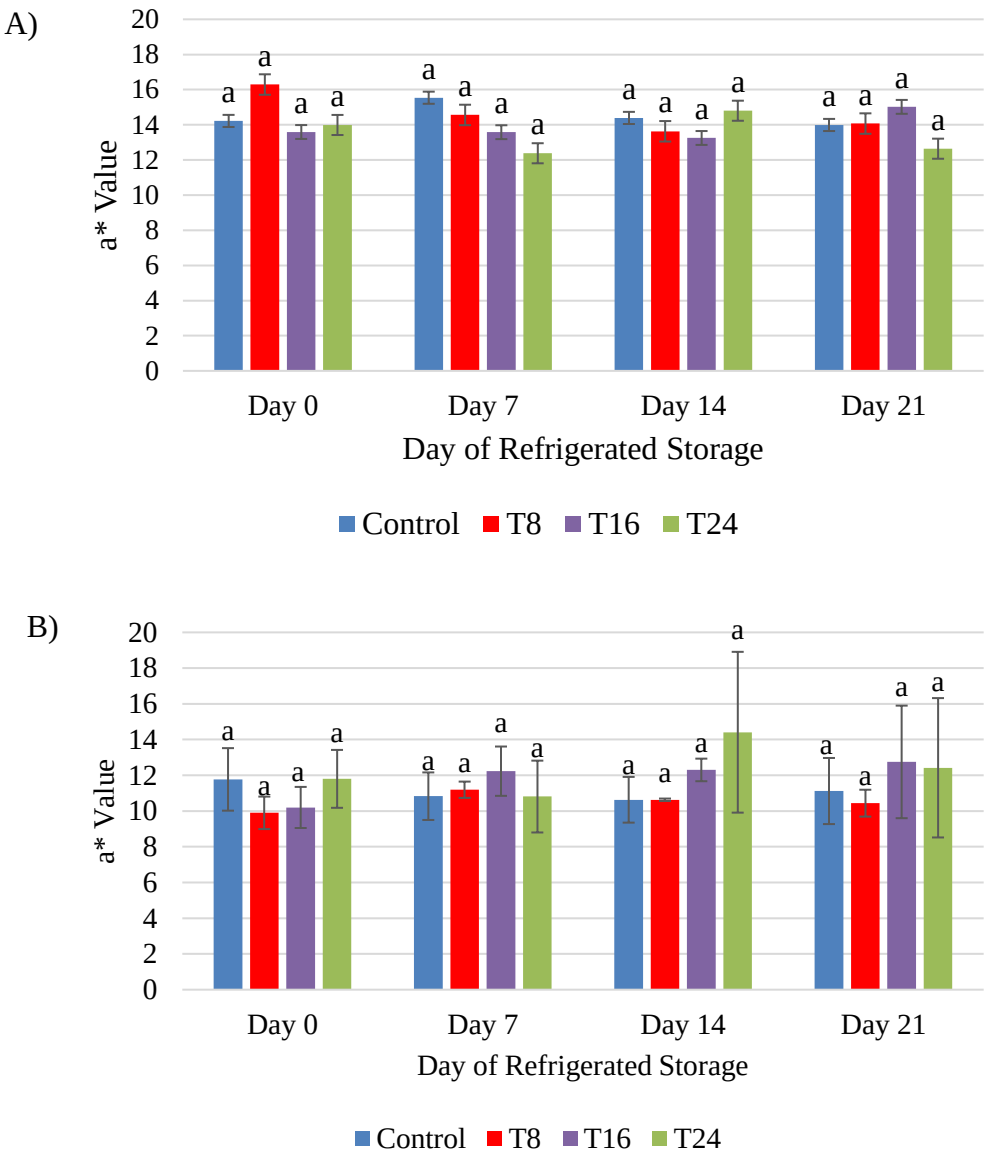


Figure 3.6: The a* values of whole (A) and shredded (B) red cabbages exposed to temperature abuse conditions of 25 °C for 0 (control), 8 (T8), 16 (T16), and 24 (T24) h then stored for 21 days under refrigeration conditions at 4 °C. All measurements were taken in triplicate, with one reading from three individual cabbages held at the same storage conditions. Different letters indicate significant differences ($P < 0.05$).

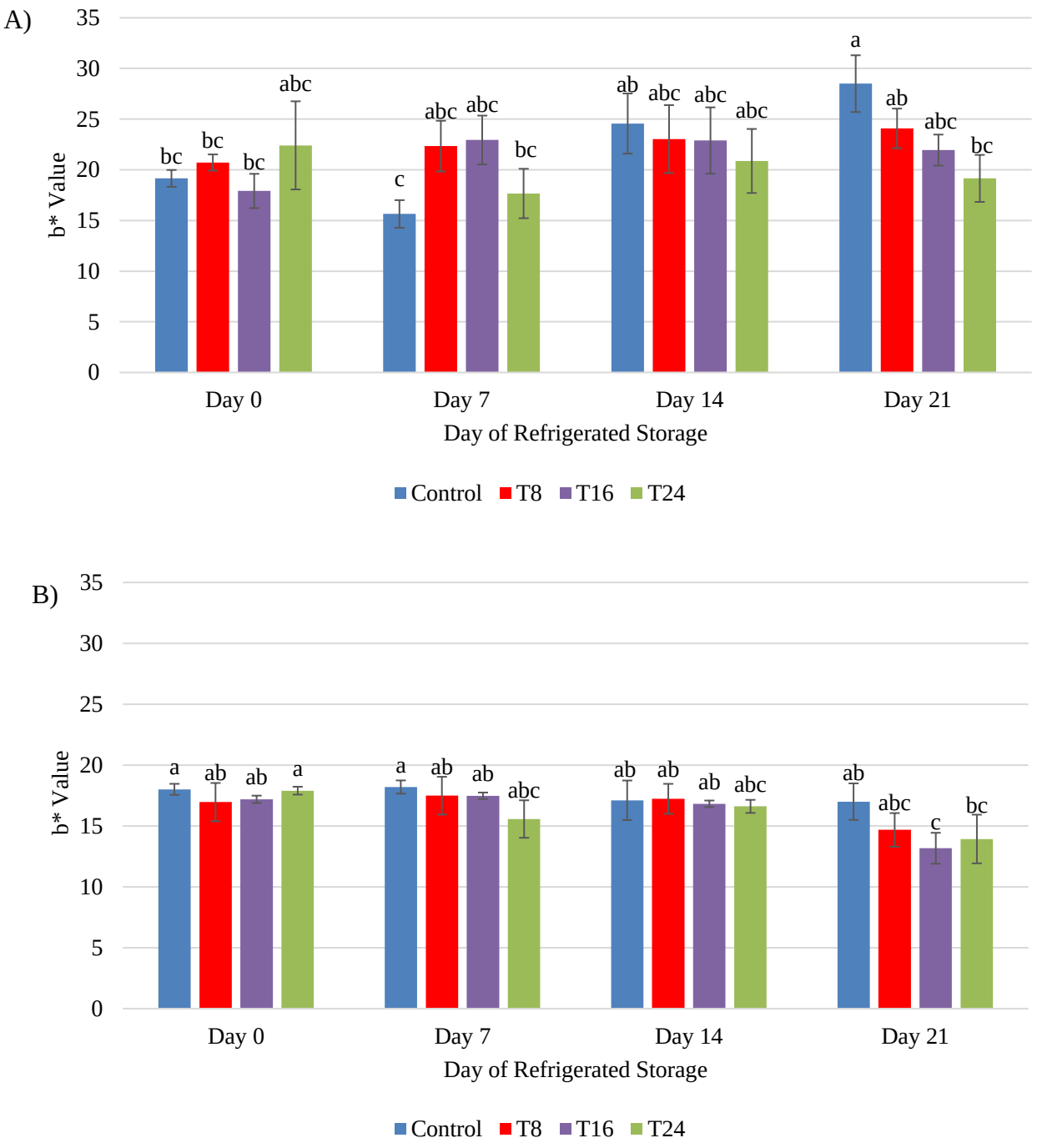


Figure 3.7: The b* values of whole (A) and shredded (B) green cabbages exposed to temperature abuse conditions of 25° C for 0 (control), 8 (T8), 16 (T16), and 24 (T24) h then stored for 21 days under refrigeration conditions at 4°C. Different letters indicate significant differences ($P < 0.05$).

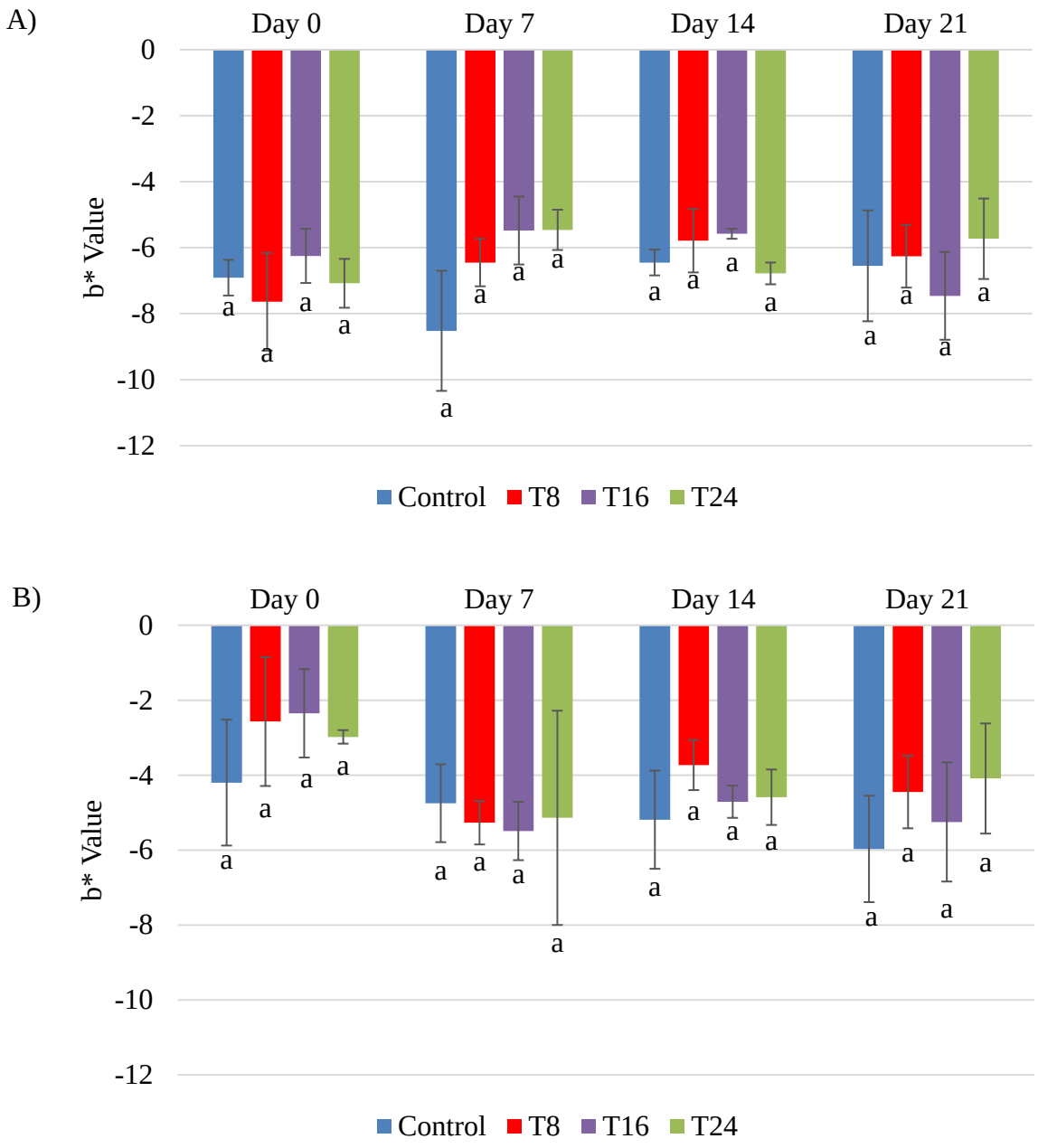
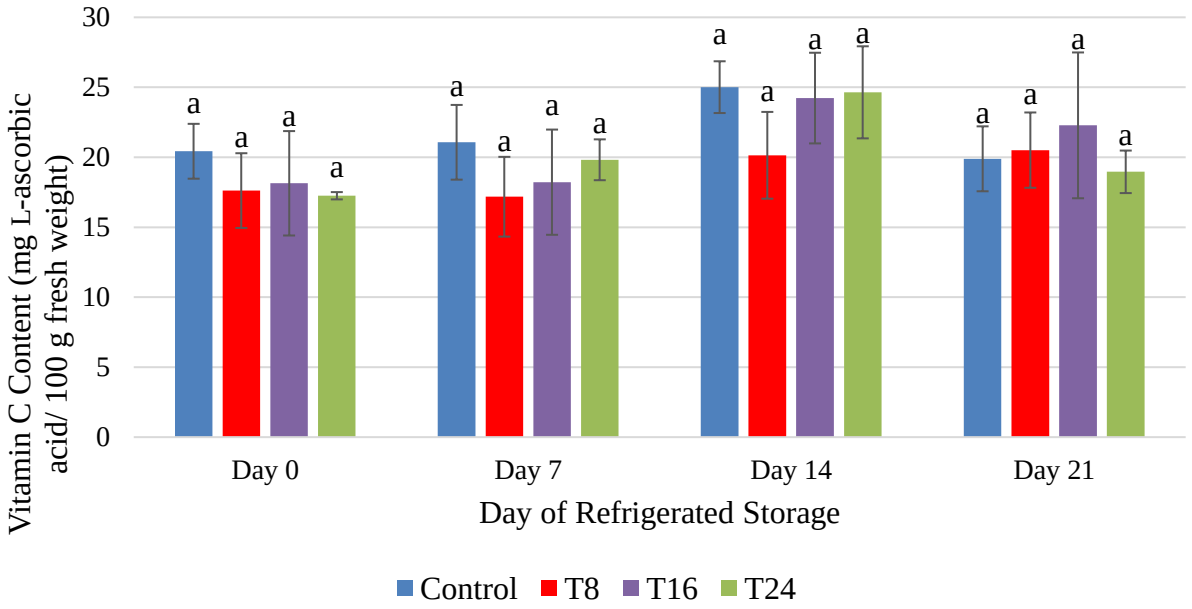


Figure 3.8: The b* values of whole (A) and shredded (B) red cabbages exposed to temperature abuse conditions of 25° C for 0 (control), 8 (T8), 16 (T16), and 24 (T24) h then stored for 21 days under refrigeration conditions at 4°C. All measurements were taken in triplicate, with one reading from three individual cabbages held at the same storage conditions. Different letters indicate significant differences ($P < 0.05$).

A)



B)

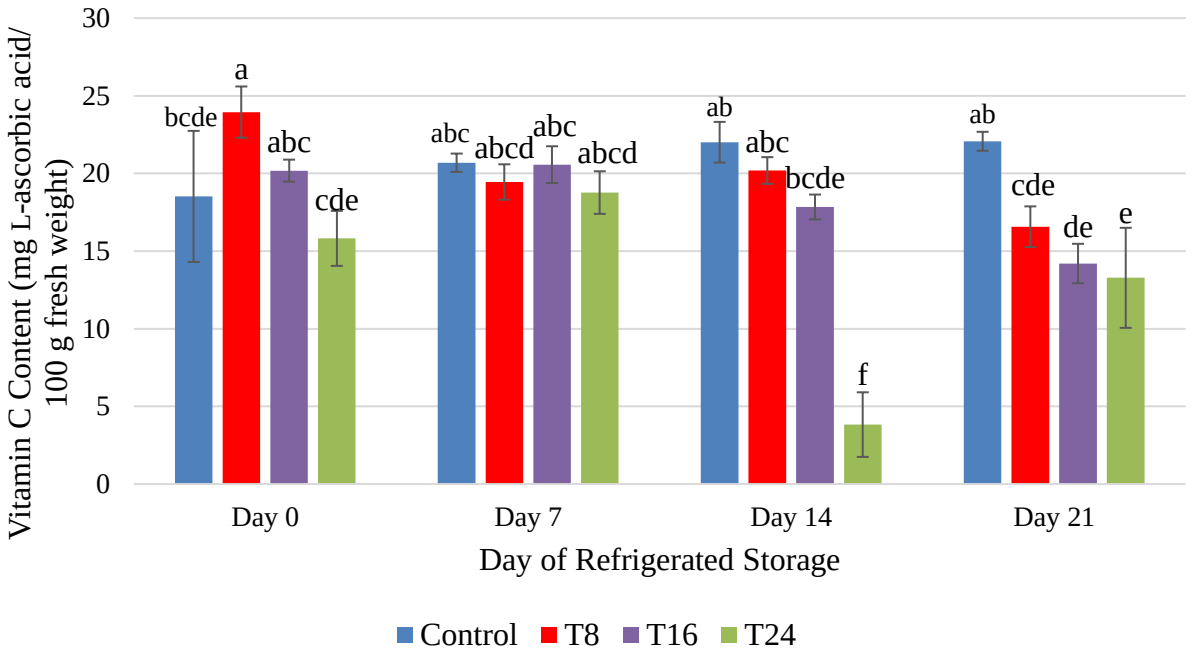
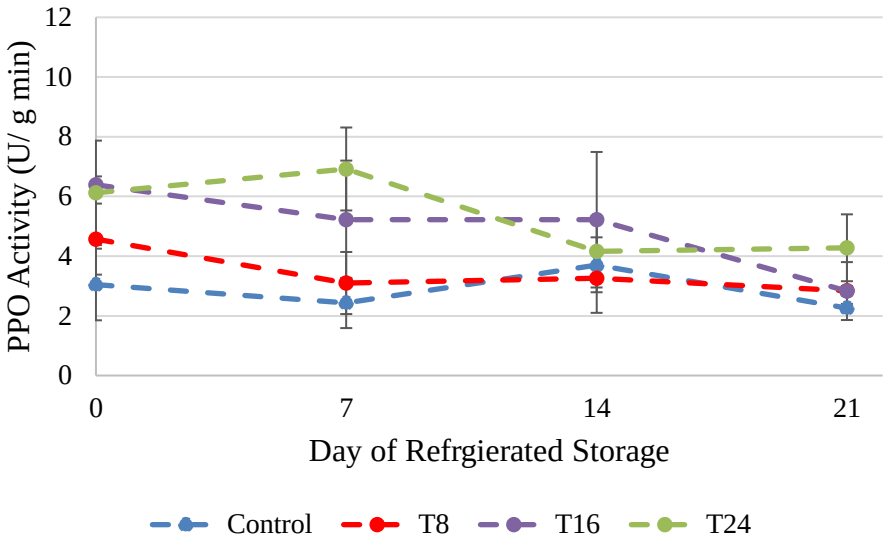


Figure 3.9: L-ascorbic acid content of whole (A) and shredded (B) green cabbages exposed to temperature abuse conditions for 0, 8,16, and 24 h then stored for 21 days at 4°C. Samples were measured in triplicate and expressed as mg L-ascorbic acid per 100 grams of fresh weight cabbage. Different letters indicate significant differences ($P < 0.05$).

A)



B)

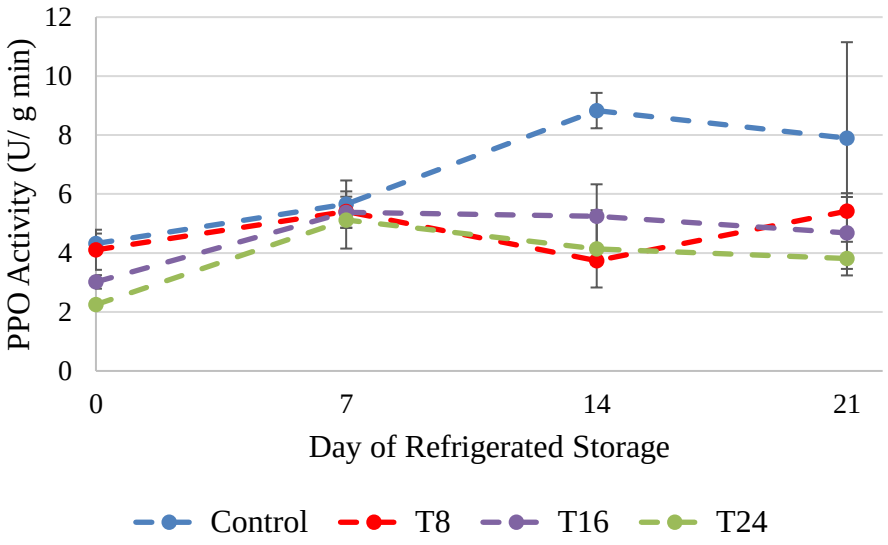
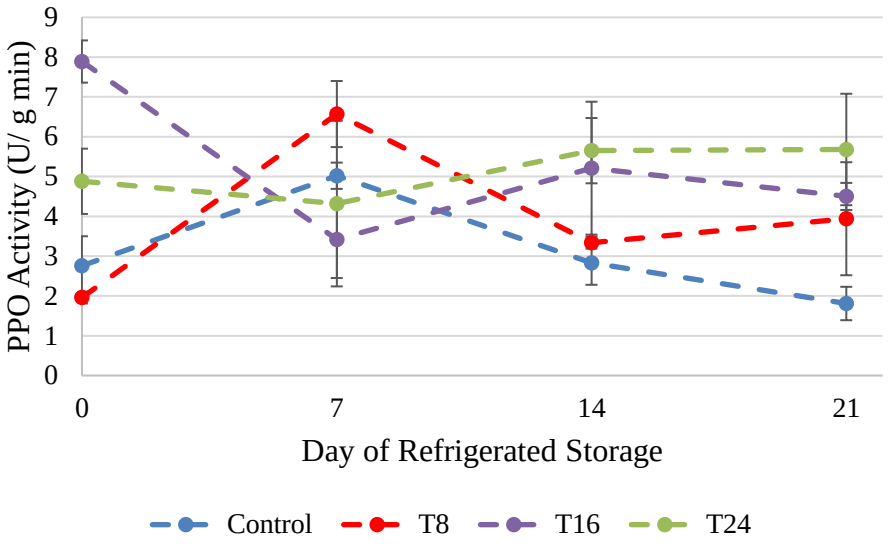


Figure 3.10: Polyphenol oxidase (PPO) activities of whole (A) and shredded (B) green cabbages. Measurements were taken in triplicate from three individual cabbages exposed to the same temperature conditions and storage time. U is an arbitrary enzyme activity until equal to an increase in absorbance of 0.001. Error bars represent standard deviation from three samples at the same conditions.

A)



B)

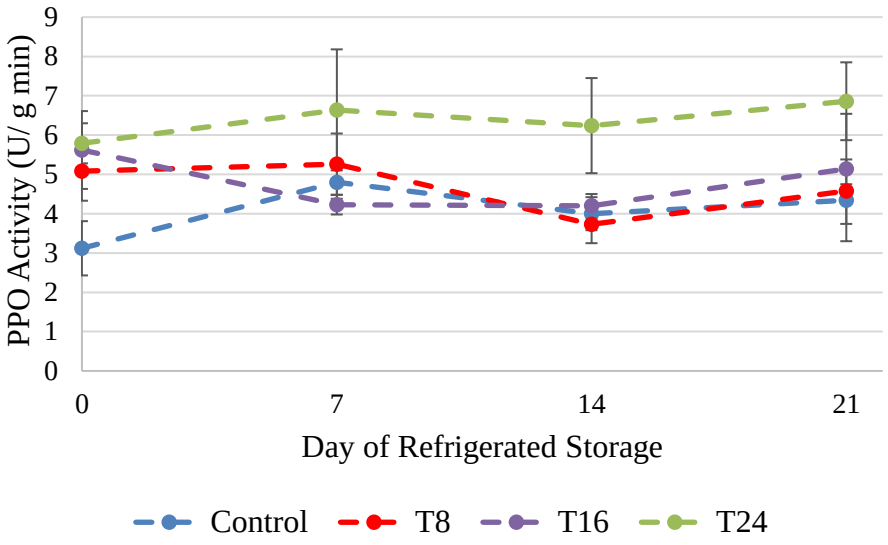


Figure 3.11: Polyphenol oxidase (PPO) activities of whole (A) and shredded (B) red cabbages. Measurements were taken in triplicate from three individual cabbages exposed to the same temperature conditions and storage time. U is an arbitrary enzyme activity until equal to an increase in absorbance of 0.001. Error bars reflect standard deviation of three samples at the same conditions.

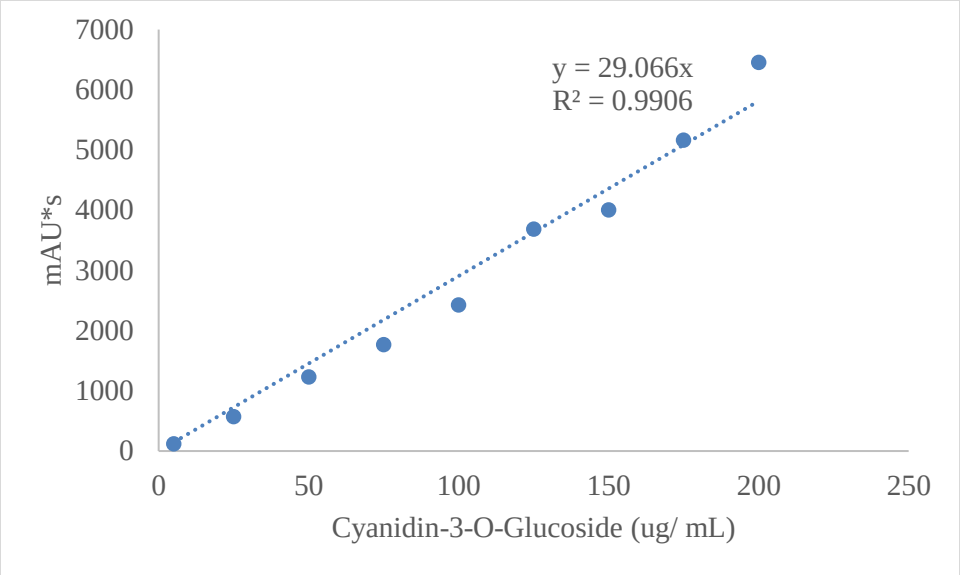


Figure 3.12: External calibration curve of cyanidin-3-*O*-glucoside for HPLC analysis of anthocyanins

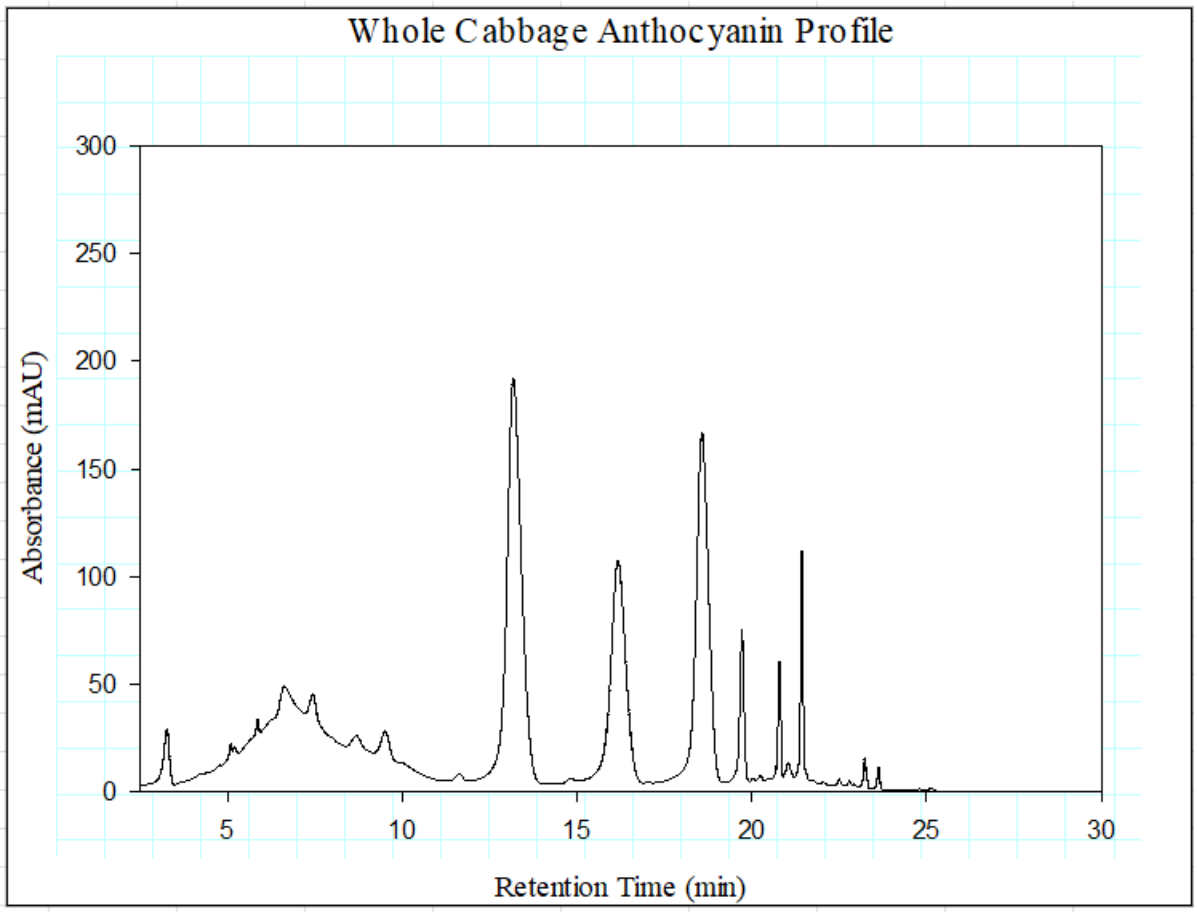


Figure 3.13: HPLC chromatogram of control (refrigerated) whole red cabbage on the first day of refrigerated storage. Retention time (t_R) in min with absorbance recorded in milli-absorbance units (mAU).

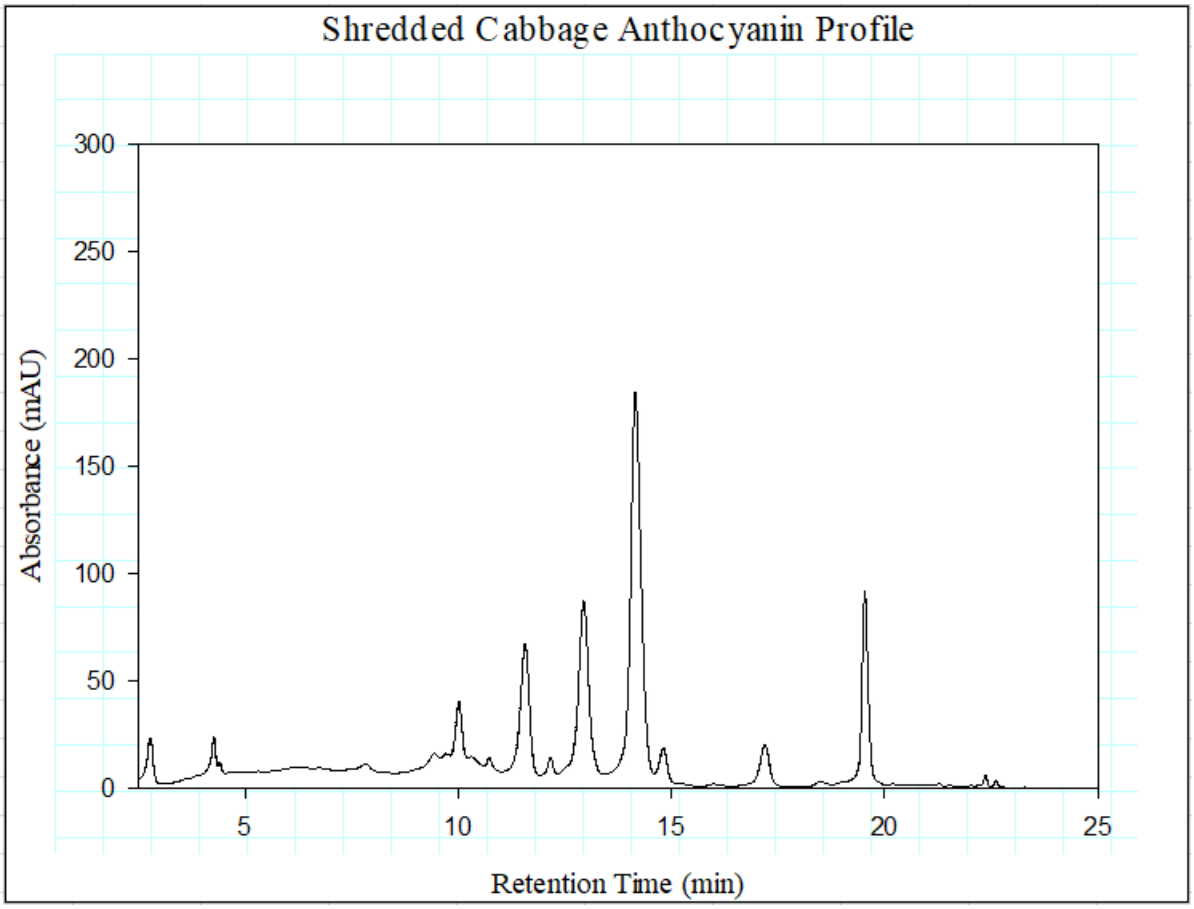


Figure 3.14: HPLC chromatogram of control (refrigerated) shredded red cabbage on the first day of refrigerated storage. Retention time (t_R) in min with absorbance recorded in milli-absorbance units (mAU).

CHAPTER 4

CONCLUSIONS

Demand for fresh cut cabbages has greatly increased due to their desirable health benefits and convenience. Modified atmosphere packaging has enabled shredded cabbages to retain sensory characteristics for longer durations, but there is a lack of literature on the potential consequences of loss of refrigeration conditions on the quality of cabbages. Cabbages can be exposed to loss of refrigeration conditions at many points postharvest, including during processing, distribution, and retail. Faulty cooling units on transportation trucks, extended time sitting on loading docks during distribution, and inadequate storage conditions during retail display may all introduce cabbages to temperature abuse for varying periods of time. Once shredded, there is additional concern as shredding releases enzymes that accelerate degradation reactions and make produce more susceptible to microbial activity. The desirable health characteristics of cabbages as well as the color and sensory attributes that attract consumers may be negatively affected by temperature abuse conditions. Therefore, it is important to understand the effects of potential storage temperature abuse conditions on the quality characteristics of cabbages, particularly cabbages that have undergone shredding processes.

During this study, cabbages were exposed to durations of 8, 16, and 24 h of temperature abuse at 25 °C, then returned to refrigerated storage at 4 °C. Quality characteristics evaluating the color, health, and flavor components of cabbages were then evaluated over a three-week storage period. The main focus of this study was the shredded green cabbages, as green cabbages are more

widely consumed than red cabbages and there has been increased demand for shredded produce in recent years. Red cabbages were also analyzed for their color properties and anthocyanin contents. Another emerging topic in the food industry is food waste valorization, so there has been interest in extracting anthocyanins from spent cabbages to utilize as food colorants.

The whole heads of cabbage showed no obvious visible signs of deterioration throughout the study regardless of temperature abuse duration or length of refrigerated storage. However, the shredded cabbages that were temperature abused for 16 and 24 h started showing signs of spoilage by day 14. These cabbages lost structural integrity, had a sour smell, and were sticky to the touch. The shredded cabbages that were temperature abused for 8 h, however, had only slightly deteriorated in quality by day 21 of the study, and the control shredded cabbages kept in refrigeration conditions remained fresh in visible quality throughout the duration of the study.

Statistical analysis was performed on both the whole and shredded cabbages to determine the effect of temperature abuse duration and length of refrigerated storage on the quality characteristics of cabbage. Overall, whole cabbages were less susceptible to quality degradation due to temperature abuse conditions. The lightness values as well as the L-ascorbic acid, glucosinolate, and anthocyanin contents of the whole cabbages were not affected by temperature abuse. This is promising for farmers who field package, as cabbages can be harvested and left temporarily on trucks without undergoing significant loss of quality as long as the cabbages are maintained in the whole form.

However, shredded cabbages were negatively affected by temperature abuse for 24 h at 25 °C. Visible spoilage occurred by day 14, and L* values decreased in the T24 cabbages as compared to the refrigerated cabbages ($P < 0.05$). Additionally, the L-ascorbic acid content decreased in the T24 cabbages in comparison to the other temperature groups ($P < 0.05$). The PPO activity of the

red shredded cabbages also increased with temperature abuse, with the control, T8, and T16 cabbages having significantly lower PPO values than the T24 cabbages ($P < 0.001$). The glucosinolate content of the refrigerated cabbages were significantly higher than that of the T24 cabbages as well ($P < 0.05$). These results emphasize the importance of maintaining the cold chain throughout cabbage storage and transport. Efforts should be made to ensure there are short holding times on loading docks and that all refrigeration units on transport trucks and retail displays are functioning at the proper temperature. Failure to do so results in cabbages spoiling prematurely as well as loss of nutritive properties, both of which have negative consequences for cabbage consumers.

Anthocyanins, the colorants in red cabbage, were lost due to shredding ($P < 0.001$) but not temperature abuse. The spoiled samples had comparable anthocyanin contents to the non-spoiled samples at the same temperature and time conditions. This is promising because of the potential to extract anthocyanin colorants from spent cabbages to salvage some of the lost product.

Future studies should focus on the microbiological safety of temperature abused cabbages. The bacteria responsible for food spoilage can grow under different conditions than food pathogens, so the cabbages may not look spoiled but may pose a food safety threat. Of particular interest is *Listeria monocytogenes*, which can grow under refrigeration conditions and has been associated with previous recalls in cabbage products.

Overall, shredded cabbages were significantly affected by storage temperature abuse of 25 °C for 24 h. Temperature abused cabbages spoiled quicker than the refrigerated samples and underwent a loss in desirable micronutrients and flavor compounds. The results of this study emphasize the importance of maintaining the cold chain throughout cabbage storage and transport,

particularly for fresh cut cabbages. However, there is potential to extract anthocyanins from spent cabbages to use as food colorants and help reduce food waste.