The Effect of pH and Temperature on Cabbage Volatiles during Storage

THESIS

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Hacer Akpolat, B.S.

Graduate Program in Food Science and Technology

The Ohio State University

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Master's Examination Committee:

Dr. Sheryl Barringer, Advisor

Dr. John H. Litchfield

Dr. Luis Rodriguez-Saona

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ABSTRACT

During storage of shredded cabbage, characteristic sulfurous volatile compounds are formed which can affect cabbage aroma both negatively and positively. Selected ion flow tube-mass spectrometry (SIFT-MS) was used to measure the concentration of cabbage volatiles in the headspace during storage. Shredded cabbage was added to buffers at pH 3, 4.6, 6.4, and 8. The volatile levels of cabbage samples were measured at all pH levels at 4 °C for 14 days and pH 3.3 at 25 °C for 5 days in order to determine the effect of pH and temperature. A sensory test was conducted to investigate whether consumers can distinguish the difference between samples stored at different pH levels and the similarity between stored and freshly shredded samples. The samples in lower pH buffers (pH 3.3 and 4.6) generated significantly lower amounts of off odors. Allyl isothiocyanate creates hotness and pungency and is one of the desirable compounds in cabbage. While allyl isothiocyanate was lower in high pH samples (pH 7.4 and 6.4) higher amounts of allyl isothiocyanate were detected in low pH samples. Lipoxygenase (LOX) volatiles, which provide a fresh green and leafy aroma in cabbage, were generated in very low amounts at any pH value. Dimethyl disulfide, dimethyl trisulfide and methyl mercaptan are off odors, and have a significant negative effect on the aroma quality of cabbage. The samples in buffer solutions with higher pH levels generated significantly higher concentrations of off-odors during storage. Sensory tests showed that higher pH samples had significantly stronger off odor and lower desirable cabbage aroma than lower pH samples. Thus, sensory results matched the volatile results where samples at

higher pH levels formed the highest amount of undesirable volatiles and the least amount of desirable volatiles. Higher amounts of all volatiles were formed at 25 °C than 4 °C. Off-odor formation increased over time, with the increase starting sooner at 4 °C than 25 °C. Shredded cabbage products should be stored in low pH dressings to minimize formation of volatiles with off odors and maximize formation of volatiles with characteristic, desirable cabbage odor.

Practical Application

Elucidation of the aromatic flavor development of shredded cabbage with a SIFT-MS instrument provides information on undesirable flavor release and offers better understanding of off flavor formation. Use of SIFT-MS for aroma evaluation of cabbage may be a faster method and as reliable as conventional aroma evaluation techniques for understanding sensory quality at different temperatures and pH levels. Low pH samples (pH 3.3 and 4.6) formed less undesirable and more desirable volatile compounds overall. The natural pH of cabbage is around 6.4, and at this pH, higher amounts of off odors are formed during storage, so the use of low pH dressings is necessary to reach a desirable aroma. Moreover, higher concentrations of all volatile compounds were formed at 25 °C than 4 °C. Thus, low pH salad dressings are more suitable storage way for ready-to use cabbage products in terms of sensory quality, and high temperature is not suitable for cabbage storage, since undesirable aroma compound formation is high, desirable aroma loss occurs, and microbial growth occurs at very early stage of storage.

Dedicated to my family and friends

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VITA

January 1987 Born, Sivas, Turkey
June 2004 Cumhuriyet Anadolu High School
June 2008 B.S. Food Engineering, Pamukkale University

FIELDS OF STUDY

Major Field: Food Science and Technology

TABLE OF CONTENTS

ABSTRACT.....	ii
Dedication.....	iv
ACKNOWLEDGEMENTS.....	v
VITA.....	vi
FIELDS OF STUDY.....	vi
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
Chapter 1 : INTRODUCTION	1
Chapter 2 : LITERATURE REVIEW	4
2.1 Cabbage	4
2.1.2 Glucosinolates in cabbage	4
2.1.3 Volatile compounds in cabbage.....	7
2.2 Effect of storage and processing on glucosinolates and volatile compounds	15
2.3. Effect of different conditions on glucosinolate degradation and myrosinase activity and volatile compounds.....	16
2.3.1 Effect of pH and temperature.....	16
2.3.2 Effect of growing conditions, maturity and cultivar	19
2.3.3 Effect of processing.....	21
2.4. Lipxygenase derived volatile compounds	21
2.4.1 Effect of pH on lipxygenase activity and lipxygenase derived volatile compounds	22
2.5 SIFT-MS	22
Chapter 3 : MATERIALS AND METHODS	26
3.1 SIFT-MS Analyses	26
3.2 Headspace Measurements	27
3.3 Sensory Test	29
3.4 Statistical Analysis.....	29
Chapter 4 : RESULTS AND DISCUSSION.....	31

4.1 Fresh Odor Formation in Different pH Solutions	31
4.2 Off Odor Formation at Different pHs.....	35
4.3 pH Effect on other Volatiles	38
4.4 Sensory Results	40
4.5 Fresh and Off Odor Formation at Different Temperatures	41
4.6 Conclusion.....	43
References	44
APPENDIX: Additional volatile information and pH change of cabbage.....	54

LIST OF TABLES

Table	Page
Table 3.1. Major cabbage volatiles, reagent ions and masses of the compounds used in this study.....	27
Table 4.1. Sensory test results of samples which were stored for 5 days at 4 °C.....	40
Table A.1. pH values of the samples in different buffers in the last day of SIFT-MS experiment.....	54
Table A.2. Concentrations of desirable and undesirable volatile compounds in cabbage at 25 °C during 5 day storage period.....	54
Table A.3. Presence of major cabbage volatiles in common studies, reagent ions and masses of the compounds used in this study.....	55

LIST OF FIGURES

Figure	Page
Figure 2.1. Basic structures of glucosinolates.	5
Figure 2.2. Schematic diagram of hydrolysis of glucosinolates. R can represent an aliphatic, aromatic or indolyl group (Jahangir and others 2009).	5
Figure 2.3. Formation of volatile compounds from hydrolysis of sinigrin (allyl glucosinolate) in cruciferous plants (Chin and others 1996).	8
Figure 2.4. Schematic diagram of selected ion flow tube (Spanel and others 1996).	25
Figure 4.1. Allyl isothiocyanate levels during storage in different pH solutions at 4 °C.	32
Figure 4.2. Allyl cyanide levels during storage in different pH solutions at 4 °C.	33
Figure 4.3. Formation of some of the lipoxygenase volatiles, (<i>E</i>)-2-hexenal, 3-hexen-1-ol, and their change during storage in different pH solutions at 4 °C.	34
Figure 4.4. Dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, and methyl mercaptan formation during storage in different pH solutions at 4 °C.	38
Figure 4.5. Hydrogen sulfide and carbon sulfoxide concentration during storage in different pH solutions at 4 °C.	39
Figure 4.6. Allyl isothiocyanate levels during storage in pH 3.3 buffers at 4 °C and 25 °C.	41
Figure 4.7. Off odor formation during storage in pH 3.3 buffers at 4 °C and 25 °C.	42

Chapter 1 : INTRODUCTION

Cruciferous plants such as cabbage, broccoli, mustard and cauliflower has been recognized for their curative, medicinal and culinary uses for a very long time (Fenwick and Heaney 1983). Cabbage (*Brassica oleracea* var. *capitata*) and other cruciferous vegetables are characterized by their sulfurous aroma which is produced by enzymatic hydrolysis of glucosinolates (Yen and Wei 1993; Chin and others 1996). High consumption of *Brassica* vegetables is becoming increasingly popular as glucosinolates are defined as anticarcinogenic compounds by scientific research (Jahangir and others 2009). Freshly shredded leaves of cabbage are available in the market and commonly used in salads such as coleslaw (Banerjee and others 2014).

Allyl isothiocyanate is the most important volatile compound for cabbage since it gives a characteristic, desirable aroma and produces fresh cabbage flavor. Allyl isothiocyanate is derived from a precursor glucosinolate (sinigrin) by myrosinase action (MacLeod and MacLeod 1968; Chin and Lindsay 1993). Dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide and methyl mercaptan are off odors in cabbage (Chin and Lindsay 1993; Banerjee and others 2014). Dimethyl sulfide is derived from methionine (Casey and others 1963). Its odor is 'decayed cabbage' (Ruth 1986) and its concentration is frequently higher than other volatile compounds in cabbage (MacLeod and Nussbaum

1977). Dimethyl disulfide is another predominant volatile compound which produces off odor, and it is derived from S-methylcysteine sulfoxide by the action of cysteine sulfoxide lyase. Dimethyl trisulfide is also an important volatile for sensory quality of cabbage and derived from S-methylcystein and S-methylcysteine sulfoxide, and has a spoiled and cooked cabbage-like odor (Kubec and others 1998). Methyl mercaptan is a highly volatile off odor compound in cabbage described as “rotten cabbage” (Chin and Lindsay 1993).

Allyl isothiocyanate, dimethyl disulfide, dimethyl trisulfide and methyl mercaptan form enzymatically, and pH affects the activity of the enzymes responsible for formation of these volatiles (Rungapamestry and others 2006). Myrosinase is the enzyme which is responsible for allyl isothiocyanate formation. The optimum pH for myrosinase varies between 4 and 9 depending on the type of vegetable (Travers-Martin and others 2008). Optimum cabbage myrosinase activity was near neutral pH (West and others 1977) or at pH 8 (Yen and Wei 1993). Cysteine sulfoxide lyase is the enzyme which is responsible for the formation of dimethyl disulfide, dimethyl trisulfide and methyl mercaptan (Chin and Lindsay 1993; Stoewsand 1995). The activity of cysteine sulfoxide lyase enzyme extracted from cabbage is optimum at around pH 8.5 to 9 (Hall and Smith 1983)

Enzyme activity is also affected by temperature. Cabbage myrosinase activity increases starting from 20 °C to a maximum at 60 °C, then activity decreased above 60 °C (Yen and Wei 1993). For radish myrosinase, optimum temperature was 37 °C, and the enzyme deactivated completely at 45 °C (Jwanny and others 1995).

While there is a lot of research about glucosinolate content of cabbage and other cruciferous vegetables, there is limited information on volatile compound formation

under different conditions. Optimum pH and temperature for the formation of volatile compounds, which can affect cabbage aroma both negatively or positively, can be determined by SIFT-MS during storage of shredded cabbage. The objective of this study was to determine how storage at different temperature and pH levels effects desirable and undesirable aroma development and sensory quality of shredded cabbage products such as coleslaw during storage.

Chapter 2 : LITERATURE REVIEW

2.1 Cabbage

Cabbage (*Brassica oleracea* var. *capitata*) and other Cruciferous vegetables such as broccoli, mustard and cauliflower are characterized by their sulfurous aroma which is produced by thioglucosidase hydrolysis of glucosinolates (Chin and Lindsay 1993; Chin and others 1996). High consumption of *Brassica* vegetables is becoming increasingly popular as glucosinolates are defined as anticarcinogenic compounds (Jahangir and others 2009).

2.1.2 Glucosinolates in cabbage

Glucosinolates are thioglucosides containing a cyano and a sulfate group. Basic structures of glucosinolates were shown in Figure 2.1. They are important secondary metabolites in *Brassica* vegetables and derived from amino acid biosynthesis (Rungapamestry and others 2006; Jahangir and others 2009). 12 distinct glucosinolates are present in *Brassica* family out of 100 previously identified glucosinolates (Stoewsand 1995). In cabbage, sinigrin, glucoiberin and glucobrassicin are predominant glucosinolates (Jahangir and others 2009).

Figure 2.1. Basic structures of glucosinolates.

By enzymatic and non-enzymatic changes, glucosinolates breaks down into numerous bioactive compounds and volatile compounds (Ciska and Kozłowska 2001). Myrosinase hydrolyzes glucosinolates, once cabbage tissue is disrupted by slicing, shredding, cooking or chewing (Chin and others 1996; Ciska and Kozłowska 2001). Hydrolysis of glucosinolates is shown in Figure 2.2.

Figure 2.2. Schematic diagram of hydrolysis of glucosinolates. R can represent an aliphatic, aromatic or indolyl group (Jahangir and others 2009).

The myrosinase-glucosinolate system is influenced by several factors, namely myrosinase inactivation, loss of myrosinase cofactors, thermal breakdown of glucosinolates, leaching of glucosinolates into the aqueous cooking medium, and hydrolysis products of glucosinolates. The researchers reported that total glucosinolate concentration significantly decreased by cooking depending on cooking time and method. Myrosinase activity was lost after 7 min steaming, and after 2 min microwave cooking. They also showed that while allyl isothiocyanate release was minimum in raw and near-raw (microwaved 45 s or steamed 210 s) cabbage, surprisingly it gave the highest yield when cabbage was slightly cooked (microwaved 210 s or steamed 420 s) (Rungapamestry and others 2006).

A study analyzing the post-harvest increase of glucosinolates in *Brassica* vegetables, namely red cabbage, white cabbage and broccoli reported an increase in glucosinolates in these vegetables after 48 h storage at room temperature (Verkerk and others 2001). The cabbages were cut into pieces of 1 cm² and stored at room temperature for 48 h. Then, the samples were freeze dried for HPLC analysis. Nine different glucosinolates for white and red cabbage was revealed in control samples, which were not stored, in HPLC analysis and five of them were aliphatic and four were indolyl glucosinolates. The major glucosinolates were 3-methylsulphinylpropyl (glucoiberin) (25%) and 2-propenyl (sinigrin) (45%) in white cabbage. 19% of the glucosinolates were indolyl glucosinolates in white cabbage, particularly 3-indolylmethyl (glucobrassicin). The researchers also reported that storage of chopped white cabbages in ambient temperature did not affect the aliphatic glucosinolate content, while indolyl glucosinolates were affected significantly by storage. The amount of indolyl glucosinolates increased to 61%

from 19% after 48 h. On the other hand, a decrease in aliphatic and indolyl glucosinolates after 24 h of storage was observed when the tissue damage was increased by pulping of white cabbage in water (Verkerk and others 2001).

Cooked cabbage was analyzed to investigate the effect of cooking time on glucosinolate content. Two aliphatic glucosinolates, sinigrin and glucoiberin, and two indole glucosinolates, glucobrassicin and 4-methoxyglucobrassicin were four major glucosinolates in cabbage. In the first five min of cooking, glucosinolate amount decreased 35 %. After first five min, glucosinolate amount decreased 10-15 % in every five min during 30 min of cooking time. Among all glucosinolates, glucoiberin was found to be more thermolabile, as a higher rate of change was observed for it (Ciska and Kozłowska 2001).

In another study 30 min boiling of *Brassica* vegetables caused a significant decrease, 58-77 %, on glucosinolates (Song and Thornalley 2007). When boiling water was analyzed, it was shown that glucosinolates leached out into the boiling water. The other cooking methods, stir-fry cooking for 0-5 min, steaming for 0-20 min and microwave cooking for 0-3 min did not significantly affect glucosinolate content of *Brassica* vegetables (Song and Thornalley 2007).

2.1.3 Volatile compounds in cabbage

Allyl isothiocyanate is the major volatile compound which gives cabbage its hotness and pungency, so it is defined as fresh cabbage flavor (MacLeod and MacLeod 1968; Chin and Lindsay 1993). Formation pathway of allyl isothiocyanate is shown in Figure 2.3.

Figure 2.3. Formation of volatile compounds from hydrolysis of sinigrin (allyl glucosinolate) in cruciferous plants (Chin and others 1996).

1-cyano-2,3-epithiopropene is also a major volatile compound in freshly disrupted cabbage (Chin and others 1996). However, in their earlier research Chin and Lindsay (1993) did not report the presence of 1-cyano-2,3-epithiopropene, since their focus was different. When the aroma profile of the compound is evaluated in aqueous and anhydrous mediums, it revealed musty, sulfurous, mustard-like notes, but it did not give sharp, pungent sensations, so the researchers reported that it does not have a significant contribution to the flavor of cabbage because it has a weak flavor (Chin and others 1996). In 1982, Petroski and Tookey worked on cruciferous vegetables to investigate the formation of 1-cyano-2,3-epithiopropene and its dependence on epithiospecifier protein (ESP). They reported that 1-cyano-2,3-epithiopropene formed in the presence of ESP as well as myrosinase and sinigrin in all cruciferous vegetables, while allyl isothiocyanate

formation needs only the presence of myrosinase and sinigrin. When there was a reducing agent such as 2-mercaptoethanol in the model system, allyl cyanide formed as well as 1-cyano-2,3-epithiopropene and allyl isothiocyanate. However, in the absence of 2-mercaptoethanol, the only breakdown product of sinigrin was allyl isothiocyanate (Petroski and Tookey 1982; Chin and others 1996).

There is a difference between inner and outer leaves in terms of the amount of compounds present. 1-cyano-2,3-epithiopropene amount was higher in the inner leaves of cabbage, but it did not contribute to cabbage aroma significantly. It may be because of its weak flavor properties and high detection threshold value (Chin and others 1996).

Green leaf volatiles (GLVs) that give a green odor to the fresh vegetables are C6 aldehydes and alcohols and their corresponding esters (Banerjee and others 2014). The unsaturated fatty acids, linoleic and linolenic acid, release these compounds by enzyme hydrolysis. Lipxygenase pathway causes the release of GLVs by lipxygenase (LOX) and hydroperoxide lyase (HPL) enzymes. These compounds are *n*-hexanal, giving green odor and related freshness perception, *E*-2-hexanal and *Z*-3-hexenol, providing fresh green and leafy aroma in cabbage. The researchers investigated volatile composition in two known cultivars and one market sample. They found *n*-hexanal and *trans*-hex-2-enal highest in market samples than two known cultivars of cabbage. They did not find any significant difference of *cis*-hex-3-enol between different cultivars. Allyl isothiocyanate was the major volatile compound of cabbage and the amount was different depending on variety, being highest in the market sample. They reported but-3-enyl isothiocyanate and 3-(methylthio) propyl isothiocyanate as other isothiocyanate compounds, but they were in low concentrations, therefore they claimed that these compounds possibly do not affect

the overall odor quality of cabbage. Dimethyl disulfide, dimethyl trisulfide and dimethyl tetrasulfide were defined as off flavors and they varied among cabbage cultivars. These compounds have significant effect on aroma quality (Banerjee and others 2014).

In another study, the volatile compounds of fresh, dehydrated and rehydrated cabbage samples were investigated (Bailey and others 1961). Volatiles of fresh cabbage reported in this study were isothiocyanates (methyl, *n*-butyl, butenyl, allyl, and methylthiopropyl), sulfides (hydrogen, carbonyl, dimethyl, diethyl and dibutyl), disulfides (carbon, dimethyl, methyl ethyl, diethyl, ethyl propyl, dipropyl, propyl butyl, propyl allyl and diallyl) and dimethyl trisulfide. Among those volatiles, only dimethyl sulfide was detected in dehydrated samples, and only *n*-butyl, butenyl, allyl isothiocyanates were detected in rehydrated and enzyme treated cabbage samples. When compared to dehydrated cabbage, fresh cabbage had much more volatile compounds, as processing caused partial or complete loss of these compounds. Moreover, when myrosinase and water were added to dehydrated cabbage, some volatile compounds, allyl, *n*-butyl and 3-butenyl isothiocyanates were released proving that some of the precursor glucosinolate compounds were not lost during processing. All of the volatile compounds existed in fresh cabbage were not detected in rehydrated and enzyme treated cabbage samples, and this may be because quantities were not enough for detection in rehydrated samples (Bailey and others 1961).

Cabbage volatiles were reported as sulfurous compounds, disulphides, isothiocyanates, ketones, short-chain alcohols, aldehydes and monoterpenes by Lonchamp and others (2009). The researchers worked on how cabbage volatile compounds change by storage over a certain time period with active and passive

modified atmosphere packaging (MAP). 1.5 in² cut pieces of inner cabbage leaves were disinfected with chlorine solution, washed and dried to use in the analyses. Until day 7, all the fresh aromas decreased and off-odors and yellowing increased in passive MA-packaged Irish York cabbage. Irish York cabbage remained acceptable until day 11 in active MA-package, while passive MA-packaged samples were unacceptable from day 7 regarding sensory and visual test results when looked at appearance and off odors. They also reported higher quality samples remained their aroma better in first 7 days (Lonchamp and others 2009).

Limonene is a terpene and it was identified as quality loss factor as it is a possible oxidation product of chlorophyll. Thus, green color loss was associated with formation of limonene by the authors (Lonchamp and others 2009).

Cabbage volatiles were investigated in a study of Chin and Lindsay (1993) in thirty eight cultivars of cabbage using GC headspace technique. Blended cabbage in distilled water was held in 30°C water bath at various times (10 to 100 minutes), and analyzed every 30 minutes. The detected volatile compounds in cabbage were hydrogen sulfide, carbonyl sulfide, methanethiol (methyl mercaptan), dimethyl disulfide, dimethyl trisulfide, and allyl isothiocyanate (Chin and Lindsay 1993).

Hydrogen sulfide was formed in some of the cultivars at very early stages of analysis. It is a quite reactive compound and assumed to be participated in the formation of dimethyl trisulfide (Maruyama, 1970) and carbonyl sulfide in cabbage. The researchers claimed that its disappearance can be related to the formation of off flavors (Chin and Lindsay 1993).

Carbonyl sulfide was the most abundant compound in all cultivars. Its inactivation property with glyoxal was used to prove its existence in the samples. Its formation is not clear, but two mechanisms have been proposed by previous research. One is that isothiocyanate hydrolysis may lead the formation of carbonyl sulfide (Bailey and others 1961), and the another mechanism is that hydrogen sulfide could oxidize to SO_2 and then may equilibrate with CO_2 to form carbonyl sulfide and water (Chin and Lindsay 1993).

Methyl mercaptan is a degradation product of methyl methanethiosulfinate (MMTSO), which is generated from S-methyl-L-cystein sulfoxide by the activity of cysteine sulfoxide lyase (Dan and others 1997). Other researchers also reported that the major pathway for the formation of methyl mercaptan in freshly disrupted tissues of cabbage is the reaction of hydrogen sulfide with methyl methanethiosulfinate or methyl methanethiosulfonate (Chin and Lindsay 1994a). Methyl mercaptan was present in 24 of 38 cabbage cultivars, and it was detected in 28 cultivars at the end of 100 min. However, some of the cultivars which produced methyl mercaptan at the beginning did not show detectable amount of the compound after 100 min. Although the presence of methyl mercaptan was not reported in earlier research, the authors detected the compound and proposed that it is a quite volatile compound and that is why other researchers could not detect it. Methyl mercaptan odor was described as “rotten cabbage” (Chin and Lindsay 1993).

Dimethyl trisulfide was also one of the volatiles detected in all cultivars in the study. However, its production was slow, so only 6 cultivars produced detectable amount of dimethyl trisulfide after 10 min, and the amount increased over the time (Chin and Lindsay 1993).

All of the cultivars except five varieties produced detectable amounts of allyl isothiocyanate during 100 min (Chin and Lindsay 1993). The cultivar groups from different companies showed different amount of allyl isothiocyanate; therefore, the breeding history could be important for volatile profile (Chin and Lindsay 1993).

Although previous research done by Bailey and others (1961) and MacLeod and MacLeod (1968) reported the existence of dimethyl sulfide in freshly minced, dehydrated and cooked cabbage, Chin and Lindsay (1993) did not detect dimethyl sulfide in any of the blended cabbage samples. Overall, the off flavors were defined as methyl mercaptan, dimethyl disulfide and dimethyl trisulfide in cruciferous vegetables in this study (Chin and Lindsay 1993).

In cooked cabbage, the first six abundant volatiles were found to be dimethyl sulphide, methyl alcohol, acetone, allyl isothiocyanate, *cis*-hex-3-en-1-ol, and allyl cyanide (MacLeod and MacLeod 1968).

Cooked dried cabbage and cooked fresh cabbage was investigated to determine the volatile content by MacLeod and MacLeod in 1970. While fresh cabbage had 4.5% of *cis*-hex-3-en-1-ol, it was not detected in cooked dried cabbage samples. In this research, dimethyl sulfide was found to be most abundant volatile compound in cooked fresh cabbage, while some of the cooked dried samples produced much less dimethyl sulfide (MacLeod and MacLeod 1970).

In another study, among all volatile compounds identified, allyl isothiocyanate, allyl cyanide and *cis*-3-hexenol were found to be at highest amounts (Itoh and others 1985).

S-Methyl-L-cystein sulfoxide (SMCSO) and *S*-Methyl-L-cystein (MeCys) are amino acids that lead to the formation of various odors in *Brassica* vegetables. SMCSO degradation was investigated under different conditions, namely water content, temperature and time of heating. SMCSO broke down almost completely in the presence of 10-40% water during 1 h at 120 °C. Dimethyl disulfide was the predominant volatile compound (almost more than 60% of total volatiles). The second most abundant volatile compound was dimethyl trisulfide and its amount increased with temperature. The amount of dimethyl disulfide and dimethyl trisulfide increased with increasing time of heating. Water content affected the rate of degradation and the structure of the breakdown products. Higher amounts of volatiles were released in the presence of 20-80% of water. Dimethyl disulfide was also found to be highest among all volatiles in different water contents. It was claimed that the formation of dimethyl disulfide starts with breakdown of SMCSO into methanesulfenic acid and α -aminoacrylic acid. When methanesulfenic acid self-condensates, it leads to formation of dimethyl thiosulfinate and this compound decomposes into dimethyl disulfide and some other compounds. Although the formation of dimethyl trisulfide was proposed to be from the decomposition of disulfides in previous research, the researchers reported that dimethyl disulfide decomposition was limited at temperatures below 200 °C (Kubec and others 1998).

Degradation of *S*-Methyl-L-cystein (MeCys) was investigated in terms of the volatile compounds formed. MeCys was found to be more heat stable than SMCSO, since significant amounts of volatiles formed only at higher temperatures than 180 °C (Kubec and others 1998).

Dimethyl trisulfide was found to be most important compound that has a significant sensory effect among the flavors derived from MeCys and SMCSO, while dimethyl disulfide was found to have a minor role on sensory characteristics. This finding was also in agreement with previous research. Odor description of dimethyl trisulfide was indicated as spoiled and cooked cabbage-like (Kubec and others 1998).

2.2 Effect of storage and processing on glucosinolates and volatile compounds

Effect of storage was investigated in broccoli, Brussel sprouts, cauliflower and green cabbage (Song and Thornalley 2007). Glucosinolate concentrations of different *Brassica* vegetables stored at 12-22 °C did not decreased significantly; however, when the vegetables stored in a refrigerator at 4-8 °C, there was a decrease during 7 days of storage. While the change was minor in the first 3 days, glucosinolate amount decreased 11-27% after 7 days of storage. Glucoiberin, glucoallyssin and glucoraphanin losses were higher than sinigrin, gluconapin and progoitrin losses. It was also reported that storage at -85 °C caused higher loss than storage at 4-8 °C due to freeze-thaw affect and change in accessibility of myrosinase. The researchers also showed the effect of shredding reporting that loss of glucosinolates was up to 75% in shredded vegetables during 6 h at ambient temperature. When vegetables shredded into larger pieces, glucosinolate loss decreased to 10 % and lower. Also when isothiocyanate formation was investigated, it was reported that isothiocyanates represented 30-50 % of the glucosinolate loss which indicates some other hydrolysis products formed (Song and Thornalley 2007).

In order to investigate the effect of storage on glucosinolates, cabbage samples were stored at 1 ± 1 °C and analyzed between 5 and 215 d. Between day 5 and 40, a moderate increase was observed in cabbage glucosinolates. Until day 131, there was a

little or no change in cabbage glucosinolates. After day 131, there was a sharp increase and in day 173 glucosinolates reached the maximum level. A rapid decline was observed from day 173 to 215. Moreover, when volatile isothiocyanates were measured, one cultivar (F1 Mercury) had higher levels of volatile isothiocyanates with a different formation pattern than other 2 cultivars, Hidena and Safekeeper (Chong and Berard 1983).

2.3. Effect of different conditions on glucosinolate degradation and myrosinase activity and volatile compounds

Myrosinase activity is affected by several factors such as cofactors, ascorbic acid, epithiospecifier protein (ESP), ferrous ions, pH and temperature (Rungapamestry and others 2006).

2.3.1 Effect of pH and temperature

2.3.1.1 Myrosinase activity

The optimum pH found for myrosinase varies between 4 and 9 depending on the type of vegetable (Travers-Martin and others 2008).

Gil and MacLeod (1980) conducted a study to investigate the effect of pH on glucosinolate degradation by looking at nitrile and isothiocyanate formation. Mustard enzyme preparation and pure glucosinolates were used to determine the degradation of glucosinolates. Citrate-phosphate buffer solution at pH 7 did not make any significant difference than distilled water samples. At pH 3, the reaction rate decreased but the enzyme, mustard thioglucosidase, was still active. Between the pH ranges 4.0-8.5, relative amounts of reaction products were constant. They also claimed that nitrile formation may not be pH dependent (Gil and MacLeod 1980).

Endogenous myrosinase activity was found to be maximum at 25 °C from broccoli seeds (Shen and others 2010). Sulforaphane, a glucosinolate degradation product was used to determine the enzyme activity endogenously and exogenously. The researchers reported that endogenous enzyme stopped working below pH 2 and above pH 8. Maximum enzyme activity was found at pH 4, and when pH increased above 5, enzymolysis rate decreased. On the other hand, exogenous myrosinase activity was tested in different conditions and found to have high activity in a wide temperature range compared to endogenous enzyme. When temperature increased from 5 °C to 35 °C, enzyme activity significantly increased and reached maximum level and remained high until 55-60 °C with a slight decrease. The pH effect on exogenous myrosinase was found also significant as reported to be maximum at pH 5. Further increase of pH affected enzyme activity adversely (Shen and others 2010).

In another study, myrosinase from horseradish was investigated (Li and Kushad, 2005). Maximum myrosinase activity was found to be increased starting from 23 °C and maximum between 37-45 °C. A significant decrease was observed above 50 °C. On the other hand, the researchers found maximum enzyme activity at pH 5.7. Enzyme activity started to increase from pH 3.4, reached its maximum at pH 5.7, and remained high up to pH 8 (Li and Kushad, 2005).

Myrosinase from radish roots was found to have maximum activity at pH 6.0-6.5 and half of the maximum activity at pH 5.2. Myrosinase activity sharply decreased at pH 7. Optimum temperature for the enzyme was found to be 37 °C. Over 45 °C, enzyme deactivated completely (Jwanny and others 1995).

Optimum pH was reported to be around neutral pH for cell-free cabbage myrosinase. L-ascorbic acid increased the activity of myrosinase 13.6 times at pH 7.0 and 10.3 times at pH 4.45. When d-ascorbic acid was used, activation increase was lower than l-ascorbic acid. Cole slaw (pH 4.45) was found to have six times greater allyl isothiocyanate than freshly shredded cabbage (pH 6.4). Allyl isothiocyanate was found to be ten times greater than allyl cyanide in coleslaw, while the amount of these two compounds were the same in shredded cabbage (West and others 1977).

Effect of pH on myrosinase activity was investigated in cabbage produced in Taiwan (Yen and Wei 1993). Optimum pH was found to be at pH 8.0 in white and red cabbages. However, enzyme activity was also high at pH 4, slightly lower than at pH 8. The researchers also analyzed enzyme stability at different pH levels (3-9), and they reported that the enzyme was stable at pH values between 6.0 and 9.0. Below pH 5.0, enzyme stability rapidly decreased (Yen and Wei 1993).

Cabbage myrosinase has shown maximum activity at 60 °C. Temperature increase starting from 20 °C caused an increase in myrosinase activity up to 60 °C, then activity decreased above 60 °C (Yen and Wei 1993).

In a study in which enzymolysis conditions were investigated, optimum pH was found to be pH 5.0 for formation of isothiocyanates from broccoli sprouts (Guo and others 2013).

2.3.1.2 Cystein sulfoxide lyase activity

The activity of cysteine sulfoxide lyase enzyme extracted from cabbage is optimum at around pH 8.5 to 9 (Hall and Smith 1983).

Dimethyl disulfide formation and precursor compounds were investigated in cooked cabbage. S-methylcystein sulfoxide decomposition to form dimethyl disulfide in basic fractions of cooked cabbage extract was found higher than in acidic fractions (Dateo and others 1957). Dimethyl disulfide formation in broccoli florets was found to be higher at higher pH values such as pH 9 than pH 5 (Tulio and others 2002). When the fresh tissues of broccoli florets were homogenized, high amounts of dimethyl disulfide and trace amounts of methyl mercaptan were formed. When tissue pH was changed to pH 8.0 with buffer solution, the amount of dimethyl disulfide increased 3 times, but methyl mercaptan amount remained insignificant (Tulio and others 2002).

Freeze-thawing caused an inhibition in the formation of dimethyl disulfide and methyl mercaptan in broccoli florets. Moreover, when buffer solution with a pH 8.0 was added into frozen-thawed tissues, methyl mercaptan remained unchanged, but dimethyl disulfide increased from 0.6 nmol/g to 9.1 nmol/g (Tulio and others 2002).

Methyl mercaptan is a degradation product of methyl methanethiosulfinate (MMTSO), which is generated from S-methyl-L-cystein sulfoxide by the activity of cysteine sulfoxide lyase. MMTSO amount is dependent on pH, and it was found in higher concentrations in basic than in acidic conditions (Dan and others 1997).

2.3.2 Effect of growing conditions, maturity and cultivar

MacLeod and Nussbaum (1976) showed that there are differences between different cabbage cultivars in terms of relative percentages of the volatile compounds. The researchers confirmed their results by sensory tests. Summer Monarch (SM) and Elsoms New Hybrid (ENH) cultivars released higher amounts of acetaldehyde, dimethyl sulfide, propanal, trans-hex-2-enal and allyl isothiocyanates and lower amounts of

ethanol and methanol compare to other cultivars, Ennes Cros (EC), Autumn Monarch (AM) and Winter Monarch (WM). SM and ENH were found to have better flavor in sensory analyses. They also observed one particular cultivar during different seasons and reported the seasonal differences of volatiles. While a particular cultivar, SM, had a good flavor with a moderate amount of allyl isothiocyanate in mid-season, EX showed a different pattern and was not the best in the same season as SM (MacLeod and Nussbaum 1976).

Maturity was also reported to be one of the factors affecting the cabbage aroma. Three cultivars were analyzed while they were not yet mature, mature and when almost going to seed. In mature plants a peak was observed for generally desirable compounds. Allyl isothiocyanate amount was the lowest in over-mature plant, while it was the highest in immature plant (MacLeod and Nussbaum 1976).

Crop spacing was also found to make a significant difference in flavor composition of cabbage. Apart from normal spacing, 60 cm, the effect of wide (75 cm) and close (45 cm) spacing was investigated. Normal spacing gave the best flavor composition and it was preferred in sensory tests. 45 cm spacing increased the percentage of sulfurous compounds. Allyl isothiocyanate amount increased from 1% at wide spacing to 24% at close spacing. It was claimed that allyl isothiocyanate amount was mainly responsible from the strength of cabbage flavor (MacLeod and Nussbaum 1976).

Refrigerated storage of cabbage at 1 ± 1 °C between 5 and 215 d gave different results for 3 different cultivars. While F1 Mercury had higher amount of volatile isothiocyanates, Hidena and Safekeeper cultivars had lower amounts of volatile

isothiocyanates than F1 Mercury. F1 Mercury had also higher quality than others during the storage (Chong and Berard 1983).

2.3.3 Effect of processing

In broccoli florets, dimethyl disulfide and methyl mercaptan were formed when fresh tissues were crushed manually. On the other hand, only dimethyl disulfide was found to be formed when broccoli floret tissues were homogenized in distilled water. Formation of dimethyl disulfide was inhibited by freeze-thawing of the tissues (Tulio and others 2002). The activity of cysteine sulfoxide lyase enzyme, which leads to the formation of dimethyl disulfide, in frozen-thawed and fresh broccoli florets was not significantly different from each other (Tulio and others 2002).

2.4. Lipoxygenase derived volatile compounds

Lipoxygenases have a significant effect on the formation of volatile compounds that have a major role on flavor characteristics of fruits and vegetables. Oxidation of polyunsaturated fatty acids (PUFA) is catalyzed by these enzymes. Lipoxygenase (LOX) uses linoleic and linolenic acids as major substrates and transform them into hydroperoxides in the presence of oxygen. Different isoenzymes of LOX insert oxygen in carbon-9 or carbon-13. Thus, 13S-ROOH, 13R-ROOH, 9S-ROOH and 9R-ROOH can be synthesized from linoleic and linolenic acid. All of these compounds are used as substrates of hydroperoxide lyase and *cis-trans* isomerases producing aldehydes and alcohols that are responsible for off flavors. LOX isoenzymes can be classified according to their characteristics under different conditions such as pH, activation and inhibition properties by calcium and co-oxidation properties of carotenoids. LOX I, LOX II and

LOX III have optimum pH around 9.0-9.5, 5.5-6.5, and neutral pH respectively (Jacobo-Velázquez and others 2010).

2.4.1 Effect of pH on lipoxygenase activity and lipoxygenase derived volatile compounds

In a study regarding avocado lipoxygenase activity, pH 6.5 was found to be optimum pH value at 40 °C for avocado lipoxygenase. pH 6.5 was also reported as natural pH value of avocado pulp (Jacobo- Velázquez and others 2010).

Lipoxygenase from tomato fruit was found to have an optimum pH of 6.5 (Bowsher and others 1992).

2.5 SIFT-MS

The principle of SIFT-MS technique is based on the reactions between ions and molecules which are measured in the gas phase. It is a sensitive method for real-time measurement of concentrations of trace gases and vapors of volatile compounds in air sample. SIFT-MS is based on chemical ionization (CI) using selected reagent ions, H_3O^+ , NO^+ , and O_2^+ , coupled with fast flow tube technology and quantitative mass spectrometry. The selected reagent ions do not react rapidly with the major components of air such as N_2 , O_2 and Ar, CO_2 and water vapor. However, they react rapidly with most volatile organic compounds and many inorganic molecules that are of interest in research (Spanel and Smith 1999).

The SIFT-MS process contains five major steps as shown in Figure 5. (1) SIFT-MS generates reagent ions by using a microwave discharge or radio frequency source. (2) Other than selected precursor ions, generally H_3O^+ , NO^+ , and O_2^+ , all ions are removed by a quadrupole mass filter in an upstream chamber. (3) After selecting ions, the precursor ions pass through a venturi to the reaction chamber (the flow tube) and react

with the volatiles in sample. (4) The reaction products are selected in a downstream chamber by a second quadrupole mass filter. (5) The selected product ions are detected and quantified by a particle multiplier (Syft Technologies Ltd. 2007). The instrument can quantify trace volatiles to ppb levels (Spanel and Smith 1999).

5 different types of reactions occur between the reagent ions and the analyte in the sample being measured. The first one is the proton transfer. In this type of reaction there is a hydrogen transfer to the analyte. The product then is the analyte plus one proton.

The second type of reaction is the charge transfer reaction which occurs when the reagent removes a charge from the analyte. The product is the analyte with a positive charge and the reagent ion is neutralized.

The third type of reaction is the dissociative charge transfer reaction where a charge transfer takes place with fragments formed due to the reaction.

This reaction generally occurs with O_2^+ , but NO^+ can also participate the reaction when those compounds have low ionization energy. The fourth type of reaction is an association reaction. In this type of reaction, there is a three body collision between the reagent ion, analyte, and either the carrier gas (helium), nitrogen, or oxygen (symbolized by M).

NO^+ is the common reagent ion in this type of reaction, but it can be seen with H_3O^+ .

Hydride extraction is the last class of reaction seen between the reagent ions and sample analyte. In this case, negative hydrogen ion is removed by the reagent ion.

The type of reaction depends on the chemical structure of the analyte and which reagent ion it reacts with. For best results, a mass scan mode is used to determine which compounds to be analyzed specifically for the target product (Smith and Spänzel 1999, 2005).

SIFT-MS has a compound library including the rate coefficients and the product ions generated from the particular precursor ion/trace gas compound reactions. The library has been created by using previous SIFT studies of the reactions of numerous compounds such as alcohols, aldehydes, ketones, and hydrocarbons with the reagent ions, H_3O^+ , NO^+ , and O_2^+ (Spänzel and Smith 2011).

The SIFT-MS instrument has two operation modes. The first mode of operation is full-scan mode. When the instrument is used in this manner, sweeping the detection quadrupole ion over a selected mass-to-charge ratio for a set amount of time is used while the sample which is analyzed is introduced to the system. The result of this scan is a complete mass spectrum of the analyzed sample. From the number of counts and total sampling time for each ion, the count rates are calculated. The count rate is stored and displayed as a linear/semi-logarithmic scale, and the mass spectra are interpreted by relation of product ion peaks to trace gas samples of the analyte. The second mode of operation in SIFT-MS is the multiple ion monitoring mode (MIM) or selected ion mode (SIM). In this mode, the count rates of the precursor ions and those of selected product

ions are monitored. For this purpose, the downstream mass spectrometer is rapidly switched between the masses of all the primary ions and the selected product ions. Then, each of these masses is examined for a certain amount of time. The fast time response of SIFT-MS (approximately 20 ms) makes possible real-time monitoring of the analytes. The machine's specifically-designed interface driver computer program performs the switching between different ions and count rates (Smith and Spanel 2005). Schematic diagram of SIFT-MS is shown in Figure 2.4.

Figure 2.4. Schematic diagram of selected ion flow tube (Spanel and others 1996).

Chapter 3 : MATERIALS AND METHODS

3.1 SIFT-MS Analyses

Headspace measurements were taken using selected ion mode (SIM) with a selected ion flow tube-mass spectrometer (SIFT-MS) (Voice 200, Syft Technologies Ltd., Christchurch, New Zealand). Selected compounds were measured in headspace analysis with Syft VOICE-200 software (v.1.4.9.17754, Syft Technologies Ltd., Christchurch, New Zealand). Headspace measurements had a scan time of 60 sec. The headspace volatile compounds were sampled directly by coupling the inlet port of the instrument with an 18 gauge 3.8-cm-long stainless steel piercing the septa. A hot water sample (above 50 °C) was run after each measurement in order to clean compounds remaining from the previous run from the flow tube. The volatiles selected for headspace measurement along with SIFT-MS settings are listed in Table A.3. The major cabbage volatiles important for aroma are presented in Table 3.1.

Table 3.1. Major cabbage volatiles, reagent ions and masses of the compounds used in this study

Compound	Reagent	Mass (m/z)	Product	Reference
(E)-2-hexenal	NO ⁺	97	C ₆ H ₉ O ⁺	c, e, f
3-hexen-1-ol	NO ⁺	82	C ₆ H ₁₀ ⁺	d, e
allyl cyanide	H ₃ O ⁺	68	C ₄ H ₅ N.H ⁺	a, e
allyl isothiocyanate	H ₃ O ⁺	100	C ₄ H ₅ NS.H ⁺	a, b, c, d, e
carbon sulfoxide	O ₂ ⁺	60	COS ⁺	a, b
dimethyl disulfide	NO ⁺	94	(CH ₃) ₂ S ₂ ⁺	a, b, c, e
dimethyl sulfide	H ₃ O ⁺	63	(CH ₃) ₂ S.H ⁺	a, b, e
dimethyl trisulfide	NO ⁺	126	C ₂ H ₆ S ₃ ⁺	a, b, c
hydrogen sulfide	H ₃ O ⁺	35	H ₃ S ⁺	a, b
methyl mercaptan	H ₃ O ⁺	49	CH ₄ S.H ⁺	b, e

^a Bailey and others 1961, ^b Chin and Lindsay 1993, ^c Banerjee and others 2014, ^d MacLeod and MacLeod 1968, ^e MacLeod and Nussbaum 1977, ^f MacLeod and MacLeod 1970

Allyl isothiocyanate was analyzed as a mixture of three volatiles together with allyl thiocyanate and 1-cyano-2,3-epithiopropene. 1-cyano-2,3-epithiopropene does not have a significant effect on cabbage flavor because of its weak flavor properties and high threshold detection value (Chin and others, 1996).

3.2 Headspace Measurements

Green cabbage (*Brassica oleracea* var. *capitata*) was purchased from a local market (Kroger, Columbus, OH, USA). After outer leaves were removed, cabbage heads were cut into half and the cores were removed. Half of the cabbage halves were promptly cooled to 4 °C and the rest of the halves were kept at 25 °C until shredding. The halves were shredded with a food chopper (Hobart, ITW Food Equipment Group North America, Troy, OH, USA) and mixed to obtain a homogenous mixture of the inner and outer parts of cabbage. A 1/2" shredder plate was used for shredding. Immediately after shredding, 50 g samples were weighed and transferred into a 500 ml Pyrex media storage bottle, and capped with open top screw caps fitted with polytetrafluoroethylene (PTFE)-faced silicone septa.

Buffer solutions with pH 8, 6.4, 4.6 and 3 were prepared to measure the effect of pH on volatile formation. Three different stock solutions, 0.1 M citric acid, 0.2 M $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ and 0.2 M monobasic sodium phosphate were prepared previously. Volumes of 15.4, 26.7 and 39.8 ml of 0.1 M citric acid solution and 34.6, 23.3 and 10.2 ml of 0.2 M $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ solution were mixed together and diluted to a total of 100 ml with distilled water to prepare buffer solutions that have pH levels 6.4, 4.6 and 3, respectively. For pH 8 buffer preparation, 5.3 ml of 0.2 M monobasic sodium phosphate solution and 94.7 ml of 0.2 M $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ solution were mixed together and diluted to a total of 200 ml with distilled water.

Two hundred ml of buffer solution and 50 g of shredded cabbage were transferred into Pyrex bottles, and put into a refrigerator to keep the temperature at 4 °C or were kept at 25 °C for 1 h until SIFT-MS analysis started. Buffer solution and cabbage mixtures' final pH levels were 7.4, 6.4, 4.6 and 3.3 (Table A.1, Appendix).

Five replicates were prepared for each buffer solution. Cabbage-buffer mixtures were covered with an open top cap coupled with polytetrafluoroethylene (PTFE)-faced silicone septa. The headspace was measured immediately after removing the samples from the refrigerator. Headspace scan measurements had a scan time of 60 sec to measure the volatiles of cabbage. The volatile analyses were repeated for all samples each day to monitor the volatile levels. The samples were stored in the refrigerator until the analyses were completed. The samples were analyzed during 14 d at 4 °C and 5 d at 25 °C. During SIFT-MS analyses, pH levels of control samples were measured daily without blending the samples to monitor if there was any unexpected change in the samples. At the end of the SIFT-MS experiment, the cabbage-buffer mixtures were blended and pH levels were

measured to determine the final pH of the samples with a pH meter (Denver Instrument Company, Arvada, CO, USA).

3.3 Sensory Test

Sensory analysis of shredded cabbage in different pH buffers was performed by a 102 member untrained panel in the sensory lab at the Ohio State University. Panelists were aged 18 to 60. Samples were prepared on the same day of SIFT-MS analysis and stored in the refrigerator until the sensory test. On the 5th day, sensory analysis was performed. This time was chosen based on preliminary results (data not shown), since there was a significant increase on that day in cabbage volatiles. Also a fresh cabbage was shredded and put into 200 ml of distilled water on the day of sensory analysis and panelists were asked to determine which sample was closest to the fresh cabbage sample. Thus, the effect of volatiles was studied to determine if they have a significant effect on sensory characteristics. Panelists were asked to smell each sample and determine the aroma intensity, as well as which sample has the best aroma, the freshest aroma, and the strongest off odor. Aroma intensity was evaluated by ranking the samples from 1 (least) to 5 (most). There was no dependence of sensory results on gender, age or cabbage consumption frequency.

3.4 Statistical Analysis

The mean and standard deviation values of 5 replicates of shredded cabbage samples were calculated by 2010 Microsoft Excel (Microsoft, Redmond, WA, USA). Sensory data were analyzed by one-way analysis of variance (ANOVA) using Fisher's Least Significant Difference (LSD) procedure to determine significant difference among shredded cabbage samples in different pH buffer solutions. Significance was defined as p

≤ 0.05 acceptance of null hypothesis by using Compusense Sensory Analysis Software (2013, Compusense, Guelph, Ontario, Canada).

Chapter 4 : RESULTS AND DISCUSSION

4.1 Fresh Odor Formation in Different pH Solutions

Allyl isothiocyanate has a pungent aroma and it is the characteristic aroma compound in cabbage (MacLeod and MacLeod 1968; Chin and Lindsay 1993). Allyl isothiocyanate formation was higher in pH 4.6 and 3.3 samples than pH 6.4 and 7.4 samples (Figure 4.1). This may be due to the effect of pH on myrosinase activity.

During storage, pH 3.3 samples had the highest level of allyl isothiocyanate for the first 2 days, but then decreased to the level of the higher pH samples (Figure 4.1). Allyl isothiocyanate in pH 4.6 samples reached its maximum on day 6 and remained constant until day 11. At pH 6.4 and 7.4, allyl isothiocyanate amounts were low during storage. Therefore, allyl isothiocyanate formation was pH dependent, being higher at low pH levels, pH 3.3 and 4.6, than at neutral pH levels, pH 6.4 and 7.4.

Cabbage myrosinase has a high enzyme activity from pH 4 to 8 (Yen and Wei 1993). Although researchers reported optimum activity at pH 8, enzyme activity is high starting from pH 4 and it is almost as high as at pH 8 (Yen and Wei 1993). In our study, at higher pH levels, pH 6.4 and 7.4, much less allyl isothiocyanate was formed than at pH 4.6 and 3.3. However, this result is not in accordance with Yen and Wei's (1993) results of enzyme activity since volatile levels were very low at higher pH. On the other hand,

coleslaw with dressing at pH 4.45 had six times greater allyl isothiocyanate than freshly shredded dry slaw mix at pH 6.4 (West and others 1977). Therefore, myrosinase activity may not be the only factor for the formation of allyl isothiocyanate as there was a weak correlation between enzyme activity reported in previous research and the level of allyl isothiocyanate formed.

Figure 4.1. Allyl isothiocyanate levels during storage in different pH solutions at 4 °C.

Allyl cyanide is derived from the same pathway as allyl isothiocyanate, and it has been reported to be one of the most abundant volatiles in cabbage (MacLeod and MacLeod 1968; Itoh and others 1985). However, no odor description has been reported in the literature for allyl cyanide, likely because it is odorless. West and others (1977)

reported that allyl isothiocyanate level in coleslaw (pH 4.45) was ten times greater than allyl cyanide. Similarly, allyl isothiocyanate concentration was approximately 10 to 20 times higher than allyl cyanide concentration in pH 4.6 samples in this study (Figure 4.1, 4.2). Although allyl cyanide and allyl isothiocyanate are derived from the same pathway, the pH response of the formation of the volatiles was slightly different. While allyl isothiocyanate level increased and remained high at pH 4.6 but not 3.3, allyl cyanide increased and remained high at pH 3.3 but not 4.6. Both levels were low at pH 6.4 and 7.4. The pH response of allyl cyanide has not been presented in the literature.

Figure 4.2. Allyl cyanide levels during storage in different pH solutions at 4 °C.

Green leaf volatiles derived from the lipoxygenase pathway give a fresh green and leafy aroma in cabbage (Banerjee and others 2014). Detection thresholds in water for the volatiles discussed were shown in Table A.4. The green leaf volatiles (*E*)-2-hexenal and

3-hexen-1-ol were not generated in higher amounts than their detection thresholds at any pH level or generated just above the threshold values (Figure 4.3). Their amounts did not change much with storage. There was little difference among pH 3.3, 6.4 and 7.4 samples, while pH 4.6 samples had a much lower amount of (*E*)-2-hexenal than other pH levels. (*Z*)-3-hexen-1-ol was found to be produced in higher amounts at pH 5.6 and 5.9 than pH 7.0 and 7.5 in a previous study analyzing cabbage volatiles (Itoh and others 1985). There is limited available information in the literature regarding the change in green leaf volatiles of cabbage during storage or at different pH. Since the concentrations of green leaf volatiles did not exceed the detection threshold values or were just above the detection thresholds, they may not have an important effect on cabbage aroma.

Figure 4.3. Formation of some of the lipoxygenase volatiles, (*E*)-2-hexenal, 3-hexen-1-ol, and their change during storage in different pH solutions at 4 °C.

4.2 Off Odor Formation at Different pHs

Dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide and methyl mercaptan are defined as off odors in cabbage (Casey and others 1963; Chin and Lindsay 1993; Banerjee and others 2014). These off odors are different from allyl isothiocyanate and allyl cyanide in terms of pH dependence, since the enzymes responsible for their formation are different.

Dimethyl sulfide is derived from the S-methylated form of methionine, S-methylmethionine (81 mg/kg in cabbage) (Casey and others 1963; Cerny 2014). S-methylmethionine easily degrades into dimethyl sulfide during storage and/or thermal treatment of foods (Scherb and others 2009). An enzyme fraction from leaves of cabbage cleaves the S-methylmethionine sulfonium salt to dimethyl sulfide and homoserine. The maximum enzyme activity is at pH 7.8 for this reaction (Lewis and others 1971). Dimethyl sulfide odor was described as ‘decayed cabbage’ (Ruth 1986), and boiled cabbage-like (Morisaki and others 2014). In the present study, dimethyl sulfide was the highest in pH 7.4 samples followed by pH 6.4 and lowest in pH 4.6 and 3.3 samples, and it increased continually during storage (Figure 4.4). Similarly, Lewis and others (1971) reported a maximum activity at pH 7.8 for the enzyme responsible for dimethyl sulfide production. Dimethyl sulfide concentration is frequently higher than other volatile compounds in cabbage (MacLeod and Nussbaum 1977), and is the most abundant volatile of fresh cabbage in certain cultivars (MacLeod and MacLeod 1970). However, its concentration was lower than other sulfur compounds in this study.

Dimethyl disulfide is derived from S-methylcysteine sulfoxide by the action of cysteine sulfoxide lyase enzyme and is also an off odor compound (Kubec and others 1998). At high pH levels, pH 7.4 and 6.4, dimethyl disulfide concentrations were

significantly higher than at low pH levels, pH 3.3 and 4.6 (Figure 4.4). This result is in accordance with the literature. The activity of cysteine sulfoxide lyase enzyme extracted from cabbage is optimum at around pH 8.5 to 9 (Hall and Smith 1983). Similarly, dimethyl disulfide formation in broccoli florets was found to be higher at pH 9 than pH 5 (Tulio and others 2002). Therefore, dimethyl disulfide formation can be expected to be higher at high pH levels than low pH levels.

Dimethyl trisulfide also has a significant sensory effect and is derived from *S*-Methyl-L-cystein (MeCys) and *S*-methylcysteine sulfoxide by the action of cysteine sulfoxide lyase. The odor descriptions of dimethyl trisulfide are spoiled and cooked cabbage-like (Kubec and others 1998; Banerjee and others 2014). Dimethyl trisulfide formation was also high at pH 7.4 and 6.4, while lower pH samples, pH 3.4 and 4.6, produced the compound at very low levels (Figure 4.4). Since the optimum activity of cabbage cysteine sulfoxide lyase is pH 8.5 to 9 (Hall and Smith 1983), it was expected that both dimethyl disulfide and dimethyl trisulfide to be generated in higher amounts at higher pH levels.

Methyl mercaptan is a highly volatile off odor compound in cabbage described as “rotten cabbage” (Chin and Lindsay 1993). *S*-methyl-L-cystein sulfoxide is hydrolyzed by cysteine sulfoxide lyase to form methyl methanethiosulfinate (MMTSO) forms. Methyl mercaptan is a degradation product of MMTSO (Dan and others 1997). Other researchers also reported that the reaction of hydrogen sulfide with MMTSO is the major pathway for the formation of methyl mercaptan (Chin and Lindsay 1994a). Methyl mercaptan is converted to dimethyl disulfide and dimethyl trisulfide (Chin and Lindsay 1994b). Methyl mercaptan was completely converted to dimethyl disulfide by a

cell-free enzyme extract of broccoli after 2 h of incubation (Di Pentima and others 1995). Again, high pH levels trigger methyl mercaptan formation (Figure 4.4). Methyl mercaptan formation and its pH dependence was similar to other off odors, being higher at pH 6.4 and 7.4 and lower at pH 3.3 and 4.6, except the dip on day 6 before increasing gradually again until the end of storage. Since the optimum activity of cabbage cysteine sulfoxide lyase is pH 8.5 to 9 (Hall and Smith 1983), it was expected for methyl mercaptan to be generated in higher amounts at higher pH levels.

Methyl mercaptan is oxidized into dimethyl disulfide in the presence of oxygen (Miller and others 1973). There was no obvious correlation between dimethyl disulfide formation and methyl mercaptan destruction in this study (data not shown). However, the cabbage samples were in buffer solutions and the bottle caps were tightly closed during storage and analysis in this study, so there was minimum oxygen exposure. Since methyl mercaptan concentrations increased to day 3 then and there was a decrease from day 3 to 6 in methyl mercaptan concentration, it is possible that some of the methyl mercaptan was oxidized into dimethyl disulfide.

Figure 4.4. Dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, and methyl mercaptan formation during storage in different pH solutions at 4 °C.

4.3 pH Effect on other Volatiles

Hydrogen sulfide and carbon sulfoxide are also volatiles present in cabbage.

Hydrogen sulfide has a rotten egg odor in durian fruits (Li and others 2012), and sulfur, fetid odor in lychee (Mahattanatawee and others 2007); however, there is limited information about its odor in cabbage. Hydrogen sulfide can be generated from cysteine or sulfite in plants by the enzymatic actions of O-acetylserine (thiol) lyase or sulfite

reductase, respectively (Rausch and Wachter 2005). Hydrogen sulfide formation by the action of O-acetylserine (thiol) lyase was reasonable only at pH values higher than pH 8.0 (Burandt and others 2002). Reported optimum activity for sulfite reductase is at pH 7.4 (Nowak and others 2004). Similarly, hydrogen sulfide formation was highest at pH 7.4 and lowest at pH 3.3 and 4.6 (Figure 4.5). Hydrogen sulfide is a very reactive compound. In a previous study, it was claimed that its disappearance can be related to the formation of off flavors in cabbage (Chin and Lindsay 1993). However, there was no obvious correlation between hydrogen sulfide destruction and off odor formation in this study (data not shown). The mechanism of formation for carbon sulfoxide has not been described in the literature. It was reported to be the most abundant volatile in cabbage in a previous study (Chin and Lindsay 1993). Similarly, carbon sulfoxide was the most abundant volatile in the present study although it is an odorless compound (Figure 4.5). While carbon sulfoxide was highest at pH 3.3 and 4.6 on first days, pH 6.4 and 7.4 samples had the highest concentration of carbon sulfoxide after day 6.

Figure 4.5. Hydrogen sulfide and carbon sulfoxide concentration during storage in different pH solutions at 4 °C.

4.4 Sensory Results

Panelists were asked to rank cabbage samples from 1 (least) to 5 (most) by evaluating them on the strongest off odor, aroma intensity, the freshest aroma, and the best aroma. Freshly shredded cabbage and lower pH (pH 3.3 and 4.6) samples had significantly less off odor, lower total aroma intensity and higher fresh aroma than higher pH (pH 6.4 and 7.4) samples (Table 4.1). Thus, sensory results matched the volatile results where samples at lower pH levels formed the lowest amount of off odors and the most amount of desirable volatiles. The rankings for aroma intensity and strongest off odor were almost parallel, indicating that a greater concentration of off odor may be correlated with greater aroma intensity. Surprisingly, there was no significant difference in terms of having the best aroma. The only sample significantly different from the freshly shredded sample was the pH 7.4 sample. This result was also in accordance with volatile results, as pH 7.4 samples had the highest levels of off odors.

Table 4.1. Sensory test results of samples which were stored for 5 days at 4 °C.

Sample	Strongest off odor (average rank)	Aroma intensity (average mean out of 5)	Freshest aroma (average rank)	Best aroma (average rank)	Closest to freshly shredded sample (average mean out of 5)
Freshly shredded cabbage	2.58 ^c	1.98 ^c	3.11 ^{bc}	2.92 ^a	NA
Stored at pH 3.3	3.05 ^{ab}	2.28 ^b	3.24 ^{ab}	2.99 ^a	2.57 ^a
Stored at pH 4.6	2.72 ^{bc}	2.11 ^{bc}	3.56 ^a	3.01 ^a	2.63 ^a
Stored at pH 6.4	3.20 ^a	3.37 ^a	2.67 ^{cd}	2.86 ^a	2.61 ^a
Stored at pH 7.4	3.44 ^a	3.28 ^a	2.40 ^d	3.21 ^a	2.46 ^b

*numbers with the same superscript letters indicates there is no significant difference between samples in the same column ($p \leq 0.05$)

4.5 Fresh and Off Odor Formation at Different Temperatures

Allyl isothiocyanate is derived from sinigrin by myrosinase activity, and myrosinase activity is dependent on temperature. Cabbage myrosinase activity was maximum at 60 °C, being higher at higher temperatures up to 60 °C (Yen and Wei 1993). Similarly, more allyl isothiocyanate were generated at 25 °C than 4 °C on day 0, but its concentration was same on day 1 and higher at 4 °C after day 2 until the end of the storage (Figure 4.6). Allyl isothiocyanate amount in the headspace decreased over storage time at 25 °C (Table A.2, Appendix). Although allyl isothiocyanate concentration was higher at 25 °C only on day 0, there was a rapid decrease during the storage. The degradation of the compound at 4 °C was slower when compared to 25 °C. Therefore, characteristic fresh cabbage aroma was maintained better at lower temperature.

Figure 4.6. Allyl isothiocyanate levels during storage in pH 3.3 buffers at 4 °C and 25 °C.

All volatile compounds with off odors were generated in significantly higher amounts at 25 °C than at 4 °C (Figure 4.7). Optimum activity for cysteine sulfoxide lyase, which is the enzyme that produces dimethyl disulfide and dimethyl trisulfide, was found to be between 36-40 °C in *Allium* species (Krest and others 2000). Therefore, it is expected to have higher concentrations of these volatiles at higher temperatures. On the other hand, hydrogen sulfide and carbon sulfoxide levels were lower at 25 °C than at 4 °C (data not shown).

Figure 4.7. Off odor formation during storage in pH 3.3 buffers at 4 °C and 25 °C.

4.6 Conclusion

In cabbage, volatile compound quantification with SIFT-MS correlated with sensory odor changes overall. From the sensory test results, freshly shredded cabbage and low pH (pH 3.3 and 4.6) samples had significantly less off odor, lower total aroma intensity and higher fresh aroma than high pH (pH 6.4 and 7.4) samples. In the SIFT-MS results, the desirable cabbage odor, allyl isothiocyanate, formation was higher in lower pH samples, than higher pH samples. Off odors (dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide and methyl mercaptan) were formed in lower concentrations in low pH samples than high pH samples. Low pH samples formed less undesirable and more desirable volatile compounds overall. Higher concentrations of all volatile compounds were formed at 25 °C than 4 °C except from allyl isothiocyanate. Allyl isothiocyanate concentration was higher at 25 °C only on day 0, after that, there was a rapid decrease during the storage and it was higher in 4 °C samples. The degradation of the compound at 4 °C was slower when compared to 25 °C; therefore, characteristic fresh cabbage aroma was maintained better at lower temperature. High temperature is not suitable for cabbage storage, since undesirable aroma compound formation is high, loss of desirable aroma occurs rapidly, and microbial growth occurs at very early stage of storage. Thus, low pH salad dressings at low temperature are the most suitable for storage of ready-to use cabbage products in terms of sensory quality.

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APPENDIX: Additional volatile information and pH change of cabbage

Table A.1. pH values of the samples in different buffers in the last day of SIFT-MS experiment (The pH of the aqueous part of the samples were measured during 14 days and pH values were close to blended samples' last day pH values).

Buffer solution	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
pH 3	3	3.27	3.32	3.32	3.33	3.33	3.35	3.32	3.30	3.33	3.32	3.37	3.37	3.35
pH 4.6	4.6	4.55	4.55	4.62	4.61	4.60	4.73	4.62	4.64	4.63	4.66	4.68	4.67	4.69
pH 6.4	6.4	6.43	6.45	6.42	6.39	6.26	6.38	6.39	6.42	6.42	6.42	6.42	6.41	6.42
pH 8	8	7.65	7.57	7.53	7.5	7.47	7.37	7.39	7.36	7.38	7.40	7.38	7.38	7.39

Table A.2. Concentrations of desirable and undesirable volatile compounds in cabbage at 25 °C during 5 day storage period.

Compound	Concentration (ppb)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Allyl isothiocyanate	377	243	152	83	26	20
(<i>E</i>)-2-hexen-1-ol	12	11	8	17	300	1452
(<i>Z</i>)-3-hexen-1-ol	80	55.5	32	41	30	31
Dimethyl disulfide	96	106	62	224	665	1901
Dimethyl trisulfide	98	83	50	138	66	168
Methyl mercaptan	27	37	25	109	5914	9533
Carbon sulfoxide	2787	4423	3565	11337	19003	17254
Hydrogen sulfide	6	4	3	7	21	71

Table A.3. Presence of major cabbage volatiles in common studies, reagent ions and masses of the compounds used in this study

Compound	Reagent	Mass (m/z)	Product	Reference
(E)-2-hexen-1-ol	NO ⁺	83	C ₆ H ₁₁ ⁺	e, f
(E)-2-hexenal	NO ⁺	97	C ₆ H ₉ O ⁺	c, e, f
(E)-2-pentenal	NO ⁺	83	C ₅ H ₇ O ⁺	e
(E)-3-hexen-1-ol	NO ⁺	82	C ₆ H ₁₀ ⁺	g
(Z)-2-penten-1-ol	H ₃ O ⁺	69	C ₅ H ₉ ⁺	d
(Z)-3-hexen-1-ol	NO ⁺	82	C ₆ H ₁₀ ⁺	d, e
(Z)-3-hexenyl acetate	NO ⁺	82	C ₆ H ₁₀ ⁺	g
1-butanol	NO ⁺	73	C ₄ H ₉ O ⁺	h
1-hexanol	NO ⁺	101	C ₆ H ₁₃ O ⁺	f
1-octen-3-one	H ₃ O ⁺	127	C ₈ H ₁₄ O.H ⁺	g
1-pentanol	NO ⁺	87	C ₅ H ₁₁ O ⁺	g
1-penten-3-ol	H ₃ O ⁺	69	C ₅ H ₉ ⁺	g
2,3-butanedione	NO ⁺	86	C ₄ H ₆ O ₂ ⁺	g
2-butenal	NO ⁺	69	C ₄ H ₅ O ⁺	h
2-methoxyethanol	NO ⁺	75	CH ₃ OC ₂ H ₄ O ⁺	g
2-methyl-3-buten-2-ol	NO ⁺	69	C ₅ H ₉ ⁺	g
3-methyl-1-butanol	NO ⁺	87	C ₅ H ₁₁ O ⁺	g
3-methylindole	H ₃ O ⁺	132	C ₉ H ₁₀ N ⁺	h
acetaldehyde	O ₂ ⁺	44	C ₂ H ₄ O ⁺	e, f
acetic acid	NO ⁺	90, 108	NO ⁺ .CH ₃ COOH, NO ⁺ .CH ₃ COOH.H ₂ O	h
acetoin	NO ⁺	118	C ₄ H ₈ O ₂ .NO ⁺	e
acetone	NO ⁺	88	NO ⁺ .C ₃ H ₆ O	e
acetonitrile	NO ⁺	71	NO ⁺ .CH ₃ CN	h
acrolein	NO ⁺	55	C ₃ H ₃ O ⁺	e
allyl cyanide	H ₃ O ⁺	68	C ₄ H ₅ N.H ⁺	a, e
allyl isothiocyanate	H ₃ O ⁺	100	C ₄ H ₅ NS.H ⁺	a, b, c, d, e
benzyl alcohol	NO ⁺	107	C ₇ H ₇ O ⁺	g
butanal	NO ⁺	71	C ₄ H ₇ O ⁺	e
butanone	NO ⁺	102	NO ⁺ .C ₄ H ₈ O	e
carbon sulfoxide	O ₂ ⁺	60	COS ⁺	a, b
carvone	NO ⁺	180	C ₁₀ H ₁₄ O. NO ⁺	g
diethyl ether	NO ⁺	73	C ₄ H ₉ O ⁺	f
dihydrocarveol	NO ⁺	137	C ₁₀ H ₁₇ ⁺	g
dimethyl disulfide	NO ⁺	94	(CH ₃) ₂ S ₂ ⁺	a, b, c, e
dimethyl sulfide	H ₃ O ⁺	63	(CH ₃) ₂ S.H ⁺	a, b, e
dimethyl trisulfide	NO ⁺	126	C ₂ H ₆ S ₃ ⁺	a, b, c

continued

Table A.3:Continued

ethyl acetate	NO ⁺	118	NO ⁺ .CH ₃ OOC ₂ H ₅	g
ethyl methanoate	NO ⁺	104	NO ⁺ .HCOOC ₂ H ₅	g
formaldehyde	H ₃ O ⁺	31, 49, 67	CH ₃ O ⁺ , H ₂ CO.H ⁺ .H ₂ O, H ₂ CO.H ⁺ .(H ₂ O) ₂	f
guaiacol	NO ⁺	124	C ₇ H ₈ O ₂ ⁺	g
hydrogen sulfide	H ₃ O ⁺ , O ₂ ⁺	35, 53, 34	H ₃ S ⁺ , H ₃ S ⁺ .H ₂ O, H ₂ S ⁺	a, b
indole	O ₂ ⁺	117	C ₈ H ₇ N ⁺	h
isopropyl benzene	O ₂ ⁺	105	C ₈ H ₉ ⁺	g
menthol	NO ⁺	155	C ₁₀ H ₁₉ O ⁺	g
methanol	H ₃ O ⁺	33, 51, 69	CH ₅ O ⁺ , CH ₃ OH ₂ ⁺ .H ₂ O, CH ₃ OH.H ⁺ .(H ₂ O) ₂	d, e
methyl acetate	NO ⁺	104	NO ⁺ .CH ₃ COOCH ₃	g
methyl mercaptan	H ₃ O ⁺	49	CH ₄ S.H ⁺	b, e
octanal	NO ⁺	127	C ₈ H ₁₅ O ⁺	f
pentanal	NO ⁺	85	C ₅ H ₉ O ⁺	h
R-limonene	NO ⁺	88	C ₁₀ H ₁₆ ⁺	h

^a Bailey and others 1961, ^b Chin and Lindsay 1993, ^c Banerjee and others 2014, ^d MacLeod and MacLeod 1968, ^e MacLeod and Nussbaum 1977, ^f MacLeod and MacLeod 1970, ^g Itoh and others 1985, ^h Ikeura and others 2012

Table A.4. Detection thresholds (in water) of some of the major cabbage volatiles.

Volatile	Odor Threshold (mg/kg) in Water	Reference
Allyl isothiocyanate	0.375	van Gemert 2011
(E)-2-hexanal	0.017	van Gemert 2011
3-hexen-1-ol	1.63	van Gemert 2011
Hydrogen sulfide	0.0001	van Gemert 2011
Methyl mercaptan	0.00002	van Gemert 2011
Dimethyl sulfide	0.003	Buttery and others 1990
Dimethyl disulfide	0.012	Buttery and others 1976
Dimethyl trisulfide	0.00001	Buttery and others 1976
Carbon Sulfoxide	0.055	Nagata 2003