Managing Quality of Shredded Cabbage during Storage

Miss Benjamaporn Matulaprungsan

Doctoral Program in Environmental Science and Technology
Graduate School of Science and Technology
Niigata University

2020

Abstract

The quality of shredded cabbage during storage was studied by dividing into two major topics. Firstly, the browning characteristics of shredded cabbage from different leaf layers were investigated. Cabbage leave from outer, middle and inner layers were selected and cut in vertically half separated into upper and lower parts prior to be scliced to 3 mm thickness. Six parts of shredded cabbage were obtained, then were packed in foam trays, and stored at 10 °C for 6 days. The results clearly showed that the shredded cabbage from upper parts of the outer and middle layers had obviously browning symptoms during storage while the lower part of these two layers and both parts of the inner layer was observed as less. The polyphenol oxidase (PPO) activities of these two parts were also significantly higher than the other parts. The polyphenol ammonia lyase (PAL) and peroxidase (POD) were found to be remained constant in all parts throughout storage period. The chlorophyll and ascorbic acid contents gradually decreased by time. Secondly, the feasibility of determination of foodborne microbe contamination of fresh- cut shredded cabbage using short wavelength near infrared spectroscopy (SW-NIR) was determined. Good quality of cabbage heads was selected and shredded before separated into two groups by simulating the different method of sample preparation. Then, SW-NIR spectroscopy was applied on two types of solutions, a drained solution from the outer surface of the shredded cabbage (SC) and a ground solution of shredded cabbage (GC) which were inoculated with a mixture of two bacterial suspensions, *Escherichia coli* and *Salmonella typhimurium*. NIR spectra (700 to 1100 nm) were collected from the samples after 0, 4, and 8 h at 37 °C incubation, along with the growth of total bacteria, *E. coli* and *S. typhimurium*. The raw spectra were obtained from both sample types, clearly separated with the increase of incubation time. The first derivative, a Savitzky–Golay pretreatment, was applied on the GC spectra, while the second derivative was applied on the SC spectra before developing the calibration equation, using partial least squares regression (PLS). The obtained correlation (r) of the SC spectra was higher than the GC spectra, while the SECV was lower. The RPD of the SC spectra was higher than the GC spectra, especially in total bacteria, quite fair for the *E. coli* but relatively low for the *S. Typhimurium*. The prediction results of microbial spoilage were more reliable on the SC than on the GC spectra. Total bacterial detection was best for quantitative measurement, as *E. coli* contamination could only be distinguished between high and low values. Conversely, *S. typhimurium* predictions were not optimal for both sample types. Managing the quality of shredded cabbage could be accomplished by both controlling the quality of first product and monitoring the deterioration of finished product. Selecting the lower part of leave of any layers may help reducing the problem from browning. However, the upper part could also be used in the case that the product will be used instantly regardless of storage time. The rapid method to detect and monitor bacteria contamination should be more investigated in order to differentiate the different types of microbe or increasing the sensitivity of detection.

CONTENTS

	PAGE
Chapter 1 Introduction	**1**
1.1 Introduction	1
1.2 Objectives of the study	3
Chapter 2 Review of literature	**4**
2.1 Cabbage	4
2.2 Fresh-cut fruits and vegetables	5
2.3 Browning of fresh-cut fruit and vegetables	8
2.3.1 Browning	9
2.3.1.1 Non-enzymatic browning	9
2.3.1.2 Enzymatic browning	10
2.3.2 Browning mechanisms in plants	11
2.3.2.1 Phenolic compounds	12
2.3.2.2 Phenylalanine ammonialyase (PAL)	15
2.3.2.3 Polyphenol oxidase (PPO)	15
2.3.2.4 Peroxidase (POD)	15
2.4 Microorganisms of fresh cut fruit and vegetables	16
2.4.1 Spoilage organisms associated with fresh cut produced	17
2.4.2 Food borne pathogens	17
2.4.3 Enteric Pathogens (Family *Enterobacteriaceae*)	18
2.4.3.1 *Escherichia coli.*	18
2.4.3.2 *Salmonella spp.*	19
2.5 Near Infrared Spectroscopy	20
2.5.1 Portable Near-Infrared Spectrometers.	22
2.5.2 Near-Infrared Spectral Processing.	23
2.5.3 Applications in the agro-food industry	24
2.5.4 Use of NIR for microbial determination	24
Chapter 3 Difference in leaf positions and locations associated with browning characteristics of the shredded cabbage	**26**
3.1 Introductions	26
3.2 Materials and methods	27
3.3 Results and discussion	32

CONTENTS

	PAGE
Chapter 4 Feasibility of determination of foodborne microbe contamination of fresh-cut shredded cabbage using SW-NIR	**37**
4.1 Introduction	38
4.2 Materials and Methods	39
4.3 Result and discussion	44
Chapter 5 Summery	**53**
5.1 Difference in leaf positions and locations associated with browning characteristics of the shredded cabbage	53
5.2 Feasibility for the determination of foodborne microbe contaminated in fresh-cut shredded cabbage using SW-NIR	53
Acknowledgements	54
References	55

LIST OF TABLES

PAGE

Table 2.1	World Top 10 of cabbage producing countries in 2016	4
Table 2.2	Effect of unit operation of commercial processing lines on aerobic microbial plate counts (APC) form various vegetables (Ahvenainen, 1996)	18
Table 2.3	Microbiological limits for assessment of microbiological quality of ready to-eat foods (Centre for Food Safety, 2007).	20
Table 4.1	Score of RPD (Residual Predictive Deviation), RER (Rang Error Ratio) and RPIQ (Ratio of Performance to Inter-Quartile rang).	44
Table 4.2	Descriptive statistics of microbial growth, total bacteria, *E. coli* and *S. typhimurium*, in two types of samples; drained solution of shredded cabbage (SC) and mixed solution of ground cabbage and microbial suspension (GC).	45
Table 4.3	PLS model result for predicting the microbial contamination in the drained solution of shredded cabbage (SC) and in the mixed sample of ground cabbage and bacterial suspension (GC).	51

LIST OF FIGURES

		PAGE
Figure 2.1	Typical fresh-cut process flow chart for fruits, vegetables and root crops	6
Figure 2.2	Simple diagram of enzymatic browning source	9
Figure 2.3	Comparison of enzymatic and non-enzymatic browning reactions	11
Figure 2.4	Enzymatic browning pathway	12
Figure 2.5	Structure of common phenolic compounds	14
Figure 2.6	The electromagnetic spectrum.	21
Figure 2.7	The iterative nature of NIR	22
Figure 3.1	Separated leave of cabbage to 3 layers as outer layer, middle layer and inner layer	28
Figure 3.2	Each layer was separated 2 part that upper part and lower part	29
Figure 3.3	Percentage of weight loss of each part of shredded cabbage stored at 10 °C	32
Figure 3.4	Browning pigment and browning score of each part of shredded cabbage stored at 10 °C	33
Figure 3.5	Total phenolic content, PAL, PPO and POD activity of each part of shredded cabbage stored at 10 °C	34
Figure 3.6	Total chlorophyll content of each part of shredded cabbage stored at 10 °C	35
Figure 3.7	Total ascorbic content of each part of shredded cabbage stored at 10 °C	36
Figure 4.1	Sample preparation procedures.	41
Figure 4.2	Original SW-NIR spectra of drained solution of shredded cabbage (SC) (a) and mixed solution of ground cabbage and bacterial suspension (GC); (b) at different incubation times. The second derivative Savitzky–Golay pretreated spectra of drained solution from shredded cabbage (SC); (c) and the first derivative Savitzky–Golay pretreated spectra from mixed solution of ground cabbage and bacterial suspension (GC) (d).	47
Figure 4.3	The principle component analysis (PCA) score plot (PC1 and PC2) of the pretreatment SW-NIR spectra of drained solution of shredded cabbage (SC, left) and mixed sample of ground cabbage and bacterial suspension (GC, right), in comparison between different incubation times, 0 h with 4 h, 0 h with 8 h, and 4 h with 8 h.	49

LIST OF FIGURES

PAGE

Figure 4.4. Comparison between actual measured and predicted values, using the full-cross validation of the PLS models for total bacteria, *E. coli*, and *S. typhimurium* contamination in the drained solution of shredded cabbage (SC, left) and mixed sample of ground cabbage and bacterial suspension (GC, right), measured using a portable NIR spectrometer (700–1100 nm). 52

CHAPTER 1
INTRODUCTION

1.1 Introduction

Consumption of fresh-cut (minimal processed) produce is progressively increasing nowadays. It reasons beneficially of either maximal nutrition intake by consumers or less processing by manufacturers. However, physical damage during preparation especially by cutting and trimming causes an increase of the respiration rate, biochemical changes (i.e. cell ruptures and browning), and microbial spoilage, which may result in deterioration of color, texture, and flavor quality before distribution to consumers (Watada et al., 1990; Varoquaux and Wiley, 1994; Martín-Diana et al., 2008).

Basically fresh-cut produces are very susceptible to enzymatic browning reaction when being processed (Degl Innocenti et al., 2007). There have been enormous efforts to control the browning extent; for instance, using of chemical additives to inactivate polyphenol oxidase (PPO) (Tortoe et al., 2007), using anti-browning solutions, such as citric, oxalic, malic, or phosphoric acid, to inhibit PPO activity by reducing pH and/or chelating copper in a food product (Ibrahim et al., 2004), combination of chemical dip and/or edible coating and/or atmosphere control to synergistically maintain the quality (Bico et al., 2009), as well as, atmosphere modification in packaging adapted to increase the ability of the anti-browning solutions (González-Aguilar et al., 2000).

Shredded cabbage, one of the preferable fresh-cut produce, has also been numerously attempted by the same manners of enzymatic browning control (Kaji et al., 1993; Ibrahim et al., 2004; Cliffe-Byrnes and O'Beirne, 2005). Mostly the methodology of researches was attended on studying physical and biochemical appearance of overall shredded cabbages. Recently there have been systematic studies of different biosynthesis level occurrence in different depth of cabbage heads (Solymosi et al., 2004; Kruk, 2005; Nosek et al., 2011). Distribution patterns of biochemical compounds have been revealed of the regulatory role of light, leading to differently plastid differentiation, chlorophyll precursor biosynthesis, antioxidative enzyme activities in different leaf layers. Because of this fact, we hypothesize that, shredded cabbage from different leaf layers may occur different patterns of enzymatic browning. In this work, cabbage heads are separated into 3 leaf layers; outer, central and inner layer for the investigation of weight loss, total chlorophyll content, browning pigment, total

phenolic content, browning score and acceptance score. The comparison of the study is interesting due to probably be associated with browning management relevant to shredded cabbage products.

In addition to the major problem of fresh-cut, the vegetable is contaminations from pathogenic bacteria that human generally obtains and lead to a cause of many diseases especially *Escherichia coli* (*E. coli*) and *Salmonella spp*. They will cause many preferable symptoms such as a headache, stomachache, diarrhea, and illness. So, the research about the determination of pathogen in foods is still interesting. Recently, microbial determination used plate count method disadvantages as taking too long time for detecting. Therefore, these research focuses on a rapid method with high accurate and more environmentally sensitive as near infrared (NIR) technology is prominent.

NIR has been widely used in the Ago-food Industry (Dos Santos et al., 2013). Several studies have been published concerning the application of these techniques the Ago-food industry including micro-organism determination. In previous studies, several reports about using SW-NIR for determining the quantity of total bacteria in the real sample such chicken meat (Lin et al., 2004), flounder fillet (Duan et al., 2014), raw milk (Al-Qadiri et al., 2008) and washed solution of shredded cabbage (Phunsiri et al., 2008). Although, a lot of reports about using real samples for detecting the quantity of total bacteria but report about detection of *E. coli* in real sample was few. Only, the reported about using NIR for classified *E. coli* in the in-vivo sample such as liquids media was found (Siripatrawan et al., 2010), Sterile phosphate buffer (Feng et al., 2015), NaCl solution (Rodriguez-Saona et al., 2001). The report about using NIR for detected *Salmonella sp.* was few. Study of using NIR for detecting contamination of a sample between *E. coli* and *Salmonella spp.*is interested by many food manufacture. Therefore, in this study the feasibility of using NIR for detected total bacteria, *E. coli* and *S. typhimurium* in the solution of washed cabbage and ground cabbage was evaluated. Microbial spoilage in ground cabbage sample was investigated, so that the possibility of microbial spoilage detected in shredded cabbage head was described.

1.2 Objectives of the study

1. To study characteristics of browning reactions on shredded cabbage derived from different location and position

2. To study feasibility for the determination of foodborne microbe contaminated in fresh-cut shredded cabbage using SW-NIR

CHAPTER 2

REVIEW OF LITERATURE

2.1 Cabbage

Cabbage (*Brassica oleracea*) is a biennial or annual plant with a densely-leaves and edible head. Varieties of the plant include red, white, and Savoy cabbages. The plant has a round shape and grows to a maximum of 300 cm on a short, thick stem. Its flowers are yellow while the roots are shallow and fibrous. Japan is top five of cabbage world production (2,300,000 tons/year) (Table 2.1). Shredded cabbage is famous used in that many Japanese food. However, fresh-cut vegetables are easy to deterioration caused microbials that affect to the short shelf life.

Table 2.1 World Top 10 of cabbage producing countries in 2016

World Top 10 : Cabbage producing countries : 2016		
Rank	**Country**	**Production (MT)**
1	China	32,800,000
2	India	8,500,000
3	Russia	3,309,315
4	Japan	2,300,000
5	South Korea	2,118,930
6	Ukraine	1,922,400
7	Indonesia	1,487,531
8	Poland	1,198,726
9	Romania	990,154
10	United States	964,830

Source: https://www.worldatlas.com/articles/the-world-leaders-in-cabbage-production.html [citation data Feb 4, 2020]

2.2 Fresh-cut fruits and vegetables

Fresh-cut products were previously called lightly or minimally processed products which have been trimmed, peeled and/or cut into a fully usable product (Figure 2.1). Fresh-cut products of raw fruits and vegetables have many purposes. It is important to keep the product fresh, without losing its nutritional quality; the products should have a shelf life sufficient to make distribution feasible within a region of consumption, maintaining flavor and freshness. Therefore, handling, processing, and storage operations during fruits and vegetables transformation into fresh-cut products must be carefully selected from available food preservation techniques. Fresh-cut products are important and are developing rapidly because recently, has an increasing consumption in new family or small family. Initially, the food service industry (e. g., institutions) was the main user of fresh cut products. However, they use has expanded to restaurants, supermarkets and warehouse stores. Fresh-cut products are thus convenient foods with the additional benefit of reduced waste for retail consumers. Consequently, the main purpose of fresh cut products is to meet the increasing consumers' demand for convenient food; they also like fresh fruits and vegetables with an extended shelf life. At the same time, it is crucial to ensure food safety and maintain nutritional and sensory qualities. These products also become popular because intact fruits or vegetables are large. However, their commodities are not economical to buy if they are to be consumed by only one person or by a small family (Garcia and Barrett, 2002). However, the microbiology, sensory and nutritional values, and shelf life of fresh cut products should last at least 4-7 days, preferably even longer, up to 21 days, depending on the market (Wiley, 1994). The problems of fresh cut products concern the quality. These are softening and short shelf life due to physical damage or wounds caused by slicing, peeling, cutting, shredding, trimming, coring, removal of protective epidermal cells. They can also be caused by other mechanical injuries in minimally processed fruits resulting in increased rates of respiration and ethylene (C_2H_4) production within minutes (Abe and Watada, 1991). Increases occur in biochemical reactions related to changes in color, flavor, texture, nutritional quality and susceptibility to dehydration. During storage at different temperatures (4–5 ºC, 10 ºC, 13 ºC and 20 ºC) had effect on the shelf life of minimally processed papaya (Morais and Argaños, 2010). These responses occur in disrupted tissues where cellular decompartmentation leads to intermixing of enzymes and substrates, as well as releasing acids and hydrolyzing enzymes (Watada et al., 1990). Consequently, fresh-cut products should probably be kept at a lower temperature than that recommended for intact

commodities. It requires investment in technology, equipment, management systems and strict observation of food safety principles and practices to ensure high quality of the product.

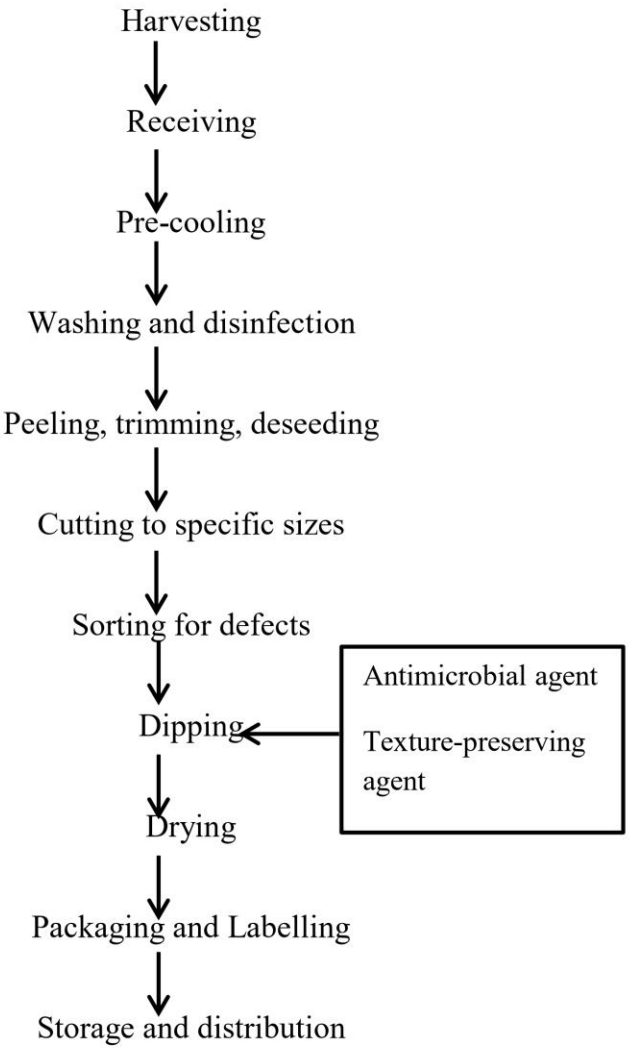

Figure 2.1 Typical fresh-cut process flow chart for fruits, vegetables and root crops (James and Ngarmsak, 2010).

Wounding is one of the primary stresses experienced by freshcut produce, which by its very definition undergoes some form of processing (e.g. slicing, dicing, chopping, trimming, peeling, coring, and/or shredding). Internal and external factors that can affect the wound

response include species, cultivar, maturity, storage/processing temperature, cutting protocols, CO_2 and O_2 levels, and water vapour pressure (Brecht, 1995; Cantwell and Suslow, 2002).

The actual cutting process results in major tissue disruption as previously sequestered enzymes and substrates mix, hydrolytic enzymes are released and signaling- induced wounding responses may be initiated, as shown by Myung et al. (2006) for strawberries (*Fragaria ananassa* L.). Wound-induced signaling is initiated at the site of injury, and then migrates into adjacent, non- wounded, tissue where it can affect suchmetabolic activities as phenolic production in fresh-cut lettuce (*Lactuca sativa* L.) (Choi et al., 2005). In wounded lettuce leaf, the signal arises within 30 min of damage, and moves into unwounded tissue at 0.5 cm h^{-1} (Ke and Saltveit, 1989). A number of volatile compounds may be released upon wounding, including phenylpropanoids, lipoxygenase derived compounds, and terpenoids as demonstrated in strawberries (Myung et al., 2006). Jasmonic acid, auxin and abscisic acid, as well as active oxygen species (AOS), have all been associated with wounding of tissues of numerous plant species (Swamy and Smith, 1999; Hodges et al., 2004; Mur et al., 2006; Takabatake et al., 2006). Salicylic acid, which can act synergistically or antagonistically with jasmonic acid (Lee et al., 2004), may also have a role in the wound response (Saltveit et al., 2005).

The jasmonic acid mediated wound signal transduction pathway requires the activation of a wound-induced protein kinase (WIPK), a defence related mitogen-activated protein kinase (MAPK) (Seo et al., 1999). Another MAPK, the salicylic acid-induced protein kinase (SIPK), is activated in response to a number of abiotic and biotic stresses (Zhang and Klessig, 1997). SIPK has been reported to function upstream of WIPK in an AOS-based signal transduction pathway (Samuel and Ellis, 2002). Enzymatic activation of both WIPK and SIPK are induced by wounding or pathogen infection (Takabatake et al., 2006). One of the most common responses to wounding in plant tissue is an increase in both respiration rate and ethylene production (Saltveit, 1997; Escalona et al., 2003; Saltveit et al., 2005). Wound-induced respiration has been associated with enhanced synthesis of enzymes involved in the respiratory pathway and to a transitory increase in aerobicrespiration infresh-cut carrots (Surjadinata and Cisneros-Zevallos, 2003). In addition, phenolic levels are also increased through wound-induction of phenylalanine ammonium lyase (PAL; EC 4.3.1.5), the committed enzyme in phenolic biosynthesis; these phenolics can be oxidized by polyphenol oxidase (PPO; EC 1.10.3.1) and peroxidase (POD; EC 1.11.1.7) to quinones, which ultimately polymerize to produce the browning appearance common to wounded lettuce (Degl'Innocenti et al., 2005).

2-Aminoindane- 2-phosphonic acid (AIP), an inhibitor of PAL and glyphosphate, an inhibitor of 5- enol- pyruvylshikimate- 3- phosphate synthase of the shikimate pathway, showed significant inhibition of the browning reaction of cut lettuce (Peiser et al., 1998; Hisaminato et al., 2001). Recent work by Saltveit et al. (2005) has implicated the phospholipid signaling pathway in wound signaling associated with phenolic metabolism in wounded lettuce leaf tissue. A common result of the fresh-cutting process is that overall activities of PAL and often POD and/or PPO increase in response to cutting. This response has been reported in fresh-cut potato strips (Cantos et al., 2002), broccoli florets (Brassica oleracea var. italica; Gong and Mattheis, 2003), jicama (*Pachyrhizus erosus* L.) cylinders (Aquino-Bolanos et al., 2000), carrots (Goldberg et al., 1985), and lettuce leaf segments (Hisaminato et al., 2001; Murata et al., 2004). Campos-Vargas et al. (2005) noted that wounding lettuce leaves increased PAL activity by approximately eightfold, and that maximum accumulation of both *PAL*mRNA and enzymatic activity occurred 24 h following wounding.

2.3 Browning of fresh-cut fruit and vegetables

When fruit or vegetable is cut, the flesh oxidizes and turns brown. This process is known as enzymatic browning, and is a reaction catalyzed by the enzyme polyphenoloxidase (PPO). In the reaction, phenolic compounds present in the apple flesh oxidize to form slightly colored o-quinones, which then polymerize to form pigments of varying hues and intensity (Le Bourvellec et al., 2004). The browning reaction is undesirable, leading to an unpleasant appearance, the possible development of off-flavors, and limiting the shelf life of fresh-cut (Sapers 1993; Gámbaro et al., 2006).

The basic enzymatic browning reaction involves the oxidation of colorless monophenols to diphenols, which are also colorless. These then further oxidize to form **o-**quinones, which are slightly colored. In the presence of amino acids and other proteins, these **o**-quinones polymerize into complex brown pigments (Grotheer et al., 2005). Additionally, quinones produced in the initial browning reaction may participate in coupled oxidation reactions, enabling them to oxidize other polyphenolsthat cannot be directly enzymatically oxidized (Cheynier et al., 1994). Figures 2.2 these reactions.

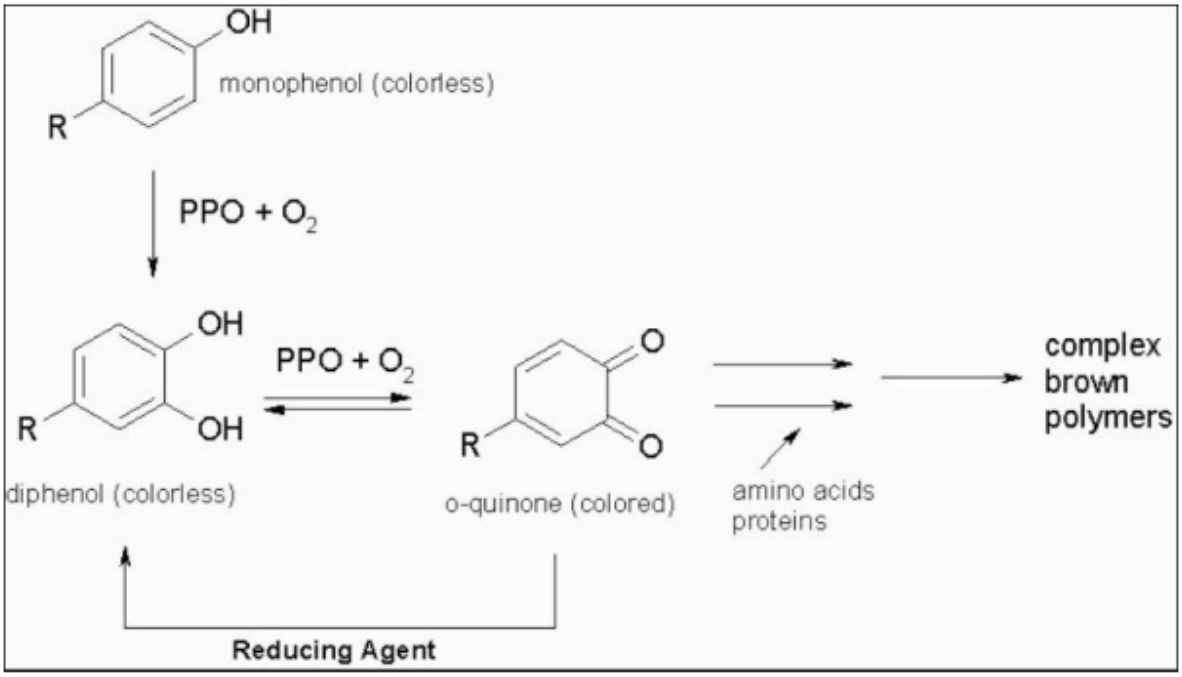

Figure 2.2 Simple diagram of enzymatic browning source (Grotheer et al., 2005)

2.2 Browning

Appearance, flavor, texture and nutritional value are four attributes considered by consumers when making food choices. The appearance is significantly impacted by color which is the first attribute used by consumers in evaluating food quality. Fruit color is influenced by naturally occurring pigments such as chlorophylls, carotenoids and anthocyanins or by some undesirable pigments resulting from both enzymatic and non-enzymatic processes (Marshall et al., 2000).

2.3.1.1 Non-enzymatic browning

Non-enzymatic browning is caused by heat degradation of sugars (caramelization), or by the reaction between reducing sugars and a free amino group, usually of an amino acid or protein. In addition, non-enzymatic browning is commonly found in food materials, and the severity of browning symptom is rapidly increased by a rise temperature and pH above 6.8. While is defect in some products, it is desirable attribute in others such as bakery products, snack foods, nuts and roasted meats (Whitfield, 1992). For example, the browning rates of aldoses, in general,

are higher than those of ketoses, those of pentoses are higher than those of hexoses, and the two-and three carbon sugar analogs are brown very rapidly (Namiki, 1988).

Non-enzymatic browning in fruit and vegetable products can be inhibited by refrigeration, controlling of water activity in dehydrated foods, reducing sugar content in potatoes by storage or glucose oxidase treatment, reducing of amino nitrogen content in juices by ion exchange, packaging with oxygen scavengers or using of sulfites.

Sulfhydryl-containing amino acids are nearly as effective as bisulfite in inhibiting non enzymatic browning in a model system (Saper, 1993). Although there are some effective alternatives of sulfites to retard enzymatic browning reactions, there are fewer effective options for non-enzymatic browning (Saper, 1993).

2.3.1.2 Enzymatic browning

Enzymatic browning is one of the most devastating reactions for many exotic fruits and vegetables, in particular tropical and subtropical varieties. It is estimated that over 50percent losses in fruits occur as a result of enzymatic browning (Whitaker and Lee,1995). Lettuce, potatoes, other green leafy vegetables, other starchy staples such as sweet potato and yam and varieties of the tropical and subtropical fruits and vegetables, for instance, breadfruit, apple, avocado, banana, grape, peache or mushrooms are all susceptible to browning and therefore, cause to the economic losses for the agriculturalists. The control of browning from harvester to consumer is therefore very critical to minimize losses and maintain economic value to the agriculturalist and food producers. Moreover, browning can also adversely affect flavor and nutritional values,

For enzymatic browning occurrence, the appropriate enzymes, substrates and an oxygen supply must be presented. Access to copper, which acts as a catalyst is, also a prerequisite (Macheix et al, 1990). However, in some particular productions such as the manufacture of tea, coffee, cocoa or cider, the enzymatic browning reaction is an essential part and must be distinguished from non-enzymatic browning resulting from the Maillard reaction that occurs when mixtures of amino acids and carbohydrates are heated together (Figure 2.3). Non-enzymatic browning in a product is very complex and numerous hypotheses have been advanced for the mechanism of these reactions (Lee, 1983).

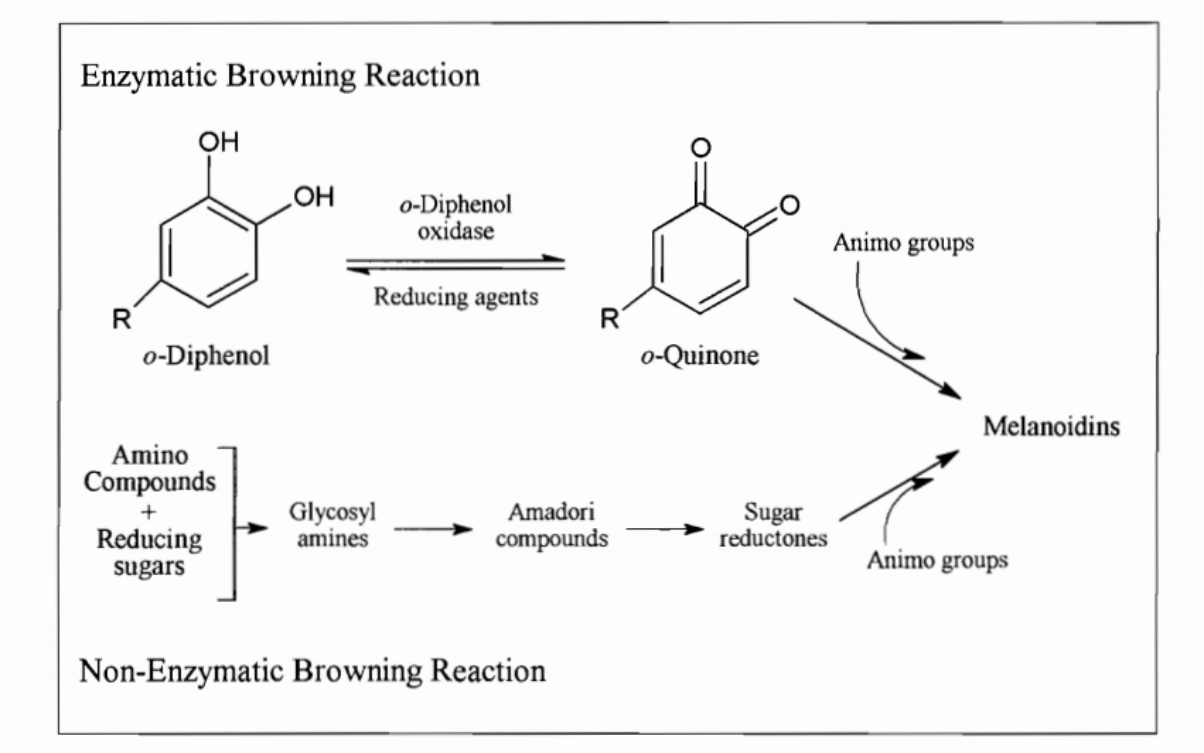

Figure 2.3 Enzymatic browning reaction (Grotheer et al., 2005)

2.3.2 Browning mechanisms in plants

Phenolic compounds are a diverse range of secondary metabolites derived from the shikimate pathway and phenylpropanoid metabolism, which consist of three early steps in the conversion of L-phenylalanine to various hydroxycinnamic acids. The enzymes catalyze the individual steps in this sequence, which are respectively phenylalanine ammonia lyase, cinnamate-4-hydroxylase and 4-coumarate CoA ligase (Haslam, 1998). Postharvest browning in fruits and vegetables has been mainly attributed by the oxidation of phenolic compounds such as polyphenol oxidase (PPO) (Walker, 1995) and/or peroxidase (POD) (Jiang. 1999; Jiang and Li, 2001). These quinones are polymerized to produce brown pigments (Mcevily et al., 1992) (Figure 2.4).

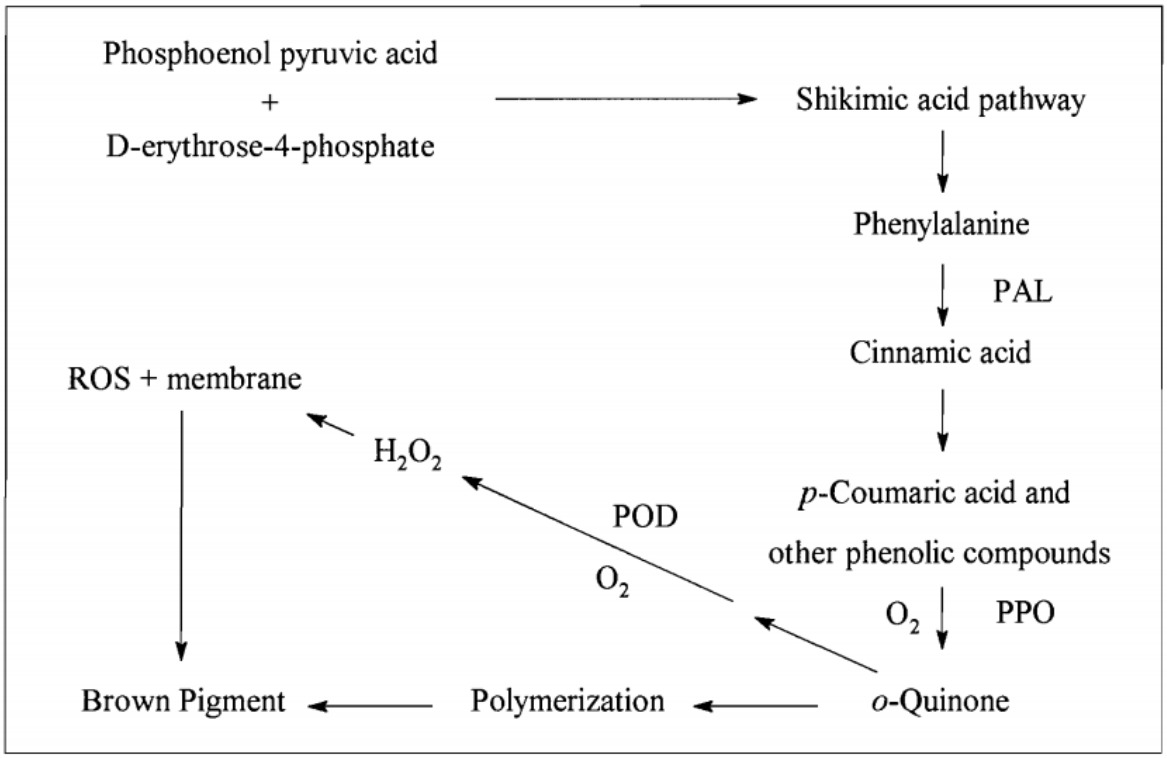

Figure 2.4 Enzymatic browning pathway (Mc Evily et al., 1992)

2.3.2.1 Phenolic compounds

Phenolic compounds are plant-based materials, phytochemicals and widely distributed in the plant kingdom. Plant tissues may contain up to several grams per kilogram of phenolic compounds. There may be 4,000 of these plant compounds, and only a few, such as Vitamin C and E, are publicly discussed to any significant degree. External stimuli such as microbial infections, ultraviolet radiation, and chemical stressors induce their synthesis Plants produce a great variety of organic compounds which are not directly involved in primary metabolic processes of growth and development. The roles of these natural products or secondary metabolites in plants have only recently come to be appreciated in an analytical context. Natural products appear to function primarily in the defense against predators and pathogens and in providing reproductive advantage as attractants of pollinators and seed dispersers. They may also act to create competitive advantage as poisons of rival species.

Most natural products can be classified into three major groups; terpenoids, alkaloids, and phenolic compounds (mostly phenylpropanoids). Terpenoids are composed of five carbon units synthesized by the acetate/mevalonate pathway. Many plant terpenoids are toxins, feeding

deterrents to herbivores and attractants of various sorts. Alkaloids, nitrogen- containing compounds, are principally synthesized from amino acids. These compounds protect plants from a variety of herbivorous animals, and many possess pharmacologically important activity. Phenolic compounds, which are primarily synthesized from products of the shikimic acid pathway, play several important roles in plants. Tannins, lignans, flavonoids, and some simple phenolic compounds serve as defenses against herbivores and pathogens (Figure 2. 5) . Flavonoids are found to comprise mainly quercetin and kaempferol, and the major dietary contributor was black tea, followed by onions and apples. Multivariate regression analysis revealed a significant relationship between flavonoid intake and the risk of mortality from coronary heart disease after adjustment for age and other risk factors (Harbbome, 2008). The phenolic compounds are found in all parts of the plants but their nature and concentration varies greatly among the various tissues contributing to their color and flavor, mainly in the astringent and bitter attributes (Macheix et al., 1990). The phenolic compounds are the diverse range of secondary metabolites derived from the shikimate

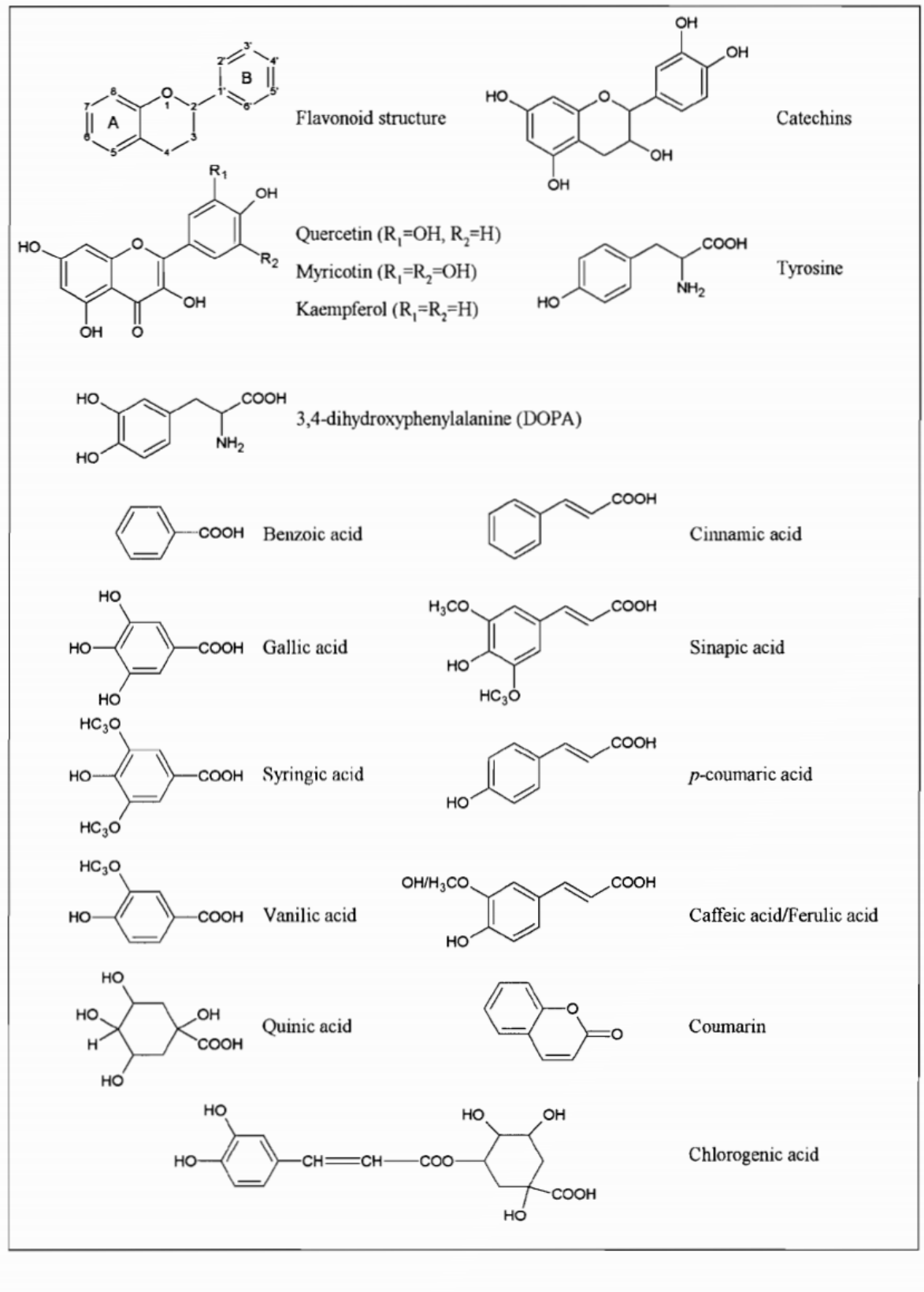

Figure 2.5 Structures of common phenolics (Marshall et al., 2000)

2.3.2.2 Phenylalanine ammonia lyase (PAL)

L-Phenylalanine ammonia lyase (PAL, EC 4.3.1.5) is considered as the key enzyme in phenolic biosynthesis since it catalyses the reductive domination of L-phenylalanine to form trans-cinnamic acid which is the first step in the biosynthesis of plant phenylpropanoid compounds, including the formation of lignins, flavonoids and hydroxycinnamic acids. L-Phenylalanine ammonia lyase activity varies with the development stage of the plant cell and tissue differentiation, as well as various stresses such as irradiation, wounding, nutrient deficiencies, herbicide treatments and viral, fungal and insect attacks. This increases PAL synthesis or PAL activity in various plants (Balsa et al., 1979; Chalker-Scott and Fuchigami, 1989; Lacroix et al., 1990; Waterman and Mole, 1994).

2.3.2.3 Polyphenol oxidase (PPO)

Polyphenol oxidase (1,2-benzenediol: oxygen oxidoreductase; EC 1.10.3.1), is a Cu containing enzyme which is also known as tyroxinase, diphenol oxidase, 0-diphenolase, catechol oxidase, catecholase and phenolase (Figure 2.5). PPO is present in some bacteria and fungi. PPOs are also found in almost all higher plants, including tea, potato, lettuce, mango and apple. PPO catalyzes the oxidation of o-phenolic substrates to o-quinones, which are subsequently polymerized to dark-colored pigments (Billaud et al., 2004).

2.3.2.4 Peroxidase (POD)

POD (EC1.11.1.7) is iron-porphyrin organic catalysts (Figure 2.9) and members of the group of enzymes described as oxidoreductases. They decompose hydrogen peroxide in the presence of a hydrogen donor. They are widespread in nature and those occurring in fruits and vegetables are iron containing. POD has been implicated in lignification, ethylene biosynthesis, praline hydroxylation, IAA degradation and other functions (Pressey, 1990). Changes in POD may be brought about by wounding, physiological stress and infections. POD can catalyze oxidation of many kind of phenols in the presence of oxygen and result in enzymatic browning of harvested fruit, such as pear (Richard and Gauillard, 1997), pineapple (Selvarajah et al., 1998) and peach (Sutte, 1989). In addition, POD are involved in several metabolic plant processes such as the catabolism of auxins, the formation of bridges between components of the cell wall

and oxidation of the cinnamyl alcohols before their polymerization during lignin and suberin formation (Quiroga et al., 2000).

2.4 Microorganisms in fresh-cut fruit and vegetables

Generally, there is a positive correlation between longer shelf life of fresh cut fruits and low aerobic plate count, low total plate count, and especially low yeast and mold counts(Table 2.2). Thus, it is very important to avoid sources of microbial contamination and to wash the fresh cut fruits and vegetables with disinfected water. A number of microorganisms have been found in fresh cut products, including mesophilic microflora, lactic acid bacteria, coliforms, fecal coliforms, yeasts and molds, and pectinolytic microflora (Nguyen and Carline, 1994). The largest19 population is the mesophilic microflora followed by the lactic acid bacteria in processed products. However, the type and population differ with commodity, sanitation and cultural practices. Product quality is found to be acceptable even with high counts of microorganisms, where growth is sustained by the product. Fresh cut products are generally rinsed in 50-200 ppm chlorine solution, but the wash does not eliminate all microorganisms (Wiley, 1994). Wiley (1994) found fresh cut spinach washed with 50 ppm chlorine to contain mesophilic aerobic bacteria, psychrotrophic bacteria, Pseudomonadaceae, Enterobacteriaceae, Vibrionaceae, coliforms, Micrococcaceae, and yeasts. Populations of a few microorganisms reached 1010 CFU/g after the spinach was kept at 10°C for 12 days. These microorganisms were found inside broken cells or cells adjacent to the broken tissue as noted in low temperature scanning electron micrographs. Although fresh cut products are washed with chlorine solution, microorganisms can survive when they are located within cells or areas not penetrated by the chemical. Microorganisms are an important factor to consider when one is working with fresh cut product. In addition, products that show obvious microbial growth even without obvious decay are visually unpleasing and represent significant economic loss to individuals involved in all aspects of the distribution chain. Relatively few types of microorganisms cause the majority of foodborne diseases. Nevertheless, fresh cut products are also occasionally linked to cases of foodborne diseases. It is therefore important that this aspect of microbiology be considered in tandem with spoilage problems.

2.4.1 Spoilage organisms associated with fresh-cut produce

Yeast and molds

Fruits are frequently affected by fungal pathogens because of their relatively high levels of acidity (low pH) while vegetables are susceptible to attack by bacterial pathogens because they are neither acidic nor basic, but are at a neutral pH. In fresh cut environment, faster growing yeasts tend to outgrow molds to cause spoilage. Yeasts are single-celled eukaryotic organisms of which many genera are associated with the fermentation and spoilage of foods. The characteristics of yeasts that allow for growth are their ability to ferment simple carbohydrates to produce alcohol, gas and flavor components, such as esters, acids and higher alcohols; and the ability of some species to grow at relatively low temperatures (10–15°C) (Heard, 2002). In some instances, specific plant pathogenic fungi may be associated with particular forms of spoilage such as the breakdown of tissues (James and Ngarmsak, 2010).

2.4.2 Food borne pathogens

Foodborne pathogens known to contaminate fresh cut products include bacteria, viruses and parasites such as protozoa. Of these, bacteria are of the greatest concern in terms of reported cases and gravity of illness (Table 2.3). The most fruits and vegetables contain nutrients required to support the growth of pathogenic and toxigenic microorganisms. Storage temperature and pH are reported to be the two principal determinants of growth for food borne pathogens associated with fresh produce. Psychrotrophic bacteria, which are organisms that can grow under conditions of refrigeration, vary widely in their acidic tolerances and are the most important spoilage group for fruits and vegetables. The most important of these from a food safety point of view are Listeria and Clostridium. The fact that these organisms can grow at refrigerated temperatures makes them very important in regard to export. Fresh cut vegetables can be occasionally contaminated with food borne pathogenic bacteria such as *E.coli* and *Salmonella* spp. (O'Mahony et al., 1990)

Table 2.2 Effect of unit operation of commercial processing lines on aerobic microbial plate counts (APC) form various vegetables (Ahvenainen, 1996)

Unit operation	**Unit operation**	**APC × 10^4 /g**	
		Before	**After**
Shredder	Cabbage	2.0	78
	Lettuce	18	140
Slicer	Onion	0.4	12
Peeler	Carrot	610	3.6
Centrifuge	Shredded cabbage	63	68
Stick cutter (4 in)	Peeled carrot	65	59
Water bath	Spinach	160	78
Chlorinated ice water	Carrot sticks	64	57
	Shredded cabbage	96	110
	Shredded lettuce	14	0.25
Conveyer belt	Shredded cabbage	78	63
	Cauliflower floret	8.0	5.2

2.4.3 Enteric Pathogens (Family *Enterobacteriaceae*)

2.4.3.1. *Escherichia coli*

Escherichia coli is a part of the natural microflora of the intestinal tract of warm blooded animals and humans. However, there are also strains capable of causing gastrointestinal diseases in humans. These strains are grouped as the enterotoxigenic, enterohemorrhagic, enteropathogenic and enteroinvasive strains of *E. coli* (Doyle et al., 1997). Survival of the organism and mechanisms of contamination in the processing environment have not been studied. Enterohemorrhagic *E. coli* O157: H7 has been recognized in recent years as a foodrelated pathogen and has been responsible for outbreaks linked to a wide range of foods, including fresh produce. It is reasonable to believe that as a result of substandard or even illegal agricultural practices, products may be contaminated with human pathogens such as *E. coli*. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF,1999) in

the United States lists 11 agents associated with product-borne outbreaks. Foremost among them are *E. coli* O157:H7 and various *Salmonella* serotypes (Heard, 2002). *E. coli* O157:H7 can survive in mango pulp for up to 13 days at 6-10°C (Leite, et al., 2002), and in mango juice kept at 7 and 25 °C for 8 and 6 days, respectively, with higher population counts at 7°C (Hisaminato et al., 2001). *E. coli* O157:H7 can grow in papaya juice held at 4 and 20°C (Yigeremu, et al., 2001).

2.4.3.2 *Salmonella* spp.

Within the genus *Salmonella*, differentiation within the species is based on antigenic differences. There are currently over 2370 serovars recognized, however, only 200 are known to cause disease in humans, including *Salmonella typhi*, the causative agent in the typhoid disease (D'Aoust, 2000). Foodborne diseases caused by nontyphoid serovars of *Salmonella* includes gastroenteritis and enterocolitis, with symptoms appearing from 8–72 h after food 22 consumption. More severe complications include septicemia and onset of reactive arthritis (Jay et al., 1997). It is now commonly accepted that fruit and vegetable consumption is a risk factor for infection with enteric pathogens. Recent examples of outbreaks related to fresh produce include cases of *E. coli* O157:H7 (spinach, lettuce), *S. typhimurium* and *S. newport* (tomatoes, lettuce), *S. thompson* (rocket) and hepatitis A (spring onion) (Heaton and Jones, 2007). Fresh products may become contaminated with *Salmonella* either from sewage and contaminated water or from handling by infected workers. A range of fresh fruit and vegetable products have been implicated in *Salmonella* infection; most commonly lettuce, sprouted seeds, melon and tomatoes. *Salmonella* spp. are often isolated from products sampled in routine surveys, including lettuce, cauliflower, sprouts, mustard cress, endive, spinach (Thunberg et al., 2002) and mushrooms (Doran et al., 2005). *Salmonella typhimurium* grew on fresh cut papayas after a six-hour storage period at 25°C (Escartin et al., 1989). *Salmonella typhimurium* and Choleraesuis inoculated in papaya juice grew slowly at 4°C and rapidly at 37°C (Yigeremu et al., 2001). Penteado et al. (2004) found *Salmonella enteritidis* growth in papaya pulp kept at 10, 20 and 30°C.

Table 2.3 Microbiological limits for assessment of microbiological quality of ready to-eat foods (Centre for Food Safety, 2007).

Criterion	**Microbiological quality Colony-forming unit (CFU) per gram unless specified**			
	Class A	**Class B**	**Class C**	**Class D**
Aerobic colony count (ACC) (30°C/48hours)				
Food Category	Level 1	$<10^3$	$<10^3$ - $<10^4$	$\geq 10^4$
	Level 2	$<10^4$	$<10^4$ - $<10^5$	$\geq 10^5$
	Level 3	$<10^5$	$<10^5$ - $<10^6$	$\geq 10^6$
	Level 4	$<10^6$	$<10^6$ - $<10^7$	$\geq 10^7$
	Level 5	N/A	N/A	N/A
Indicator organism (applies to all food categories)				
Escherichia coli (total)	< 20	20 - < 100	≥ 100	N/A
Pathogens (apply to all food categories)				
Campylobacter spp.	Not detected in 25g	N/A	N/A	Present in 25 g
E.coli O157	Not detected in 25g	N/A	N/A	Present in 25 g
Salmonella spp.	Not detected in 25g	N/A	N/A	Present in 25 g
V. cholerae	Not detected in 25g	N/A	N/A	Present in 25 g
Listeria monocytogenes	Not detected in 25g	N/A	N/A	Present in 25 g
For other ready to eat food				
V. parahaemolyticus	< 20	20 - < 100	100- $<10^3$	$\geq 10^3$
S. aureus	< 20	20 - < 100	100- $<10^4$	$\geq 10^4$
C. perfringens	< 20	20 - < 100	100- $<10^4$	$\geq 10^4$
B. cereus	<20	$<10^3$ - $<10^4$	$<10^4$ - $<10^5$	$\geq 10^5$

2.5. Near Infrared Spectroscopy

Near infrared (NIR) radiation covers the range of the electromagnetic spectrum between 780 and 2500 nm (Figure 2.6) (Herschel, 1832; Sathyanarayana, 2007). The measurement of the interaction between this radiation and a sample results in a spectrum characterized by weak absorption bands that are broad and superimposed (Agelet and Hurburgh,2010). In NIR spectroscopy, the product is irradiated with NIR radiation, and the reflected or transmitted radiation is measured. While the radiation penetrates the product, its spectral characteristics

change through wavelength dependent scattering and absorption processes. This change depends on the chemical composition of the product, as well as on its light scattering properties which are related to the microstructure. The typically observed bands in NIR spectra correspond to bonds containing the hydrogen atom, such as C–H, N–H, O–H, and S–H, that are frequently present in most organic and some inorganic compounds. (Blanco, and Villarroya, 2002) A NIR spectrum is essentially composed of overtones and combination bands containing useful chemical and even physical information. The literature on NIR spectroscopy and its applications in different fields is extensive and can be found elsewhere. (Blanco, and Villarroya, 2002)

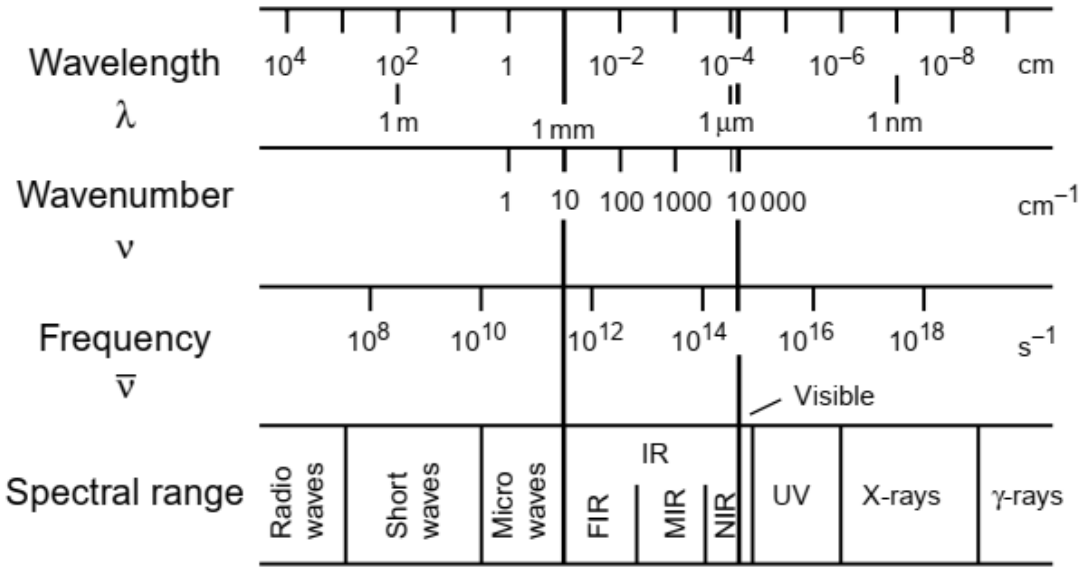

Figure 2.6 The electromagnetic spectrum. (Adapted from Naumann et al..)

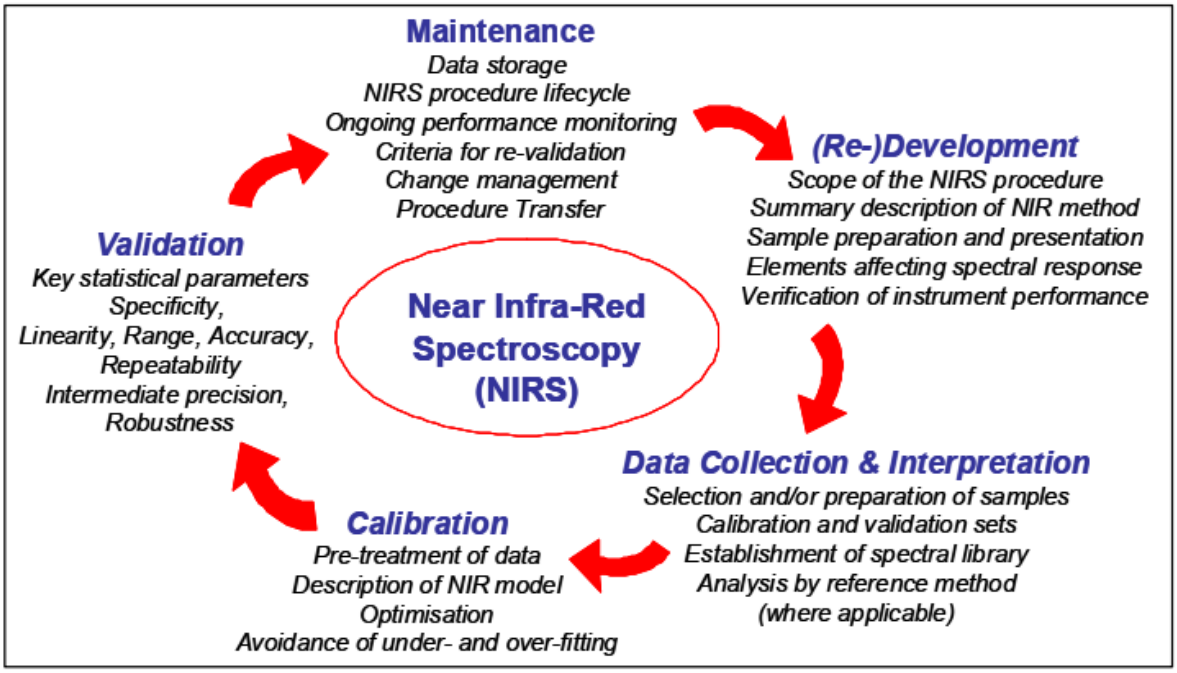

Figure 2.7 The iterative nature of NIR

2.5.1 Portable near-infrared spectrometers

Industrial applications created the need for a cost-effective and nondestructive quality-control system. This requirement increased the interest in NIR spectroscopy, leading to the transformation of large, stationary analytical instruments into lightweight tools that enabled new applications to be implemented in situ at an industrial level (crocombe, 2004). Although these portable NIR spectrometers offer several advantages, such as nondestructive and in situ analyses, their development must consider some critical factors, such as cost, size, weight, power consumption, robustness, safety, userfriendliness, durability, accuracy of measurement, and high performance reliability. Another important characteristic to be taken into account is instrument design. An ergonomic design should make these portable devices handheld, thus enhancing their ease of use. While some devices are sold as closed instruments (and cannot be adapted), others offer the possibility of interchanging measurement accessories so that the same instrument can be used for different sample requirements (Geladi, 1995). Several portable NIR spectrometers are currently available in the marketplace, varying in cost and purpose.

2.5.2 Near-Infrared spectral processing

Chemometrics, usually described as the application of mathematics, multivariate statistics, and computer science (Bosco, 2010), plays a fundamental role in the extraction of all-important relevant information hidden in NIR spectra. Because a NIR spectrum consists of weak and broad absorption bands, a straightforward interpretation is often not possible. Furthermore, there are several other factors that increase the complexity of the spectra: water absorption bands, scattering effects, instrumental noise, sample complexity, and matrix/environmental effects (Nicolaı et al., 2007). Therefore, multivariate analysis methods coupled with spectral preprocessing techniques are the fundamentals of chemometrics for the appropriate treatment of analytical information contained in NIR spectra(Geladi,1995). Essentially, spectral information captured with portable instruments is processed using standard chemometric methods that have been thoroughly described in the literature (Neas et al., 2002). Reducing instrumental noise or background information is usually performed using smoothing techniques. A detrending preprocessing technique is sometimes used to remove the effects of accumulating data sets from a trend, so that only the absolute changes are shown (Barnes et al., 1989). Derivatives are a common method used to eliminate unimportant baseline signals from samples (Savitzky and Golay, 1964). Orthogonal signal correction (OSC) was developed to reduce light-scattering effects, among other general types of interference (not interfering with the correlation with reference values). Path length effects, scattering effects, source or detector variations, and other general instrument sensitivity effects are usually corrected using normalization preprocessing methods, such as standard normal variate (SNV) (Barnes et al., 1989) and multiplicative signal correction (MSC)(Martens et al., 1983; Geladi et al., 1985). Consequently, preprocessing techniques, have been developed to unlock spectral data and enhance the robustness and predictive ability of the calibration model (Agelet and Hurburgh, 2010).Chemometric methods most commonly used to process NIR spectral information and build calibrations are available in the literature and are not developed here (Bosco et al., 2009; Flinn, 2009; Murray, 2004) . The results presented here use the root mean square error of prediction (RMSEP) or root mean square error of cross-validation (RMSECV) as indicators of NIR-based model accuracy.

The equation for root mean square error (RMSE) is

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{i=n} (y_i - \hat{y}_i)^2}{n}}$$

where ^yi is the prediction value of the ith observation, yi is the measured value of the observation, and n is the number of the observations. Equation 1 can be used to compute the root mean square error of calibration (RMSEC), RMSECV, and RMSEP (Neas et al., 2002).

2.5.3 Applications of NIR in the agro-food industry

In the following sections a full description is given of the applications of portable NIR spectrometers in the agro-food industry including the type of sample and its attributes, and, when available, the spectrometer used, its acquisition mode, and its spectral range (in most studies the visible spectral region was also included). This review considers the application of portable NIR instruments in the agro-food industry independently of the application conditions. It is clear that the great advantage of these systems is the possibility of using them under real production conditions; however, many of the studies considered here were actually performed under laboratory conditions (involving sampling and transportation of the samples to the laboratory). Therefore, these studies do not actually assess the performance of the systems under real production conditions and are not examples of taking direct in situ measurements under the uncontrolled conditions typical of production sites.

2.5.4 Use of NIR for microbial determination

NIR spectroscopy is an alternative technique for classification and prediction of microorganism in isolated systems (Feng et al., 2015; Rodriguez-Saona et al., 2001; Siripatrawan et al., 2010). Furthermore, some reports previously utilized short wavelength-near infrared (SW-NIR) techniques for determining the quantity of total bacteria in real food samples, such as chicken (Lin et al., 2004), flounder fillet (Duan et al., 2014), raw milk (Al-Qadiri et al., 2008) and shredded cabbage (Cámara-Martos et al., 2015). These results suggest that NIR is a promising potential technique for monitoring and evaluating the development of microorganisms in real food products. Although the quantitative detection of total bacteria was mostly reported, the qualitative analyses of specific types of bacteria were scarce. Cámara-Martos et al. (2015) attempted to use FT-NIR spectroscopy for evaluating the difference between two bacterial

species, *E. coli* and *Pseudomonas aeruginosa*, inoculated in ultra-high-temperature (UHT) processed whole milk.

CHAPTER 3

Differences in leaf positions and locations associated with browning characteristics of the shredded cabbage

Abstract

The aim of this study was that shredding of cabbage in different leaf layers are different characteristics to turn brown. Therefore, cabbage head was separated into 6 parts, which were multiplying of 3 multiple layers (outer layers- green, middle layers- yellowish green and inner layers- white) and 2 leaf positions (upper part and lower part). After storage of shredded cabbage at 10◦C browning pigment, browning score, total phenolic content and PPO activity shown part 1(upper parts of outer layers) and part 3 (upper part of middle layers) were higher than the other parts. Shredded cabbage from the upper part of outer and middle layer obviously turned brown in high intense during storage period compared to other parts while the lower part of outer and middle layer also both part of inner layer, generated less browning. The chlorophyll content and ascorbic content to all part decreased by storage time.

3.1. Introduction

Nowadays, consumers generally purchase ready to eat produce for their convenience, freshness nutrition safety and eating experience. Shredded cabbage has been used in many kinds of food especially in Japanese food that commonly found in salad, Gyoza, Tonkutsu or fried dishes. Shredded cabbage is produced as a ready to eat product to serve the consumer. For the production of shredded cabbage, harvested cabbage is selected for good quality and then cleaned, and cut into pieces before shredded with commercial machine. The shredded cabbage is packed in package and ready for selling.

Although fresh cut produce provide convenient life for consumer, the physical damage during preparation especially by cutting and trimming causes an increase of respiration rate, biochemical changes (i.e. cell ruptures and browning), and microbial spoilage, which may result in deterioration of color, texture, and flavor quality before distribution to consumers (Watada et al., 1990; Varoquaux and Wiley , 1994; Martín-Diana et al., 2008). Basically fresh-cut produces are very susceptible to enzymatic browning when being processed (Degl'Innocenti et al., 2007). There have been enormous efforts to control the browning extent;

for instance, use of chemical additives to inactivate polyphenol oxidase (PPO) (Tortoe et al., 2007), use anti-browning solutions, such as citric, oxalic, malic, or phosphoric acid, to inhibit PPO activity by reducing pH and/or chelating copper in a food product (Ibrahim et al., 2004), combination of chemical dip and/or edible coating and/or atmosphere control to synergistically maintain the quality (Bico et al., 2009), as well as, atmosphere modification in packaging adapted to increase the ability of the anti-browning solutions (González-Aguilar et al., 2000).

Shredded cabbage, one of the preferable fresh-cut produce, has also been numerously attempted by the same manners of enzymatic browning control (Kaji et al., 2004; Cliffe-Byrnes and O'Beirne, 2005). Mostly the methodology of researches was attended on studying physical and biochemical appearance of overall shredded cabbages. Recently there have been systematic studies of different biosynthesis level occurrence in different depth of cabbage heads (Solymosi et al., 2004; Kruk , 2005; Nosek et al., 2011).

Cabbage head anatomically comprises many layers of leaves where inner and middle layer are covered by outer layer leaves. The outer layers exhibit green leaves while there is less green in the middle of inner leaves. Distribution pattern of biochemical compounds has been revealed of the regulatory role of light, leading to differently plastid differentiation, chlorophyll precursor biosynthesis, antioxidative enzyme activities in different leaf layers. Because of this fact, objective of this study was to evaluate, browning characteristics and enzyme activity of shredded cabbage from different cabbage leaf layers. In this work, cabbage heads were separated into 3 leaf layers; outer, middle and inner layer. The comparison of the study is probably being associated with browning management relevance in shredded cabbage products.

3.2. Materials and methods

3.2.1. Plant Materials

Cabbage were bought from a local market at 'Pak Khlong Talat' (Bangkok,Thailand) and transported to the Postharvest Technology laboratory at King's Mongkut University of Technology Thonburi campus Bangkuntein. Cabbage heads were immersed in 100 ppm sodium hyprochloride for 5 min, removed of defect leaves and dried by fan. And then layers of cabbage head were separated into 3 group (Figure 3.1). The first layer was outer (green), the

second layer was middle (yellow-green) and the thirst layer was inside (white). Furthermore, as each layer was separated 2 part of upper part and lower part (Figure 3.2).

Part 1 – outer layer + upper part

Part 2 – outer layer + lower part

Part 3 – middle layer + upper part

Part 4 – middle layer + lower part

Part 5 – inner layer + upper part

Part 6 – inner layer + lower

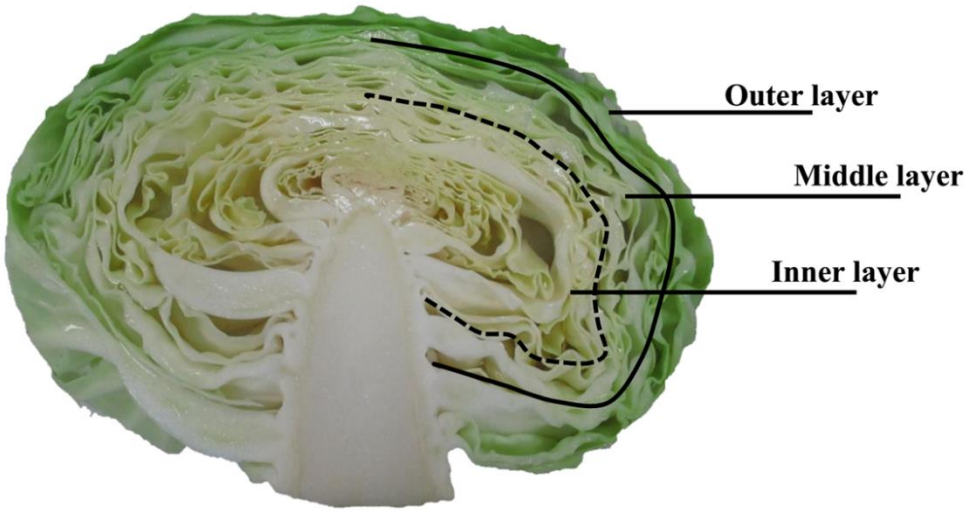

Figure 3.1 Separated leaves of cabbage into 3 layers of outer layer, middle layer and inner layer

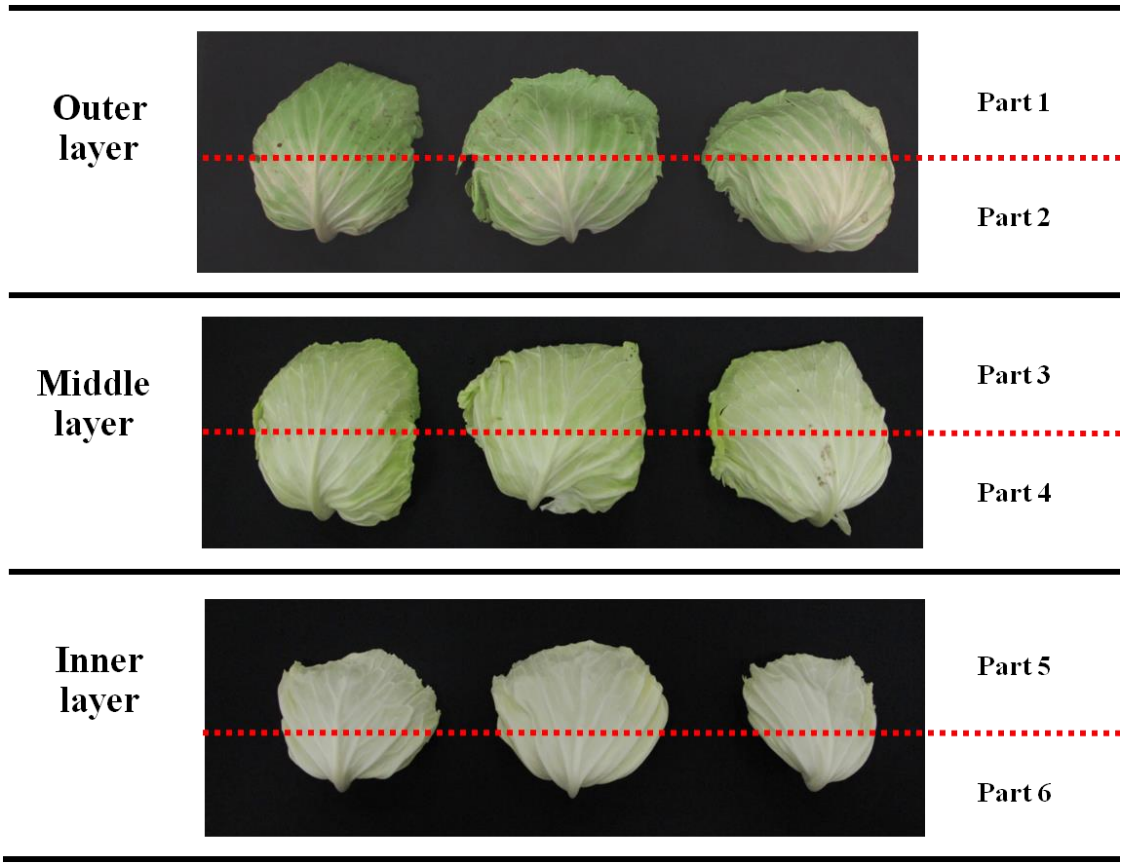

Figure 3.2. Each layer was positioned to 2 part of upper parts and lower part

Each positional part was finely shredded by knife into 3-mm width. Shredded cabbage of each part were dried by vegetable spinner before weighting into 30 grams for one plastic box. All groups were kept at 10 ℃ and 85-90% relative humidity. For parameters including weight loss, browning score, brown pigments, total phenolic, PAL PPO PAL enzyme, total chlorophyll content and total ascorbic content. The sampling was done 0, 2, 4 and 6 days after storage.

2.2 Browning score

Browning score and on cut edges of shredded cabbage was assessed by visual rating (Jame, 1986) (0 = No browning, 1 = Less than 5% of browning, 2 = 5-10% of browning on all of cut edges, 3 = 11-25% of browning on all of cut edges, 4 = 25-50% of browning on all of cut edges, 5 = More than 50% of browning on all of cut edges)

3.2.3 Brown Pigments

Brown pigments defined as browning molecules absorbing light at 410 nm were measured according to the method of Jiang et al. (1999). Five grams of shredded cabbage tissues were finely sliced, ground and extracted with 17 ml of 60% methanol (v/v) in a 0.1 M sodium phosphate buffer (pH 6.8) and 0.5 g of polyvinylpyrolidone (PVP). The extract was centrifuged at 6,000×g for 20 min. and filtered through Whatman # 1 filter paper. The supernatant was collected and diluted to a 1: 4 ratios with the phosphate buffer and measured spectrophotometrically at 410 nm.

3.2.4 Total phenolic content

Content of phenolic compounds was determined using the Folin– Ciocalteu procedure (Singleton and Rossi, 1965). Five grams of the shredded cabbage were homogenised in 100 ml of 80% ethanol. The homogenate was extracted for 4 h in a shaking water bath and filtered using filter paper (Whatman No. 2). A portion (1 ml) of the filtrate was then transferred into a test tube. Folin–Ciocalteu reagent (2 ml) was added, and mixed thoroughly. After 5 min, 2 ml of 10% Na_2CO_3 solution was added and the mixture was left for 1 h. The absorbance of the resulting solution was measured using a spectrophotometer at 640 nm. Concentration of the total phenolic compounds was determined by comparing with the absorbance of gallic acid used at different concentrations as the standard.

3.2.5 Enzyme Assay.

PAL enzyme was extraction and testing of PAL (EC 4.3.1.5), we used the methodology of Ke and Saltveit (1989) with certain modifications. The enzymatic extract was obtained by homogenization of 5 g of shredded cabbage in 20 mL of sodium borate buffer (0.1 M), pH 8.8 with β-mercaptoethanol (5 mm), EDTA (2 mm) and 1% insoluble polyvinyl pyrrolidone (PVPP) (p/v). The extract was centrifuged at 25,000 g for 20 min. with a 4ºC desalinate in a Sephadex G25 column (Sigma-Aldrich, EUA). For this assay, 2.0 mL of L-phenylalanine (Sigma-Aldrich, EUA) (60 mm) in sodium borate buffer (0.1 M), pH 8.8 with 0.5 mL of enzymatic extract was incubated at 40ºC for 1 h. after that add 2 mL of HCL. The absorbance at 290 nm was measured. Sodium borate buffer (0.1 M) was used instead of L-phenylalanine

as a blank. The activity of PAL was expressed as the increase in absorbance (ΔAbs) at 290 nm in 1 hour per milligram of protein in enzymatic extract.

PPO enzymes was extracted as described by Loiza-Velarde et al. (1997). A 2 g sample of shredded cabbage was homogenized at 4 °C with 20 mL of 50 mM phosphate buffer (pH 6.5). The homogenate was filtered and centrifuged at 20000g at 4 °C for 20 min. PPO activity was assayed as reported by Couture et al. (1993). A 0.5 mL volume of enzyme extract was incubated with 0.2 mL of 0.1 mM caffeic acid ethanolic solution, and the absorbance was recorded at 480 nm over a period of 5 min. One unit of PPO activity was defined as the amount of enzyme which causes a change of 0.01 in absorbance per minute.

Peroxidases (POD) were extracted as previously described for PPO. The assays of all POD were done in 1 mL volumes at 25 °C (Bestwick et al., 1998). The absorbance was recorded at selected wavelengths, depending on the substrate. For measurement of guaiacol peroxidase activity (A470), the assay contained 800 μL of 10 mM guaiacol in 50 mM potassium phosphate buffer, pH 6.5, 80 μL of extract and 20 μL of distilled water, and 100 μL of 35 mM H_2O_2. POD assays were initiated by the addition of H_2O_2. The activities of POD are expressed as $A\lambda$ min^{-1} g^{-1} fresh weight.

Total soluble protein was determined according to the method of Lowry et al. (1951) with bovine serum albumin as a standard.

3.2.6 Total chlorophyll content

To measure the chlorophyll content, 20 mL of 80% acetone was added to 5 g of each shredded cabbage sample and mixed with a homogenizer for 1 min at 15,500 rpm, then centrifuged at 8,000 rpm for 5 min at 4 °C (Gokmen, et al., 2005), and then filtered through Whatman No. 1 filter papers. The absorbance values were measured at 664 and 647 nm by spectrophotometer.

3.2.7 Total ascorbic acid content.

ASA and DHA were determined spectrophotometrically as described by Kampfenkel et al. (1995). Shredded cabbage (about 2 g) was transferred to 6% (w/v) trichloroacetic acid (TCA) and the mixture was stirred continuously for 15 min; the mixture was centrifuged at 15000g for 5 min at 4 °C, and the supernatant was immediately used for analysis of ascorbate.

3.2.8 Statistical analysis

Experiments were performed according to a completely randomized design with three replications. Data were analyzed by ANOVA and the means were separated by Duncan's new multiple range test (DMRT).

3.3. Result and discussion

This study was about browning characteristic on shredded cabbage. The result of weight loss shows that part 1 was highest weight loss of all part of cabbage layers but percentage of weight loss of all part were below 3 %. The maximum acceptable weight loss of shredded cabbage is 7 %(Kays,1991; Kang et al., 2002)

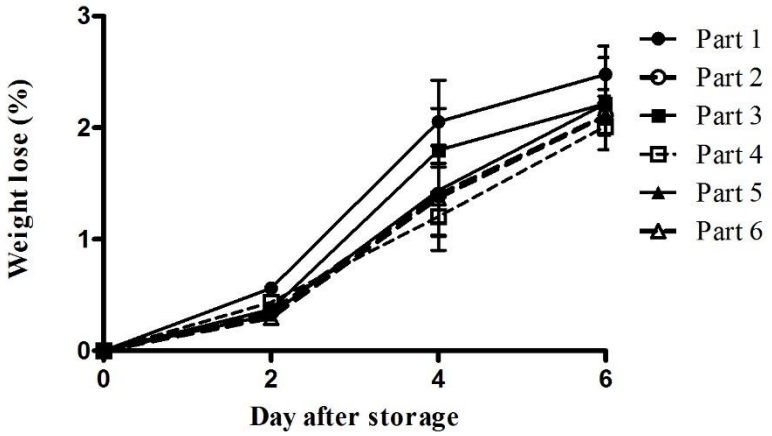

Figure 3.3 Percentage of weight loss of each part of shredded cabbage stored at 10 °C

Browning generation of shredded cabbage

The browning score of shredded cabbage show that the part 1 and part3 have a highest following part 2, 4, 5 and 6 respectively, non-difference of browning score between part 4, 5 and 6. The result of browning score correlated with browning pigment that part 1 and part3 have highest. Therefore, this research can conclusion that Part 1 and part 3 were easy to turn brown.

The browning pigment, the first day of days after storage, part 1 had highest browning pigment, non-difference between part 2, 3, 4, 5 and 6. Browning pigment of part 1 and part 3 were increased after storage at day 2 and day 4 while part 2, 4, 5 and 6 were not increased all of storage life.

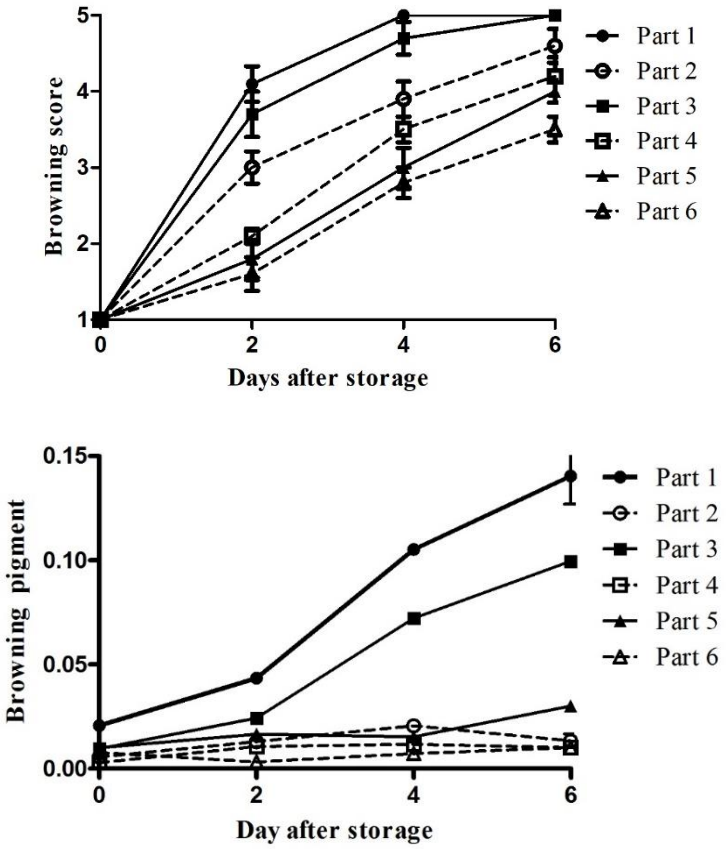

Figure 3.4. Browning pigment and browning score of each part of shredded cabbage stored at 10 °C

Total phenolic content, it was found that it continuously increased in part one and part three. While the other part is quite constant during day zero to day four before slightly increased in day six. So similarly with Seong Yeong Kim (2015) reported that leaf part of kale showed high total phenolic content than branch part. However, part 1 and 3 was higher total phenolic content than other part.

PPO activity of shredded cabbage, found that part 1 and 3 was higher activity of PPO than other part correlated with total phenolic content. PPO enzyme was importance with browning in shredded cabbage.

PAL activity, the enzyme that catalyze the production of phenolic compounds slightly increased on day two in the shredded cabbage from part one and part three, before decreased after that for the other part, PAL remain constant during storage period.

POD activity of all part of cabbage was not difference at day after storage. The POD activity the activity is in the same level for all parts.

The result shown that correlation of total phenolic content, PPO activity. The phenols may have been oxidized by PPO, inducing browning in the minimally processed leaves.

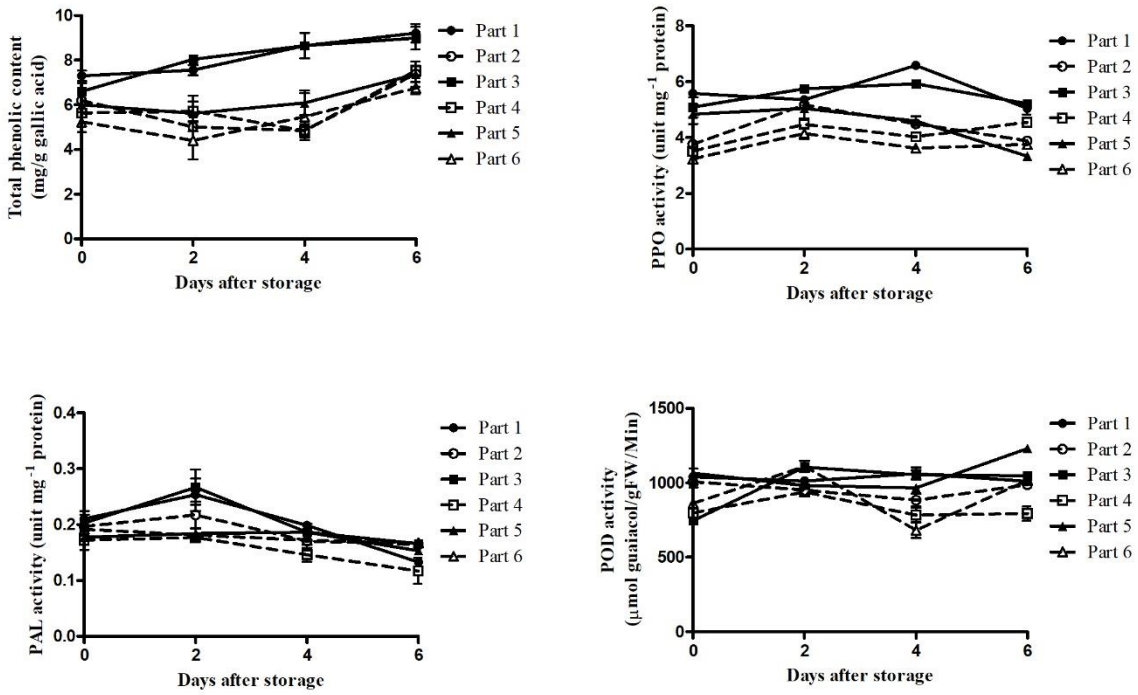

Figure 3.5. Total phenolic content, PPO PAL and POD activity of each part of shredded cabbage stored at 10 °C

The total chlorophyll content, Part 1 contained the highest chlorophyll content following part 3 and part 2 respectively. For total chlorophyll content of part 4, 5and 6 were lowest and no difference between the parts. The part 1 is outer leave of cabbage head comparing part has green color of blade leave. And part 3 is yellow-green color of blade leave. Although part 1 and part 2 are outer layers also, but total chlorophyll content of both parts were differences. The part 1 is upper part of outer layers that part is blade area but lower part (part 2) is mid rib area. As middle layer (part 3 and part 4) that total chlorophyll content of part 3 were different to part 4. For inner layers, part 5 and part 6 were not different in total chlorophyll content. However, the results of total chlorophyll content of cabbage layers were similarly with Jerk (2005) report.

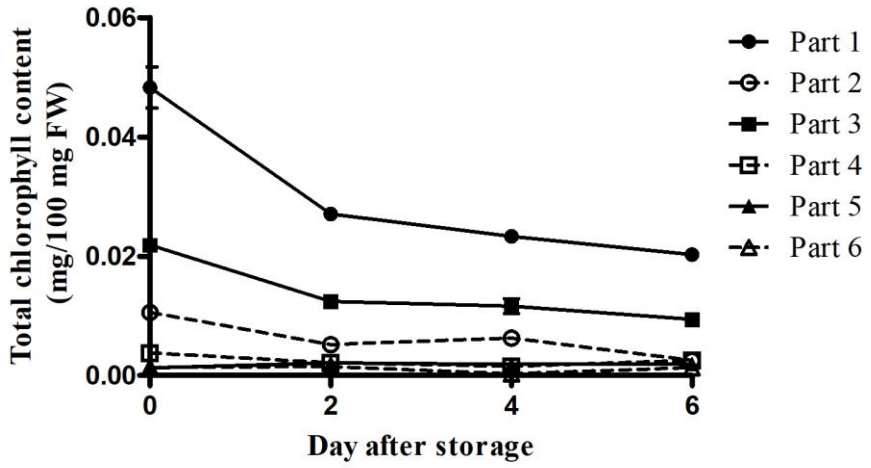

Figure 3.6. Total chlorophyll content of each part of shredded cabbage stored at 10 °C

Total ascorbic contents of part 1 and 3 were lowest compared with other parts (Figure 3.7). In addition, the lower part contained ascorbic content higher than upper part. Correlated with Michal et al.(2011), the inner side had high total ascorbic content than the outer side of cabbage. Total ascorbic content is related to the very active metabolism in young tissues and plant growth processes, such as cell division and cell elongation, as demonstrated earlier on an Arabidopsis vtc-1 mutant by Veljovic-Jovanovic et al. (2001). Our results are also in agreement with previous data (De Gara et al., 1991, 1996; Borraccino et al., 1994; Lennon et al., 1995) showing that developing tissues have higher mitochondrial activity/amount as well as a higher vitamin C level than mature tissues.

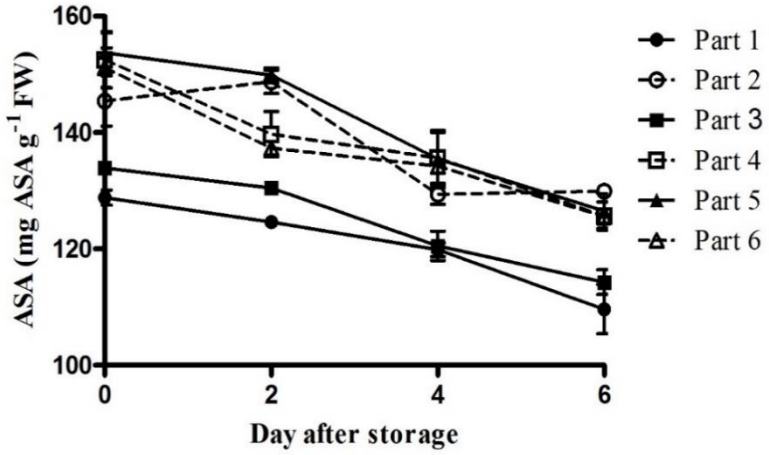

Figure 3.7. Total ascorbic content of each part of shredded cabbage stored at 10 °C

3.4. Conclusions

The lower parts of the leaf and the inner layer of the cabbage head are suitable for producing the shredded cabbage due to less browning generation. However, if the producer produces the shredded cabbage that will be used instantly the upper part of the leaf can be used to make shredded cabbage because it contains high chlorophyll content that would be benefit for the consumer. Nevertheless, this browning symptom could be protected by using some postharvest treatment such as dipping in some organic acid using modified atmosphere packaging the reduce the amount of oxygen in the package.

CHAPTER 4

Feasibility of determination of foodborne microbe contamination of fresh-cut shredded cabbage using SW-NIR

Abstract

Shredded cabbage is widely used in much ready-to-eat food. Therefore, rapid methods for detecting and monitoring the contamination of foodborne microbes is essential. Short wavelength near infrared (SW-NIR) spectroscopy was applied on two types of solutions, a drained solution from the outer surface of the shredded cabbage (SC) and a ground solution of shredded cabbage (GC) which were inoculated with a mixture of two bacterial suspensions, *Escherichia coli* and *Salmonella typhimurium*. NIR spectra of around 700 to 1100 nm were collected from the samples after 0, 4, and 8 h at 37 °C incubation, along with the growth of total bacteria, *E. coli* and *S. typhimurium*. The raw spectra were obtained from both sample types, clearly separated with the increase of incubation time. The first derivative, a Savitzky–Golay pretreatment, was applied on the GC spectra, while the second derivative was applied on the SC spectra before developing the calibration equation, using partial least squares regression (PLS). The obtained correlation (r) of the SC spectra was higher than the GC spectra, while the standard error of cross-validation (SECV) was lower. The ratio of prediction of deviation (RPD) of the SC spectra was higher than the GC spectra, especially in total bacteria, quite normal for the *E. coli* but relatively low for the *S. Typhimurium*. The prediction results of microbial spoilage were more reliable on the SC than on the GC spectra. Total bacterial detection was best for quantitative measurement, as *E. coli* contamination could only be distinguished between high and low values. Conversely, *S. typhimurium* predictions were not optimal for either sample type. The SW-NIR shows the feasibility for detecting the existence of microbes in the solution obtained from SC, but for a more specific application for discrimination or quantitation is needed, proving further research in still required.

4.1 Introduction

Public concern about the safety of fresh produce consumption has been continuously growing. The power of social media also helps to spread the news about foodborne disease infections faster than ever. The awareness of producers is high when it comes to protect the contamination problem of their products. However, many foodborne disease outbreaks in fresh and fresh-cut produce are still being reported (Yu et al, 2018). Fung et al. 2018 found that *Staphylococcus*, *Salmonella*, *Clostridium*, *Campylobacter*, *Listeria*, *Vibrio*, *Bacillus*, and *Escherichia coli* accounted for 90% of global food poisoning illnesses. *E. coli* and *Salmonella* are two pathogenic bacteria mainly concerned with fresh-cut produce (Sivapalasingam et al., 2004; Söderström et al., 2008; Nma et al., 2013; Abadias et al., 2008). This report agrees with the food poisoning statistics from the Japanese Ministry of Health, Labour, and Welfare about the most common causes of foodborne disease in Japan in 2011 (Kumagai et al., 2015). For fresh and fresh-cut producers, assuring the safety of the produce to the consumer is inescapable.

Shiina and Hasegawa (2007) reported the fast multiplication of the fresh-cut produce companies in Japan is due to the high demand of the fresh-cut vegetables, which highly relates to the increase of consumer convenience needs. Among the many kinds of fresh-cut produce, shredded cabbage holds the highest proportion of the market share. The widespread use of shredded cabbage in Japanese cuisine can be observed in restaurants, dining rooms, fast food shops, retail markets, and home-cooking. Hence, producers must ensure the consumers that their products are safe from foodborne disease microorganisms. The conventional microbiological methods, such as total plate count and coliform count, are still conducted in companies as the most reliable method, even though a duration for 48 h or more is needed to obtain the results (Suthiluk et al., 2008). Recently, it was found that the time-consuming process of conventional microbiological methods could be replaced by using Petrifilm™ (3M Company, St. Paul, MN USA), proved to be more convenient as compared to the traditional method (Jordano et al., 1995), but it is still expensive for routine identification. Therefore, many researches attempted to develop reliable and rapid nondestructive methods to determine the microorganism contamination in fresh-cut produce.

Application of near infrared (NIR) spectroscopy has been widely used as an analytical tool for measuring quality attributes of horticultural produce (Nicolaï et al., 2007) and for controlling the quality of agro-food products (Dos Santos et al., 2013). NIR spectroscopy is fast, reliable, and non-destructive, which could help reduce the analytical time of the traditional methods. For the microbial determination, NIR spectroscopy is an alternative technique for

classification and prediction of microorganism in isolated systems (Feng et al., 2015; Rodriguez-Saona et al., 2001; Siripatrawan et al., 2010). Furthermore, some reports previously utilized short wavelength-near infrared (SW-NIR) techniques for determining the quantity of total bacteria in real food samples, such as chicken (Lin et al., 2004), flounder fillet (Duan et al., 2014), raw milk (Al-Qadiri et al., 2008) and shredded cabbage(Cámara-Martos et al., 2015). These results suggest that NIR is a promising potential technique for monitoring and evaluating the development of microorganisms in real food products. Although the quantitative detection of total bacteria was mostly reported, the qualitative analyses of specific types of bacteria were scarce. Cámara-Martos et al. (2015) attempted to use FT-NIR spectroscopy for evaluating the difference between two bacterial species, *E. coli* and *Pseudomonas aeruginosa,* inoculated in ultra-high-temperature (UHT) processed whole milk. The results revealed that NIR could be applied to detect and quantify the bacteria in milk, but further study is needed for increasing the discrimination capacity of NIR. Therefore, the objective of this study is to evaluate the feasibility of using SW-NIR for detecting total bacteria, *E. coli* and *S. typhimurium,* in two different types of solutions obtained from fresh cut shredded cabbage samples.

4.2. Materials and Methods

Preparation of Shredded Cabbage

Cabbage heads were purchased from a local market and then transported to the NIR laboratory at Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom province, Thailand. Three layers of outer leaves of each cabbage head were removed before washing in 50 $mg \cdot L^{-1}$ sodium hypochlorite solution for 5 min. Then, the cabbage heads were cut into quarters by an alcohol-sterilized sharp knife, and then sliced into approximately 3-mm width fresh-cut shredded cabbage. The shredded cabbage was washed again in 50 $mg \cdot L^{-1}$ sodium hypochlorite solution and drained with a colander prior to packing into the sterilized polyethylene bags.

Preparation of Bacterial Suspension

Strains of *E. coli* TISTR527 and *S. typhimurium* TISTR1469 were obtained from the Culture Collection of Thailand Institute of Scientific and Technological Research (TISTR). The strains were sub-cultured on nutrient broth (NB; Media laboratory Mumbai, India) at 37 °C for 24h. Growth of bacterial suspension was harvested when the absorbance of a sample measured at a wavelength of 600 nm (OD_{600}) was at 0.4 in sterile saline was obtained. Each culture was centrifuged at 6000 rpm for 20 min, and the cell pallet was washed twice with a sterilized 0.85% saline solution and then resuspended in 1 mL of a sterilized 0.85% saline solution. Then, the cell suspension of *E. coli* and *S. typhimurium* were gently mixed in a 1:1 ratio and diluted in distilled water to achieve a concentration of about 3–7 log $CFU \cdot mL^{-1}$. Finally, a cell suspension of both strains was inoculated into the solution from the shredded cabbage sample.

Bacterial Inoculum Procedures

The sample preparation procedure is illustrated in Figure 4.1. Shredded cabbage was separated into two groups, non-ground and ground samples. For the non-ground sample, 10 g of shredded cabbage was weighted and packed into a sterilized polypropylene (PP) plastic bag. Then, 2 mL of bacterial suspension was inoculated into each bag and gently mixed. All bags were incubated at 30 °C for 30 min, and 10 mL of sterilized 0.85% saline solution was added into each bag to wash the surface of the sample, and then drained. The drained solution of the shredded cabbage from each bag was collected and incubated at 37 °C for further analysis. For the ground sample, shredded cabbage was ground using a commercial blender for around 30 s to 1 min, and 10 g of the ground sample was weighted. The ground sample was transferred into a sterilized PP bag and 2 mL of bacterial suspension was added. Following this, all bags were incubated at 30 °C for 30 min, prior to the adding of 10 mL of sterilized saline solution and mixed. The mixed sample of ground cabbage and bacterial suspension (GC) was incubated at 37 °C for further analysis. Sampling was done in SC and GC at 0, 4, and 8 h, for the NIR spectra acquisition and microbial growth analysis. According to the final mixture, the SC represented the microbiological composition on the surface of the shredded cabbage, while GC represented both the surface and inside.

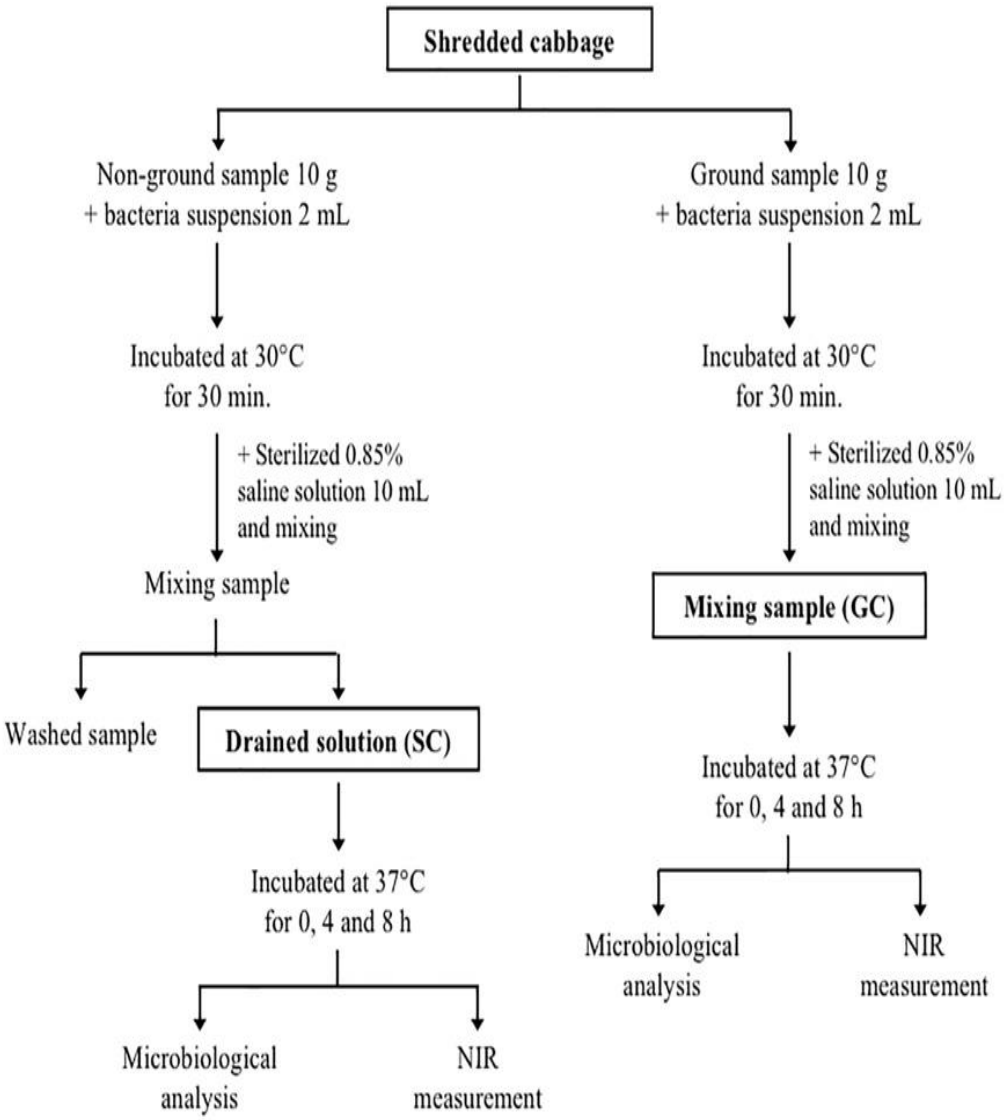

Figure 4.1. Sample preparation procedure.

SW-NIR Spectrum Acquisition

SW-NIR spectra was measured using a portable NIR spectrometer (FQA-NIR GUN, Feinted, Japan), producing a short wavelength region of 700–1100 nm, with a spectral resolution of 2 nm in reflection mode. To obtain the spectra from solution samples, the measuring cell was equipped with an aluminum block test tube holder. A Pyrex®-glass test tube [20 (∅) x 150 (h) mm] was used as the loading sample cell for the SC solution. As for the GC, a 30-mL Pyrex-glass beaker was used for the GC mixture solution and covered with a black cloth during measurement. Two spectra were recorded from two positions in each

sample. The measurement was conducted at room temperature (25 °C). There were 72 spectra in total for the SC, and 69 for the GC samples.

Microbial Analysis

The microbial growth analysis was carried out in the SC solution and GC mixture solution after the NIR measurement. One percentage sterile peptone (100 mL) was added either to 10 mL of SC or to 10 g of GC samples and then mixed well by hand shaking for SC or stomacher (IUL Instruments Masticator, Barcelona Spain) for GC. Each sample was comprised of 2 replications, each replication was managed for 3 dilutions, and each dilution was divided into 2 duplications. An aliquot (100 µL) of each duplication dilution was spread onto a petri dish of plate count agar and incubated at 37 °C for 24 h for the determination of aerobic mesophilic bacteria. While the culture of the eosin methylene blue agar was incubated at 37 °C for 24–36 h for *E. coli* counts, that of the xylose lysine deoxycholate agar was incubated at 37 °C for 24–48 h for the examination of *S. typhimurium*. These media are the standard method for enumerating our interested bacteria (AOAC, 1990). The number of bacteria were expressed as log CFU·mL^{-1} for the SC solution, and as log CFU·g^{-1} for the GC mixture solution.

Data analysis

The CA maker software (Shizuoka Shibuya Seiki, Hamamatsu, Japan) was used to acquire the SW-NIR spectra from the portable NIR spectrometer. The spectra were further analyzed with the Unscrambler® software (CAMO, Oslo, Norway). The original spectra from the different sample preparation methods (SC and GC) and incubation times (0, 4, 8 h) were compared. The pretreatment of the first and second derivatives, by means of the Savitzky–Golay method, were applied to the original spectra. The principle component analysis (PCA) was initially conducted to observe the discrimination of the data set. Then, the pretreatment spectra were analyzed using the partial least square regression (PLS) in full-cross validation method. The optimum pretreatment method for each data set was chosen from the lowest standard error of cross-validation (SECV) and the highest correlation coefficients (r). Following this, the performance of a prediction model was considered from a set of validation samples, which were based on the statistical parameters as bias (ISO12099). The significance of the bias was verified with a t-test using the following formula:

$$T_b = \pm \frac{t_{\left(1-\frac{\propto}{2}\right)SECV}}{\sqrt{n}}$$

where α is the probability of marking a type I error, *t* is the appropriate student *t*-value for a two-tailed test with degrees of freedom associated with the SECV and the selected probability of a type I error, n is the number of independent samples, and SECV is the standard error of cross-validation. If the bias value is lower than T_b, the bias is not significantly different from zero. The results of the bacteria analysis for both NIR measurement and total plate count, from the 72 spectra (SC) and 69 spectra (GC), were used for the construction model. Some samples were removed when detected as outliers. To evaluate the efficiency of the calibration model, the residual predictive deviation (RPD), ratio of performance to inter-quartile range (RPIQ), and range error ratio (RER) were applied, by following these equations below:

$$\text{RPD} = \text{SD/SECV}$$

$$\text{RPIQ} = \text{IQ/SECV}$$

$$\text{RER} = \text{Reference Range/SECV}$$

Where, SD is standard deviation, SECV is the standard error of cross-validation, IQ is an inter-quartile range: the difference between the values helps find 75% (Q_3) and 25% of the samples ($IQ = Q_3 – Q_1$), and the reference range is the difference between the maximum and minimum.

For meaning of RPD RER and RPIQ value followed Table 4.1.

Table 4.1 Score of RPD (Residual Predictive Deviation), RER (Rang Error Ratio) and RPIQ (Ratio of Performance to Inter-Quartile rang).

Indicator	Score	Meaning
RPD (William et al., 1996; Penchaiya et al., 2009; Saeys et al., 2005)	less than1.5	not usable
	1.5-2.0	possibility to distinguish between high and low values
	2.0-2.5	approximate quantitative predictions possible
	2.5-3.0, >3.0	classified as good and excellent, respectively.
RER (Blazquez et al., 2006; Williams , 2001; De Marchi,2013)	less than7	Very poor classification and not recommend for any application
	7-20	Classify the model as poor to fair and indicate it
	21-30	Good classification suggesting the model would be suitable for application in quality control
RPIQ (Bellon-Maurel et al.,2010)	more than 3	useful for screening
	more than 5	used for quality control
	more than 8	used for any application

4.3. Results and Discussion

Microbiological Analysis

The descriptive statistics of the concentrations of total bacteria, *E. coli*, and *S. typhimurium*, from the different sample preparations, are shown in Table 4.2. Samples were collected from the SC and GC solutions mixed with a bacteria suspension of *E. coli* and *S. typhimurium* at 0 (before inoculation), 4, and 8 h (after inoculation) during the incubation period at 37 °C. At 0 h (before inoculation), the total bacteria detected in the SC solution was at 2.88 log CFU·mL^{-1} and in the GC mixture solution at 3.15 log CFU·g^{-1}, while *E. coli* or *S. typhimurium* were not detected. After 8 h, the samples obviously changed from clear to an

unclear solution indicating the microbial growth. The population of total bacteria increased to approximately 6–7 log CFU·mL^{-1} (in SC) and log CFU·g^{-1} (in GC). This level was above the microbiological limits for ready-to-eat food, 6 log CFU for total bacteria, 2 log CFU for *E. coli*, and nothing was detected in the 25 g of sample for *S. typhimurium* (Risk Assessment Section,2001).

Table 4.2. Descriptive statistics of microbial growth, total bacteria, *E. coli* and *S. typhimurium*, in two types of samples; drained solution of shredded cabbage (SC) and mixed solution of ground cabbage and microbial suspension (GC).

Sample	**Bacteria**	**Microbial growth for SC in log CFU·mL^{-1}/ for GC in log CFU·g^{-1})**					
		Min	**Max**	**Mean**	**SD**	**IQ$_1$**	**IQ$_3$**
SC	Total bacteria	2.88	7.11	5.29	1.09	4.45	5.98
	E. coli	0.00	6.84	4.66	1.62	3.41	6.27
	S. typhimurium	0.00	6.18	3.53	1.26	3.00	4.29
GC	Total bacteria	3.15	7.06	5.35	1.03	4.58	6.25
	E. coli	0.00	6.59	4.60	1.88	2.85	6.00
	S. typhimurium	0.00	6.50	4.06	1.42	2.88	5.24

Remarks: SD: Standard deviation; IQ$_1$: the first quartile, the value below which we can find 25% of the samples; IQ$_3$: the third quartile, the value below which we can find 75% of the samples.

SW-NIR Spectra Analysis

The average SW-NIR spectra of the SC and GC, at different incubation times (0, 4 and 8 h), are shown in Figure 4.2a and 4.2b. For the SC spectra, each spectrum shifted upward with the increase in incubation time. For the GC spectra, each spectrum shifted downward with the increase in incubation time. This spectrum shift was also previously reported by Suthiluk et al. (2008). They suggested the growth of bacteria in the solutions, and the particles leaking from the cabbage cells were the cause of this spectrum shift. The solutions became less transparent,

which alters the light-scattering properties (Chen et al., 2002). The SC spectra separated nicely for each incubation time, while the GC spectra at 0 h was well separated from the spectrum at 4 and 8 h, which nearly overlapped. These results suggest that the SC solution had more potential for differentiating the quantity of contaminated microorganisms. However, the scattering effect was obviously found in the baseline shift of the spectra, from both the SC and GC solutions. Some pretreatment techniques, such as normalization or derivative Savitzky–Golay, have been recommended for removing this effect (Norris et al., 1984). The second derivative Savitzky–Golay, was applied to the SC spectra and the first derivative Savitzky–Golay on the GC spectra. Both methods successfully removed the baseline shift from the original spectra, as shown in Figure 4.2c and 4.2d. The strong absorption band at 962 nm due to the high amount of water in the solution was observed in both spectra. These spectra were then used for calibration.

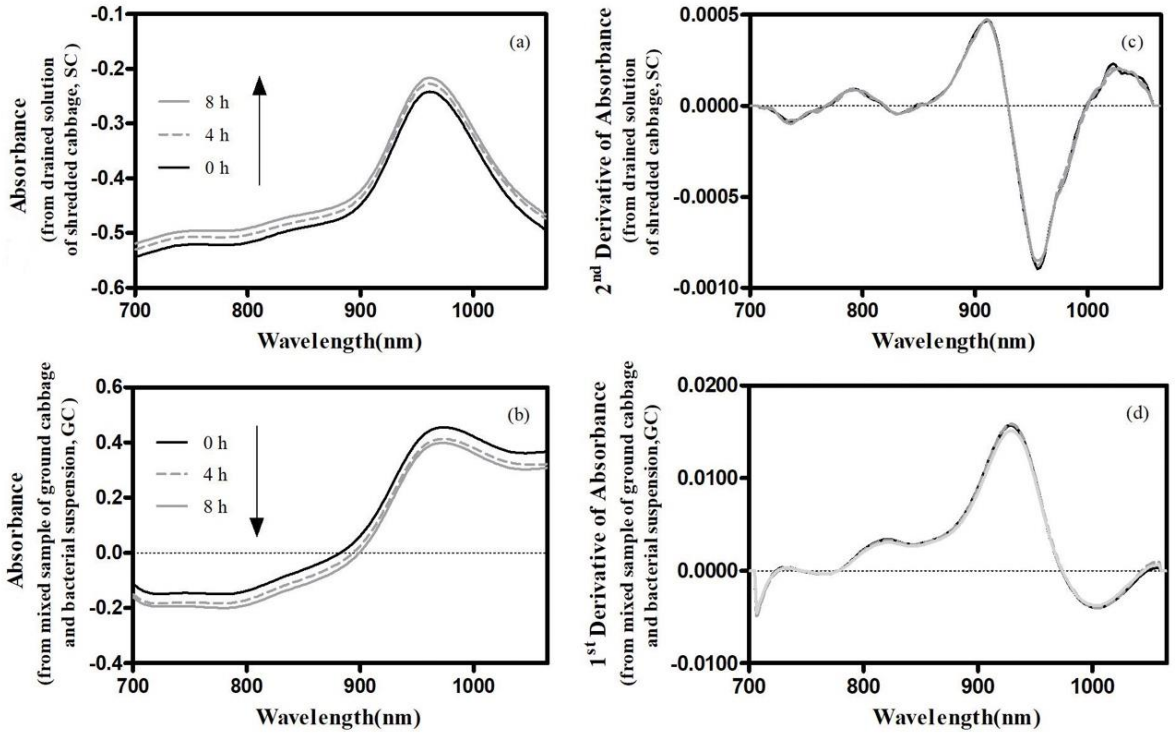

Figure 4.2. Original SW-NIR spectra of drained solution of shredded cabbage (SC) (a) and mixed solution of ground cabbage and bacterial suspension (GC); (b) at different incubation times. The second derivative Savitzky–Golay pretreated spectra of drained solution from shredded cabbage (SC); (c) and the first derivative Savitzky–Golay pretreated spectra from mixed solution of ground cabbage and bacterial suspension (GC) (d).

Feasibility of SW-NIR Used for Bacterial Detection

The principal component analysis (PCA) was applied to the SC and GC spectra treated with the Savitzky–Golay derivative, as mentioned earlier. PCA was applied to observe the clustering in the sample spectra. The score plot of the PCA of the SC and GC spectra, at different incubation times, are shown in Figure 4.3. The first two PCs (PC1 and PC2) accounted for over 90% of the total variance. The score plot of the SC samples between 0 h and 4 h, and 0 h and 8 h were completely segregated. Conversely, the score plot of 4 h and 8 h overlapped, indicating that the discrimination of the quantitative results may not be reliable. Meanwhile, the score plot of the GC samples showed inconsistency in the results. The clustering between 0 h and 4 h separated clearly but not for 0 h and 8 h, while the score plot of 4 and 8 h was well

separated. This could be related to the transparency of the samples. SC samples were more transparent than GC samples, which contained both ground shredded cabbage particles and bacteria suspension. A better NIR application is highly related to the concentration of the chemical compound in the sample. The high counts of bacterial resulted in the good separation between samples, as previously reported by Rodriguez-Saona et al. (2004) and Alexandrakis et al. (2008).

The PLS models for total bacterial were developed on the pretreatment SC and GC spectra, and the results are shown in Table 4.3. The score plot of the predicted and measured value of total bacteria, are shown in Figure 4.4. The value of *r* of total bacteria detection from SC was best at 0.91, which showed a reliable prediction of 0.91–0.95. Followed by *E. coli* of the SC samples, the value was at 0.86, considered as grading with approximation (0.81–0.90). The values of *E. coli* (0.79) and total bacteria (0.74) of GC samples and *S. typhimurium* (0.71) of SC samples could be rough-screened (0.70–0.80). The lowest *r* value was for *S. typhimurium* (0.47) from the GC sample, which indicates it is unusable (below 0.70). The *r* values of this study were based on the guidelines for interpretation of *r* (Williams, P. et al., 2006). The statistical result of the significance of bias for model performance (T_b) was higher than the biases for all parameters. T_b values suggest that bias of developed models was considered as being no different from zero.

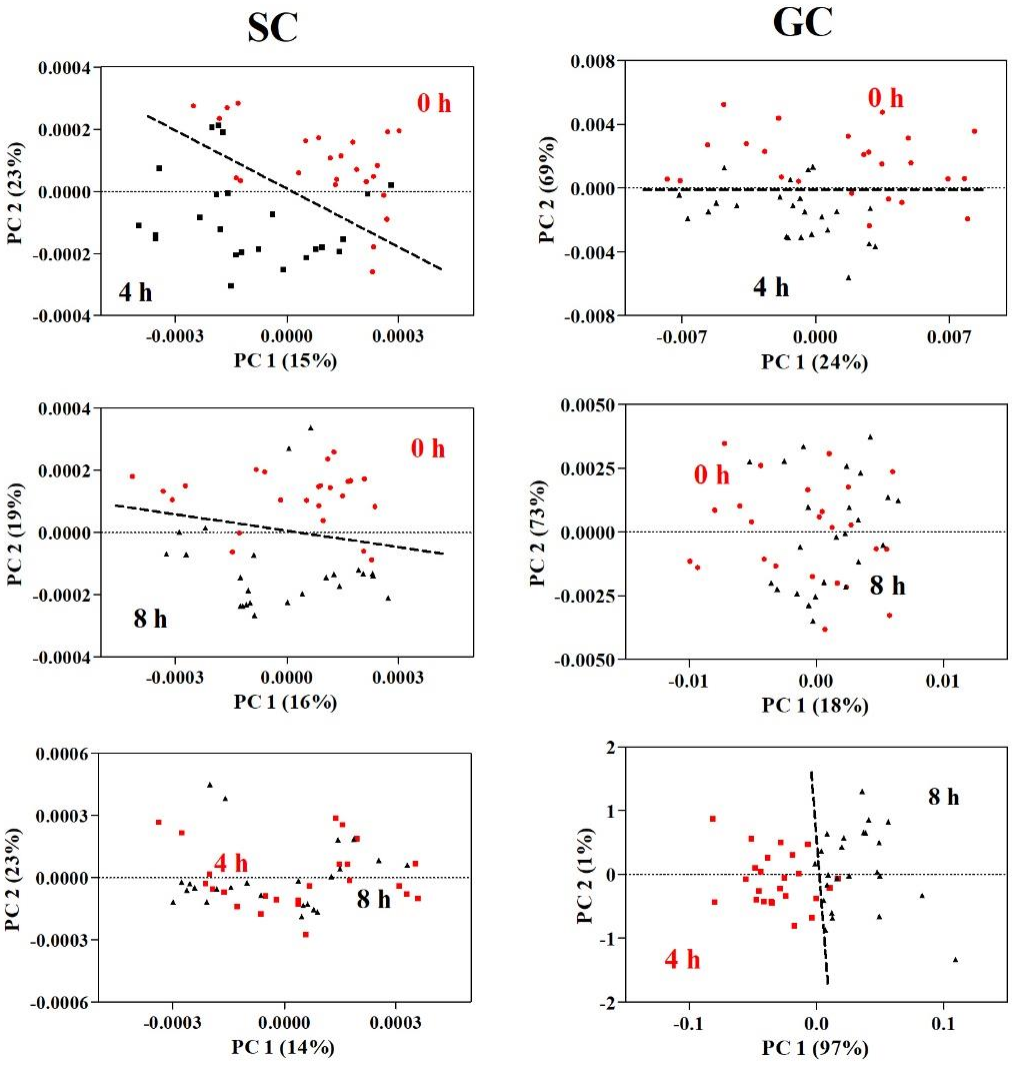

Figure 4.3. The principle component analysis (PCA) score plot (PC1 and PC2) of the pretreatment SW-NIR spectra of drained solution of shredded cabbage (SC, left) and mixed sample of ground cabbage and bacterial suspension (GC, right), in comparison between different incubation times, 0 h with 4 h, 0 h with 8 h, and 4 h with 8 h.

The ratio of prediction of deviation (RPD) is a principle indicator for considering the possibility to use NIR. RPD below 1.5 indicates that the calibration was not useable; a value between 1.5 and 2.0 reveals a possibility to distinguish between high and low values, while a value between 2.0 and 2.5 makes it possible to make predictions in an approximate quantitative propose (Penchaiya et al., 2009; Saeys et al., 2005). In our case, the SC analysis showed the highest RPD in total bacteria (2.44), followed by *E. coli* (1.95). On the other hand, for the GC, the RPD of *E. coli* was only at 1.61 and 1.44 of the total bacteria. For *S. typhimurium*, the RPD

of SC was 1.36, when it was 0.47 for the GC. According to these RPD values, the total bacteria from the SC samples could possibly be used as an approximate quantitative prediction. The detection of *E. coli* in both samples were considered to make it possible to distinguish between high and low counts.

From the *r* and RPD values, the detection of total bacteria from the SC was optimal, which is similar to the study by Suthiluk et al. (2008), who reported using the SW-NIR through a PLS regression which was capable of prediction of total bacteria in the washing solution of shredded cabbage ($r = 0.92$, SEP = 0.46). Furthermore, *E. coli* detection in SC samples was possible for the separation between the high and low counts, which was correlated using NIR for classifying *E. coli* in a phosphate buffer saline. The optimal prediction result was found in the *E. coli* concentrate, which was more than 4 log CFU·mL^{-1}. However, Kiefer et al. (2010) reported that the spectra of *E. coli* concentrations below 5 log CFU·mL^{-1} was not different. This implies that too low concentrations of bacteria are responsible for low feasibility of prediction. In this study, *E. coli* concentrations between 0 to 7 log CFU·mL^{-1} were effective in distinguishing between high and low counts.

Moreover, the ratio of performance to inter-quartile range (RPIQ) was calculated for more supporting evidence. Bellon-Maurel et al. (2010) recommended using IQ instead of SD for calculating RPD, which better represents the population spread. The statistically similar RPD should be based on the guidelines following Williams (1987), who suggested that RPIQ values higher than 3 are useful for screening, that values greater than 5 can be used for quality control, and that values greater than 8 can be used for any application. In this study, the RPIQ values were higher than 3, but less than 5 with *E. coli* (3.45) and total bacteria (3.40) in the SC samples.

The range error ratio (RER) is a method for standardizing the RMSECV, by relating it to the range of the reference data. The RER value of total bacteria was 9.55 in the SC samples, while that of *E. coli* was 8.18. RER values between 7 and 20 are classified as a poor model and can only be used for screening purposes. However, RER values of *E. coli* in GC samples (5.67), total bacteria in GC samples (5.48), and *S. typhimurium* in both samples (SC = 6.72, GC = 5.10), were less than 6 and that was not recommended for any application. RER values of this study were based on the guidelines for interpretation of RER (Blazquez et al., 2006; Williams,

2001; De Marchi,2013). The results of RPIQ and RER were similar and used to predict the total bacteria and *E. coli* in SC, a fair model for the screening propose. This confirms that the use of SC samples to predict the total bacteria and *E. coli* content was suitable.

Table 4.3. PLS model result for predicting the microbial contamination in the drained solution of shredded cabbage (SC) and in the mixed sample of ground cabbage and bacterial suspension (GC).

Sample	Bacteria	N	*r*	SECV	Bias	T_b	RPD	RPIQ	RER
SC	Total bacteria	72	0.91	0.45	−0.02	0.10	2.44	3.40	9.55
	E. coli	72	0.86	0.83	−0.12	0.20	1.95	3.45	8.18
	S. typhimurium	72	0.71	0.93	−0.01	0.22	1.36	1.39	6.72
GC	Total bacteria	69	0.74	0.72	−0.01	0.17	1.44	2.32	5.48
	E. coli	69	0.79	1.17	−0.05	0.28	1.61	2.70	5.67
	S. typhimurium	70	0.47	1.28	−0.02	0.31	1.11	1.84	5.10

Remarks: N: number of samples; *r*: correlation coefficient; SECV: standard error of cross validation; T_b: the statistic test of the significance of bias for model performance; RPD: ratio of performance to deviation; RPIQ: ratio of performance to interquartile range; RER: range error ratio.

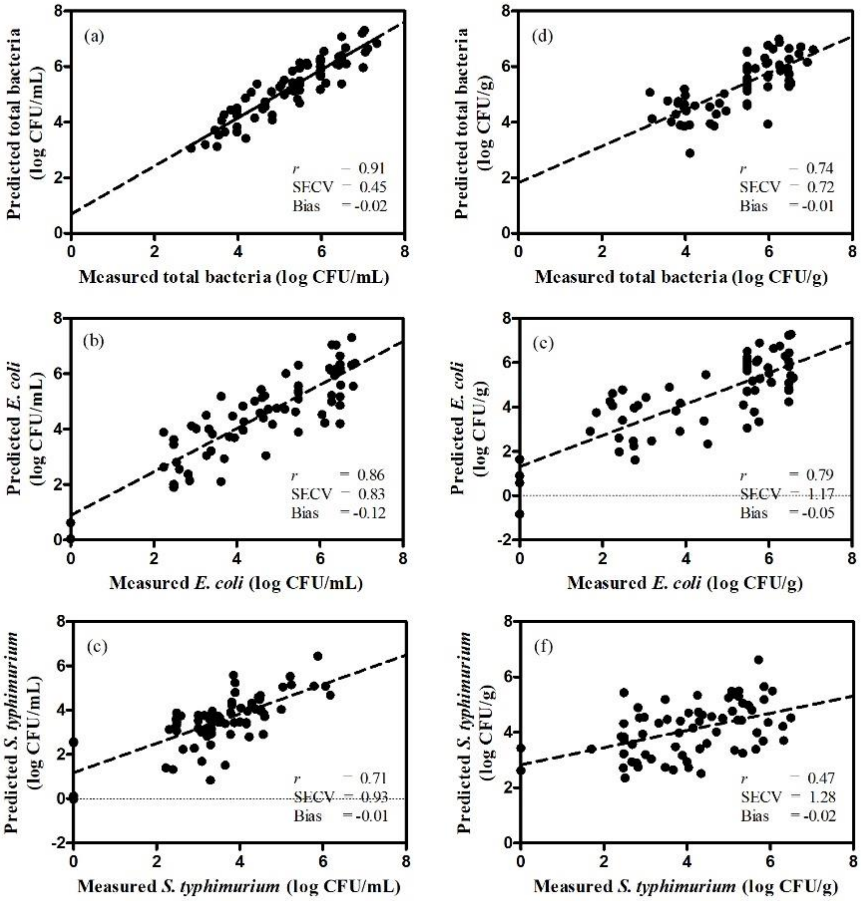

Figure 4.4. Comparison between actual measured and predicted values, using the full-cross validation of the PLS models for total bacteria, *E. coli*, and *S. typhimurium* contamination in the drained solution of shredded cabbage (SC, left) and mixed sample of ground cabbage and bacterial suspension (GC, right), measured using a portable NIR spectrometer (700–1100 nm).

4.4. Conclusions

The SW-NIR shows the feasibility for detecting the existence of microbes in the solution obtained from SC, but for a more specific application for discrimination or quantitation is needed, proving further research in still required.

CHAPTER 5

SUMMARY

5.1 Difference in leaf positions and locations associated with browning characteristics of the shredded cabbage

The lower part of the leaf and the inner layer of the cabbage head are suitable for producing the shredded cabbage if they want to store the shredded cabbage for longer time or transported for long distance. However, if the producer produces the shredded cabbage that will be used instantly the upper part of the leaf can be used to make shredded cabbage because it contains high chlorophyll content that would be benefit for the consumer. Nevertheless, this browning symptom could be protected by using some postharvest treatment such as dipping in some organic acid using modified atmosphere packaging the reduce the amount of oxygen in the package.

5.2 Feasibility for the determination of foodborne microbe contaminated in fresh-cut shredded cabbage using SW-NIR

The results suggested that the SW- NIR could be applied to detect microbial contamination in shredded cabbage. Nevertheless, the pretreatment method is recommended for application on the original spectra, in order to improve the discrimination efficiency in the PCA and PLS models. The difference of the PLS model results between the drained solution (SC) and the mixed sample of ground cabbage and microbial suspension (GC). The efficiency in detecting microbial contamination was higher in the SC, as observed from the correlation coefficient (r) and obtained RPD. The transparency of the measured sample highly affected the quality of measured spectra. The cabbage cell composition, dispersed in the GC sample, could interfere with the spectra absorbance. Although the difference at consecutive incubation times could not be clearly seen in the results, the NIR technique demonstrates potential as a rapid method for detecting or monitoring microbial contamination in fresh-cut shredded cabbage. Nevertheless, further studies are required for more precise results in terms of the amount of contamination or the capability to differentiate the types of bacteria

ACKNOWLEDGEMENTS

This thesis would not have been complete without the help, kind support, and, patience of my principal supervisor, Prof. Dr. Kazuhiro Nakano and Assoc. Prof. Dr. Sirichai Kanlayanarat, who provided valuable guidance and supervision throughout my PhD study at Niigata University and King Mongkut's University of Technology Thonburi (KMUTT) under the Double Degree Program (DDP). I am deeply grateful to Assoc. Prof. Dr. Chalermchai Wongs-Aree, Division of Postharvest Technology, School of Bioresources and Technology, King Mongkut's University of Technology, Thonburi and Asst. Prof. Dr. Shintaroh Ohashi, Faculty of Agriculture, Niigata University for their helpful advices, and valuable suggestion in this study. I also want to thank for the other members of my dissertation committee, Dr. Phonkrit Maniwara, Postharvest Technology Research Center, Faculty of Agriculture, Chiang Mai University for his useful advice for my thesis. And I want to thank Mr. Pathompong Penchaiya, Postharvest Technology Innovation Center, Office of the Higher Education Commission, Bangkok for taking the time to read this work and advice.

Several organizations contributed support to my research, and without them it would have been virtually impossible to complete this thesis. I would like to express my gratitude to The Royal Golden Jubilee (RGJ) Ph.D. program which provided a scholarship for my PhD study.

I would like to express my appreciation to all persons who have kindly offered me their time when depress, stressful, confuse, broken heart, sick, hungry and unhappiness. My sincere thanks are given to all NASER and Postharvest Technology Lab. members.

Finally, I would like to express my sincere gratitude to my family for their unfailing love, encouragement and support throughout this long and often difficult period of research and writing. My greatest respect and thanks go to my farther and my mother.

REFERENCE

Adriano do Nascimento Simões, Silvino Intra Moreira, Paulo Roberto Mosquim, Nilda de Fátima Ferreira Soares and Rolf Puschmann, 2015. “The effects of storage temperature on the quality and phenolic metabolism of whole and minimally processed kale leaves” . **Acta Scientiarum. Agronomy**. v. 37, n. 1, p. 101-107, Jan.-Mar., 2015

Agelet, L.E. and C.R. Hurburgh, 2010. ‘‘A Tutorial on Near Infrared Spectroscopy and Its Calibration’’. **Crit. Rev. Anal. Chem**., 40(4): 246-260.

Al-Qadiri, H.M.; Lin, M.; Al-Holy, M.A.; Cavinato, A.G.; Rasco, B.A.,2008. “Monitoring quality loss of pasteurized skim milk using visible and short wavelength near-infrared spectroscopy and multivariate analysis”. **J. Dairy Sci.**. 91, 950–958, doi:10.3168/jds.2007-0618.

AOAC. *Official Methods of Analysis*, 15th ed.; Helrich, K., Ed.; Association of Official Analytical Chemists, Inc.: Arlington, VA, USA, 1990; Volume 1.

Alexandrakis, D., Downey, G. and Scanell, A.G.M., 2008. “Detection and identification of bacteria in an isolated system with near-infrared spectroscopy and multivariate analysis”. **J. Agric. Food Chem.**, *56*, 3431–3437.

Abadias, M.; Usall, J.; Anguera, M.; Solsona, C.; Viñas, I.,2008. “Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments”. **Int. J. Food Microbiol**., 123, 121–129.

Ahvenainen, R., 1996, New approaches in improving the shelf life of minimally processed fruit and vegetables, **Trends in Food Science and Technology**, Vol. 7, pp. 179–187.
Brecht, J.K., 1995, Physiology of lightly processed fruits and vegetables, **HortScience,** Vol. 30, pp. 18–22.

Ahvenainen, R., 1996, New approaches in improving the shelf life of minimally processed fruit and vegetables, **Trends in Food Science and Technology**, Vol. 7, pp. 179–187.

Balsa, C., Alibert, G., Brulfert, J,, Queiroz, O, and Boudet, A.M., 1979, "Photoperiodic control of phenolic metabolism in Kalanchoe blossfeldiana", **Phytochemistry**, Vol. 18, pp.1159-1163.

Bellon-Maurel, V.; Fernandez-Ahumada, E.; Palagos, B.; Roger, J.M.; McBratney, A., 2010. "Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy". **TrAC Trends Anal. Chem**., *29*, 1073–1081, doi:10.1016/j.trac.2010.05.006.

Barnes R.J., M.S. Dhanoa and S.J. Lister, 1989. ''Standard Normal Variate Transformation and De-Trending of Near-Infrared Diffuse Reflectance Spectra''. **Appl. Spectrosc**. 43(5): 772-777.

Bico, S.L.S., Raposo, M.F.J., Morais, R.M.S.C. and Morais, A.M.M.B., 2009, "Combined Effects of Chemical Dip and/or Carrageenan Coating and/or Controlled Atmosphere on Quality of Fresh-Cut Banana", **Food Control**, Vol. 20, No. 5, pp. 508-514.

Bestwick, C. S.; Brown, J. R.; Mansfield, J. W., 1998. "Localized changes in peroxidase activity accompany hydrogen peroxide generation during the development of a nonhost hypersensitivereaction in lettuce". **Plant Physiol**., 118, 1067-1078.

Brecht, J.K., 1995. Physiology of lightly processed fruits and vegetables. **HortScience**, 30, 18–22.

Blazquez, C.; Downey, G.; O'Callaghan, D.; Howard, V.; Delahunty, C.; Sheehan, E.; Everard, C.; O'Donnell, C.P., 2006. "Modelling of sensory and instrumental texture parameters in processed cheese by near infrared reflectance spectroscopy". ***J. Dairy Res.***, *73*, 58–69, doi:10.1017/s0022029905001536.

Blanco, M. and I. Villarroya., 2002. ''NIR Spectroscopy: A Rapid-Response Analytical Tool''. TrAC, **Trends Anal. Chem**., 21(4): 240-250.

Borraccino G, Mastropasqua L, de Leonardis S, Dipierro S., 1994. "The role of the ascorbic acid system delaying the senscence of oat (Avena sativa L.) leaf segments". **J Plant Physiol** 2:161–166.

Bosco G.L., 2010. ‘‘James L. Waters Symposium 2009 on Near-Infrared Spectroscopy’’. **TrAC, Trends Anal. Chem**., 29(3): 197-208.

Centre for Food safety. 2007. Microbiological Guidelines for Ready-to-eat Food. Food and Environmental Hygiene Department, Available : http://www.cfs.gov.hk/english/whatsnew/whatsnew_act/files/MBGL_RTE%20food_e.pdf

Cantwell, M. (1992). Postharvest handling systems: Minimally processed fruits and vegetables. In: **Postharvest Technology of Horticultural Crops. 2nd Edition.** (Kader, A. A., Ed.). University of California, Division of Agriculture and Natural Resources. Oakland, CA, USA. 277-281.

Chen J.Y.; Iyo, C.; Terada, F.; Kawano, S., 2002. “Effect of multiplicative scatter correction on wavelength selection for near infrared calibration to determine fat content in raw milk”. **J. Near Infrared Spectrosc**. 2002, 10, 301–307.

Cliffe-Byrnes V. and O’Beirne, D., 2005, “Effects of Chlorine Treatment and Packaging on the Quality and Shelf-Life of Modified Atmosphere (Ma) Packaged Coleslaw Mix”, **Food Control**, Vol. 16, No. 8, pp. 707-716.

Couture R.; Cantwell, M. I.; Ke, D.; Saltveit, M. E.,1993. “Physiological attributes related to quality attributes and storage life of minimally processed lettuce”. **Hort Sci.,** 28, 723-725.

Cámara-Martos F.; Lopes, J.A.; Moreno-Rojas, R.; Pérez-Rodríguez, F., 2015. “Detection and quantification of *Escherichia coli* and *Pseudomonas aeruginosa* in cow milk by near-infrared spectroscopy”. **Int. J. Dairy Technol**., *68*, 357–365, doi:10.1111/1471-0307.12191.

Crocombe R.A., 2004. ‘‘MEMS Technology Moves Process Spectroscopy into a New Dimension’’. **Spectrosc. Eur.**, 16(3): 16-19.

Chalker-Scott, L. and Fuchigami, LH., 1989, “The role of phenolic compounds in plants stress responses, In≈ H.L. Paul (Ed.), Low-temperature Stress Physiology in Crops”, **CRC Press**, Boca Raton, FL, pp 27-40.

Choi, Y.J., Tomas-Barber ´ an, F.A., Saltveit, M.E., 2005. “Wound-induced phenolic accumulation and browning in lettuce (Lactuca sativa L.) leaf tissue is reduced by exposure to n-alcohols”. **Postharvest Biol. Technol**. 37, 47–55.

Degl’Innocenti, E., Guidi, L., Pardossi, A., Tognoni, F., 2005. “Biochemical study of leaf browning in minimally processed leaves of lettuce (Lactuca sativa L. var. Acephala)”. **J. Agric. Food Chem**. 53, 9980–9984.

De Gara L, Paciolla C, Liso R, Stefani A, Arrigoni O.,199. “Correlation between ascorbate peroxidase and some anomalies of seedlings from aged caryopses of Dasypyrum villosum (L.) Borb”. **J Plant Physiol** 137:697–700

De Gara L, de Pinto MC, Paciolla C, Cappetti V, Arrigoni O ,1996. Is ascorbate peroxidase only a scavenger of hydrogen peroxide? In: **Obinger C, Burner O, Ederman R, Penel C, Greppen H (eds) Plant peroxidases**: biochemistry and physiology. University of Geneva, Geneva, pp 157–162

De Marchi M., 2013. “On-line prediction of beef quality traits using near infrared spectroscopy”. **Meat Sci**., *94*, 455–460, doi:10.1016/j.meatsci.2013.03.003.

Degl’Innocenti, E., Pardossi, A., Tognoni, F. and Guidi, L., 2007, “Physiological Basis of Sensitivity to Enzymatic Browning in ‘Lettuce’, ‘Escarole’ and ‘Rocket Salad’ When Stored as Fresh-Cut Products”, **Food Chemistry**, Vol. 104, No. 1, pp. 209-215.

Dos Santos, C.A.; Lopo, M.; Pascoa, R.N.; Lopes, J.A.,2013**. “**A review on the applications of portable near-infrared spectrometers in the agro-food industry”. **Appl. Spectrosc.**, 67, 1215–1233, doi:10.1366/13-07228.

Duan, C.; Chen, C.; Khan, M.N.; Liu, Y.; Zhang, R.; Lin, H.; Cao, L**,**2014**.** “Non-destructive determination of the total bacteria in flounder fillet by portable near infrared spectrometer”. **Food Control**, *42*, 18–22, doi:10.1016/j.foodcont.2014.01.023.

Doyle, M.P., Zhao, T., Meng, J., and Zhao, S. 1997, Escherichia coli O157:H7, In **Food Microbiology, Fundamentals and Frontiers**, Eds, M.P. Doyle, L.R. Beuchat, and T.J. Montville, Washington DC: Amer. Soc. Microbiol Press, pp. 171–191.

D'Aoust, J.Y., 2000, "Salmonella" In **The Microbiological Safety and Quality of Food**, Lund, B. M. Baird-Parker, T. C. and Gould, G. W. Volume II, Eds, Maryland: Aspen Publishers, Inc., pp. 1233–1299.

Doran, G., Sheridan, F., DeLappe, N., O'Hare, C., Anderson, W., Corbett-Feeney, G. and Cormican, M., 2005, "Salmonella entericaserovar Kedougou contamination of commercially grown mushrooms", **Diagnostic Microbiology and Infectious Disease**, Vol. 51, pp. 73–76.

Escartin, E.F., Ayala, A.C., Lozano, J. S., 1989, Survival and growth of Salmonella and Shigellaon sliced fresh fruit, **Journal of Food Protection**, Vol. 52, pp. 471–472.

Feng, Y. Z.; Downey, G.; Sun, D. W.; Walsh, D.; Xu, J. L., 2015. "Towards improvement in classification of Escherichia coli, Listeria innocua and their strains in isolated systems based on chemometric analysis of visible and near-infrared spectroscopic data". **J. Food Eng**. , 149, 87–96, doi:10.1016/j.jfoodeng.2014.09.016.

Flinn P.C., 2009. ''Near Infrared Spectroscopy: From Sleeper to Activist''. Paper presented at: Pittcon 2009: Twentieth James L. Waters Annual Symposium: Near Infrared Spectroscopy—NIRS. Chicago, IL; March 8-13,2009.

Fung, F.; Wang, H.S.; Menon, S., 2018. "Food safety in the 21st century". **Biomed. J.** , *41*, 88–95, doi:10.1016/j.bj.2018.03.003.

Geladi P., D. MacDougall and H. Martens, 1985. ''Linearization and Scatter-Correction for Near-Infrared Reflectance Spectra of Meat''. **Appl. Spectrosc**, 39(3): 491-500.

Garcia, E. and Barrett, D., 2002, Preservative treatments for fresh-cut fruits and vegetables, In **Fresh-cut Fruits and Vegetables. Science, Technology and Market**, Lamikanra, O. Ed., CRC Press, Boca Raton, FL, USA. pp. 274-310.

Geladi P. and E. Dabakk, 1995. ‘‘An Overview of Chemometrics Applications in Near Infrared Spectrometry’’. **J. Near Infrared Spectrosc**., 3(3): 119-132.

González-Aguilar, G.A., Wang, C.Y. and Buta, J.G., 2000, "Maintaining Quality of Fresh-Cut Mangoes Using Antibrowning Agents and Modified Atmosphere Packaging" , **Journal of Agricultural and Food Chemistry**, Vol. 48, No. 9, pp. 4204-4208.

Gokmen, V. , Bahçeci, S. , & Acar, J. , 2005. Liquid chromatographic method for the determination of chlorophylls, carotenoids, and their derivatives in fresh and processed vegetables. **Journal of Liquid Chromatography & Related Technologies**, 25(8) , 1201–1213.

Grotheer, P., M. Marshall and A. Simonne. (2005). "Sulfites: separating fact from fiction." 2007, from http://edis.ifas.ufl.edu/pdffiles/FY/FY73100.pdf

Jay, L.S., Grau, F.H., Smith, K., Lightfoot, D., Mirray, C., and Davey, G.R., 1997, Salmonella, In **Foodborne Microorganisms of Public Health Significance**, 5th Ed. (A.D. Hocking, G. Arnold, I. Jenson, K. Newton and P. Sutherland, eds.) pp. 169–230, Australian Institute of Food Science and Technology, Inc., New South Wales, Australia.

James, J.B. and Ngarmsak, T., 2010, Fresh-cut processing: Physiological and microbiological impacts, In **Processing of fresh-cut tropical fruits and vegetables**: A Technical Guide, Rolle R.S. Ed., FAO, Bangkok, 25-30.

Kang H. M, K. W. Park and M. E. Saltveit,2002. “Elevated Growing Temperatures during the Day Improve the Postharvest Chilling Tolerance of Greenhouse— Grown Cucumber,” **Postharvest Biology and Technology** Vol. 24 , pp. 49-57. doi:10.1016/S09255214(01)001296

Haslam, E., 1998, “Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action”, **Cambridge University Press**, Cambridge, UK, pp. 438

Herschel W. , 1832. ‘ ‘ Investigation of the Powers of the Prismatic Colours to Heat and Illuminate Objects’’. **Philos. Trans. R. Soc**. London. 90: 255-329.

Heard, G.M., 2002, Microbiology of fresh cut produce, In **Fresh cut fruit and vegetables**, Lamikanra O. Ed., CRC press, London, New York, Washington D.C. pp. 194-255.

Heaton, J.C. and Jones K., 2007, Microbial contamination of fruit and vegetables and the behavior of enteropathogens in the phyllosphere: a review, **Journal of Applied Microbiology**, Vol. 104, pp. 613–626.

Hodges, D.M., Lester, G.E., Munro, K.D., Toivonen, P.M.A., 2004. "Oxidative stress: importance for postharvest quality". **HortScience** 39, 924–929.

Hisaminato, H., Murata, M., and Homma, S, 2001, Relationship between enzymatic browning of cut lettuce and phenylalanine ammonia-lyase activity, and prevention of browning by inhibitors of polyphenol biosynthesis, **Bioscience, Biotechnology and Biochemistry**, Vol. 65, pp. 1016-1021.

Ibrahim, R., Osman, A., Saari, N. and Rahman, R. A., 2004, "Effects of Anti-Browning Treatments on the Storage Quality of Minimally Processed Shredded Cabbage", **Journal of Food Agriculture and Environment**, Vol. 2, No. 2, pp. 54-58.

ISO. ISO12099. Animal feeding stuffs, cereals and milled cereal products. In *Guidelines for the Application of near Infrared Spectrometry*; The International Organization for Standardization: Geneva, Switzerland, 2010; p. 40, doi:10.3403/30189316.

Jerk Kruk, 2005. "Occurrence of chlorophyll precursors in leaves of cabbage heads-the case of natural etiolation", **Journal of Photochemistry and Photobiology B: Biology**,pp 187-194.

Jiang, Y.M., Fu, J.R., Zauberman, G. and Fuchs, Y., 1999. .Purification of polyphenol oxidase and the browning control of litchi fruit by glutathione and citri acid". **J. Sci. Food Agri.** 79:950-954.

Jiang, Y. M, and Li, Y. B., 2001, "Effects of chitosan coating on postharvest life and quality of longan fruit', **Food Chemistry**, Vol.73, pp. 139-143.

Jordano, R.; Lopez, C.; Rodriguez, V.; Cordoba, G.; Medina, L.M.; Barrios, J., 1995. "Comparison of Petrifilm method to conventional methods for enumerating aerobic bacteria, coliforms, *Escherichia coli* and yeasts and molds in foods". **Acta Microbiologica et Immunologica Hungarica**, *42*, 255–259.

Kampfenkel, K.; Van Montagu, M.; Inze´, D.,1995. "Extraction anddetermination of ascorbate and dehydroascorbate from plant tissue". **Anal. Biochem.**, 225, 165**-**167

Ke D.; Saltveit, M. E., 1989. "Developmental control of russet spotting, phenolics enzymes, and IAA oxidase in cultivars of Iceberglettuce"**. J. Am. Soc. Hortic. Sci.**, 114, 472**-**477.

Kumagai, Y.; Gilmour, S.; Ota, E.; Momose, Y.; Onishi, T.; Bilano, V.L.F.; Kasuga, F.; Sekizaki, T.; Shibuya, K., 2015. "Estimating the burden of foodborne diseases in Japan.". **Bull. World Health Organ. 2015**, *93*, 540–549, doi:10.2471/BLT.14.148056.

Kiefer, J.; Ebel, N.; Schlückerb, E.; Leipertza, A., 2010. "Characterization of *Escherichia coli* suspensions using UV/Vis/NIR absorption spectroscopy". **Anal. Methods**, 2, 123–128, doi:10.1039/B9AY00185A.

Kaji, H., Ueno, M. and Osajima, Y., 1993, "Storage of Shredded Cabbage under a Dynamically Controlled Atmosphere of High O2 and High Co2" , **Bioscience, Biotechnology, and Biochemistry**, Vol. 57, No. 7, pp. 1049-1052.

Kruk, J., 2005, "Occurrence of Chlorophyll Precursors in Leaves of Cabbage Heads – the Case of Natural Etiolation" , **Journal of Photochemistry and Photobiology B: Biology**, Vol. 80, No. 3, pp. 187-194.

Lee, A., Cho, K., Jang, S., Rakwal, R., Iwahashi, H., Agrawal, G.K., Shim, J., Han, O., 2004. "Inverse correlation between jasmonic acid and salicylic acid during early wound response in rice". **Biochem. Biophys. Res. Commun**. 318, 734–738.

Li, H, and Yu, T., 2001, "Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit", **Journal of the Science of Food and Agriculture**, Vol. 81, pp. 269-274.

Leite, C.C., Guimaraes, A.G., da Silva, M.D., de Assis, P.N., Cardoso, R.L., 2002. Evaluation of the behavior of Escherichia coli O157:H7 in fruit pulp. **Hig. Aliment**, Vol 16, pp. 67–73.

Lennon AM, Pratt J, Leach G, Moore AL, 1995. "Developmental regulation of respiratory activity in pea leaves". **Plant Physiol** 107:925–932

Lin, M.; Al-Holy, M.; Mousavi-Hesary, M.; Al-Qadiri, H.; Cavinato, A.G.; Rasco, B.A., 2004. "Rapid and quantitative detection of the microbial spoilage in chicken meat by diffuse reflectancee spectroscopy (600–1100° nm)". **Lett. Appl. Microbiol**. 2004, 39, 148–155, doi:10.1111/j.1472-765X.2004.01546.x.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J., 1951. **"**Protein measurements with the Folin-Phenol reagent". **J. Biol. Chem.**, 193, 265**-**275

Loiza-Velarde, J.; Toma`s-Barbera`n, F. A.; Saltveit, M. E. ,1997. "Effect of intensity and duration of heat-shock treatments on woundinduced phenolic metabolism in iceberg lettuce". **J. Am. Soc. Hortic. Sci.**, 122, 873**-**877

Marshall, M.R., Kim, J. and Wei, C., 2000, Enzymatic browning in fruits, **vegetables and seafoods. FAO**, Rome. 49 p.

Michał Nosek, Ewa Suro´wka, Stanisław Cebula, Andrzej Libik, Sylwia Goraj, Andrzej Kornas and Zbigniew Miszalski,2011. "Distribution pattern of antioxidants in white cabbage heads (Brassica oleracea L. var. capitata f. alba)", **Acta Physiol Plant**, 33: 2125–2134 DOI 10.1007/s11738-011-0752-6

Martín-Diana, A.B., Rico, D. and Barry-Ryan, C., 2008, "Green Tea Extract as a Natural Antioxidant to Extend the Shelf-Life of Fresh-Cut Lettuce", **Innovative Food Science & Emerging Technologies**, Vol. 9, No. 4, pp. 593-603.

Martens H., S.A. Jensen and P. Geladi, 1983. ''Multivariate Linearity Transformations for Near Infrared Reflectance Spectroscopy''. In: O.H.J. Christie, editor. Proceedings of the Nordic Symposium on Applied Statistics. Stokkand, Norway: Stokkand Forlag. pp. 205-234.

Morais, A.M.M.B. and Argañosa, A.C.S.J., 2010, Quality During Storage of Fresh-Cut Papaya (Carica papaya L.) in Various Shapes, **The Philippine Agricultural Scientist**, Vol. 93, pp. 88-96.

Murray I. , 2004. ‘ ‘ Scattered Information: Philosophy and Practice of Near Infrared Spectroscopy’ ’. In: A.M.C. Davies, A. Garrido-Varo, editors. Near Infrared Spectroscopy: Proceedings of the 11th International Conference. Chichester, UK: NIR Publications, pp. 1-12

Mur, L.A.J., Kenton, P., Atzorn, R., Miersch, O., Wasternack, C., 2006. “The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death”. **Plant Physiol**. 140, 249–262.

Myung, K., Hamilton-Kemp, T.R., Archbold, D.D., 2006. “Biosynthesis of trans- 2-hexenal in response to wounding in strawberry fruit”. **J. Agric. Food Chem.** 54, 1442–1448.

Næs T., T. Isakson, T. Fearn and T. Davies, 2002. A User-Friendly Guide to Multivariate Calibration and Classification. Chichester, UK: NIR Publications.

NACMCF, National Advisory Committee on Microbiological Criteria for Foods, 1999, Microbiological safety evaluations and recommendations on fresh produce, **Food Control**, Vol, 10, pp. 117-43.

Nma, O.N.; Oruese, O.M., 2013. “Prevalence of Salmonella species and Escherichia coli in fresh cabbage and lettuce sold in Port Harcourt Metropolis”, **Nigeria. Rep. Opin**. 2013, 5, 1–8.

Nguyen-the, C. and Carlin, F., 1994, The microbiology of minimally processed fresh fruit and vegetables”, **Critical Reviews in Food Science and Nutrition.** Vol. 34, pp. 371–401.

Nicolaï, B.M.; Beullens, K.; Bobelyn, E.; Peirs, A.; Saeys, W.; Theron, K.I.; Lammertyn, J., 2007. “ Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review” . **Postharvest Biol. Technol**. 2007, 46, 99– 118, doi:10.1016/j.postharvbio.2007.06.024.

Nosek, M., Surówka, E., Cebula, S., Libik, A., Goraj, S., Kornas, A. and Miszalski, Z., 2011, "Distribution Pattern of Antioxidants in White Cabbage Heads (Brassica Oleracea L. Var. Capitata F. Alba)", **Acta Physiologiae Plantarum**, Vol. 33, No. 6, pp. 2125-2134.

Norris, K.H.; Williams, P.C., 1984. "Optimization of mathematical treatments of raw near-infrared signal in the measurement of protein in Hard Red Spring wheat. I. Influence of Particle Size". **Am. Assoc. Cereal Chem**., *61*, 158–165.

Penchaiya, P.; Bobelyn, E.; Verlinden, B.E.; Nicolaï, B.M.; Saeys, W. ,2009. "Non-destructive measurement of firmness and soluble solids content in bell pepper using NIR spectroscopy". **J. Food Eng.** , *94*, 267–273, doi:10.1016/j.jfoodeng.2009.03.018.

Penteado, A.L. and Leitao, M.F.F., 2004, Growth of Salmonella Enteritidis in melon, watermelon and papaya pulp stored at different times and temperatures, **Food Control**, Vol. 15, pp. 369–373.

Phunsiri, S., Sirinnapa, S., Sumio, K., Sonthaya, N., and Takaaki, S., 2008, Possibility of using near infrared spectroscopy for evaluation of bacterial contamination in shredded cabbage. **International Journal of Food Science and Technology**, Vol. 43, 160-165.

Pressey, R., 1990,"Anions activate the oxidation of indoleacetic acid by peroxidase from tomato and other sources", **Plant Physiology**, Vol. 93, pp. 798-804.

Richard-Forget, F. and Gauillard, F.A., 1997, "Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (Pyrus communis Cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning', **Journal of Agricultural and Food Chemistry**, Vol. 45, pp. 2472-2476.

Risk Assessment Section, Food and Environmental Hygiene Department. In *Microbiological Guidelines for Ready-to-Eat Food*; Risk Assessment Section, Food and Environmental Hygiene Department: Hong Kong, China, 2001; pp. 1–6.

Rodriguez-Saona, L.E.; Khambaty, F.M.; Fry, F.S.; Calvey, E.M., 2001. “Rapid detection and identification of bacterial strains by Fourier transform near-infrared spectroscopy”. **J. Agric. Food Chem.**, *49*, 574–579.

Rodriguez-Saona, L.E.; Khambaty, F.M.; Fry, F.S.; Dubois, J.; Calvey, E.M., 2004. “Detection and identification of bacteria in a juice matrix with Fourier transform-near infrared spectroscopy and multivariate analysis”. **J. Food Prot**., *67*, 2555–2559.

Samuel, M.A., Ellis, B.E., 2002. “Double jeopardy: both overexpression and suppression of a redox-activated plant mitogen-activated protein kinase render tobacco plants ozone sensitive”. **Plant Cell** 14, 2059–2069.

Seo, S., Sano, H., Ohashi, Y., 1999. “Jasmonate-based wound signal transduction requires activation of WIPK, a tobacco mitogen-activated protein kinase”. Plant Cell 11, 289–298.

Sapers, G. M., 1993, "Browning of foods: Control by sulfites, antioxidants and other means', **Food Technology**, Vol. 47, pp. 75-84.

Savitzky A. and M.J.E. Golay,1964. ‘‘Smoothing and Differentiation of Data by Simplified Least Squares Procedures’’. **Anal. Chem**., 36(8): 1627-1639.

Saltveit, M.E., 1997. Physical and physiological changes in minimally processed fruits and vegetables. In: Tomas-Barber ´ an, F.A., Robins, R.J. (Eds.), Phyto- ´ chemistry of Fruit and Vegetables. Oxford University Press, New York, pp. 205–220

Saltveit, M.E., Choi, Y.-J., Tomas-Barber ´ an, F.A., 2005. “Involvement of ´ components of the phosopholipid-signalling pathway in wound-induced phenylpropanoid metabolism in lettuce (Lactuca sativa) leaf tissue”. **Physiol. Plant**. 125, 345–355

Saeys, W.; Mouazen, A.M.; Ramon, H., 2005. “Potential for onsite and online analysis of pig manure using visible and near infrared reflectance spectroscopy”. **Biosyst. Eng**, *91*, 393–402, doi:10.1016/j.biosystemseng.2005.05.001.

Shiina, T.; Hasegawa, Y., 2007. “Trends of fresh-cut vegetables in Japan”. **Acta Hortic.** , *746*, 39–44, doi:10.176610/ActaHortic.2007.746.3.

S. J. Kays, “Postharvest Physiology of Perishable Plant Products,” AVI Publisher, New York, 1991, pp. 356-357.

Siripatrawan, U.; Makino, Y.; Kawagoe, Y.; Oshita, S., 2010**. “**Near infrared spectroscopy integrated with chemometrics for rapid detection of *E. coli* ATCC 25922 and *E. coli* K12”. **Sens. Actuators B Chem.**, 148, 366–370, doi*:*10.1016*/*j.snb.2010.05.024.

Sivapalasingam, S.; Friedman, C.R.; Cohen, L.; Tauxe, R.V. ,2004. “Fresh produce: a growing cause of outbreaks of foodborne illness in the United States”, 1973 through 1997. **J. Food Prot.** *67*, 2342–2353.

Singleton, V. L. , Rossi, J. A. , 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. **J. Enol. Viticult**. 16, 144–158.

Söderström, A.; Osterberg, P.; Lindqvist, A.; Jönsson, B.; Lindberg, A.; Blide Ulander, S.; Welinder-Olsson, C.; Löfdahl, S.; Kaijser, B.; De Jong, B., 2008. “A large *Escherichia coli* O157 outbreak in Sweden associated with locally produced lettuce”. **Foodborne Pathog. Dis**., *5*, 339–349.

Sathyanarayana, D.N, 2007. Vibrational Spectroscopy: Theory and Applications. New Delhi, India: New Age International.

Stark E. and K. Luchter, 2005. ‘‘NIR Instrumentation Technology’’. **NIR News**. . 16(7):13-16.

Solymosi, K., Martinez, K., Kristóf, Z., Sundqvist, C. and Böddi, B., 2004, "Plastid Differentiation and Chlorophyll Biosynthesis in Different Leaf Layers of White Cabbage (Brassica Oleracea Cv. Capitata)", **Physiologia Plantarum**, Vol. 121, No. 3, pp. 520-529.

Seong Yeong Kim, 2015. “Fluctuations in Phenolic Content and Antioxidant Capacity of Green Vegetable Juices during Refrigerated Storage”. **Prev. Nutr. Food Sci**.;20(3):169-175

Suthiluk, P.; Saranwong, S.; Kawano, S.; Numthuam, S.; Satake, T., 2008. “Possibility of using near infrared spectroscopy for evaluation of bacterial contamination in shredded cabbage”. **Int. J. Food Sci. Technol**., *43,* 160–165, doi:10.1111/j.1365-2621.2006.01416.x.

Surjadinata, B.B., Cisneros-Zevallos, L., 2003. “Modeling wound-induced respiration of fresh cut carrots (Daucus carota L.)”. **J. Food Sci.** 68, 2735–2740.

Stutte, G. W, 1989, "Quantification of net enzymatic activity in developing peach fruit using computer video image analysis', **HortScience,** Vol. 24, pP. 113-115.

Swamy, P.M., Smith, B.N., 1999. “Role of abscisic acid in plant stress tolerance”. **Curr. Sci**. 76, 1220–1227.

Takabatake, R., Seo, S., Ito, N., Gotoh, Y., Mitsuhara, I., Ohashi, Y., 2006. “Involvement of wound-induced receptor-like protein kinase in wound signal transduction in tobacco plants”. **Plant J**. 47, 249–257.

Thunberg, R.L., Tran, T.T., Bennett, R.W. and Matthews, R.N., 2002, Research Note: microbial evaluation of selected fresh produce obtained at retail markets, **Journal of Food Protection**, Vol. 65, pp. 677–682.

Tortoe, C., Orchard, J. and Beezer, A., 2007, "Prevention of Enzymatic Browning of Apple Cylinders Using Different Solutions", **International Journal of Food Science & Technology**, Vol. 42, No. 12, pp. 1475-1481.

Varoquaux, P. and Wiley, R., 1994, "Biological and Biochemical Changes in Minimally Processed Refrigerated Fruits and Vegetables", In **Minimally Processed Refrigerated Fruits & Vegetables**. Wiley, R., Springer US, pp 226-268.

Veljovic-Jovanovic SD, Pignocchi C, Noctor G, Foyer CH, 2001. “Low ascorbic acid in the vtc-1 mutant of arabidopsis is associated with decreased growth and intracellular redistribution of the antioxidant system”. **Plant Physiol** 127:426–435

Waterman, P.G. and Mole, S., 1994, “Analysis of Phenolic Plant metabolites”**, Blackwell Scientific Publications**, Oxford, UK, pp 238.

Watada, A.E., Abe, K. and Yamuchi, N., 1990, "Physiological Activities of Partially Processed Fruits and Vegetables", **Food Technology**, Vol. 44, No., pp. 116-118, 120-122.

Wiley, R.C., 1994, Preservation Methods for Minimally Processed Refrigerated Fruits and Vegetables, In **Minimally Processed Refrigerated Fruits and Vegetables**, London: Chapman and Hall Inc. pp. 66-134.

Williams, P.C., 1987. "Variables affecting near-infrared reflectance spectroscopic analysis. In *Near-Infrared Technology in the Agricultural and Food Industries*; Williams, P.C., Norris, K., Eds.; American Association of Cereal Chemists: St. Paul, MN, USA, 1987; p. 158.

Williams, P.C., 2001. Implementation of near-infrared technology. In *Near-Infrared Technology in the Agricultural and Food Industries*, 2nd Ed.; Williams, P.C., Norris, K., Eds.; American Association of Cereal Chemists: St. Paul, MN, USA, 2001.

Williams, P.; Saranwong, S.; Kawano, S.; Isaksson, T.; Segtnan, V.H., 2006. "Applications to sgricultural and marine products". In *Near-Infrared Spectroscopy in Food Science and Technology*; Ozaki, Y., McClure, W.F., Christy, A.A., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2006; doi:10.1002/9780470047705.ch7.

Yigeremu, B., Bogale, M. and Ashenafi, M., 2001, Fate of Salmonella species and E. coli in fresh-prepared orange, avocado, papaya, and pineapple juices, **Ethiopian Journal of Health Sciences**, Vol. 11, pp. 89–95.

Yu, H.; Neal, J.A.; Sirsat, S.A., 2018. "Consumers' food safety risk perceptions and willingness to pay for fresh-cut produce with lower risk of foodborne illness". **Food Control**, *86*, 83–89, doi:10.1016/j.foodcont.2017.11.014.