EFFECT OF PROCESSING ON QUALITY AND SHELF LIFE OF LEAFY VEGETABLES

by

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ABSTRACT

There is a need to sustain quality of ready-to-eat (RTE) leafy vegetables in order to improve shelf life of the products during postharvest storage. The main objective of this thesis was to investigate the effect of compression towards the quality and shelf life of the RTE leafy vegetables. This thesis focused on spinach leaves as the representation of the RTE leafy vegetables. For this purpose, uniaxial compression was chosen as a method to induce damage to the leaves. The effects of compression towards qualities of RTE spinach were evaluated. The quality measures in this study focused on mechanical and microstructural properties, physical appearance, and fresh weight. Organic spinach was found to be the best type in resisting stress compared to Teen, Salad, and Baby spinach. Population test was developed to categorise the compressed leaves into degrees of leaf injuries known as Undamaged, Minor, Halfway, and Complete teared. Force 200N was suggested to be the minimum required to pick up differences of the leaves deteriorations before and after storage. Uncompressed spinach leaves were found to have a shelf life of 14 days. Higher degrees of leaf injuries which are Halfway and Complete teared showed obvious decay since before storage. Sensory test was also conducted to study consumers' evaluations towards visibility of leaf damage and perceptions in buying the product. Respiration rate of the RTE spinach stored at different films was also measured using a closed system. Michaelis-Menten and exponential models were used to study the respiration rate mechanism.

"And I bestowed upon you love from Me (Lord) that

you would be brought up under My (Lord) eye"

(Al-Quran, 20:39)

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In the name of God, The Most Gracious the Most Merciful

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AsA	*Ascorbic acid*
ATP	*Adenosine triphosphate*
AUC	*Area under the curve*
BOPP	*Biaxially oriented polypropylene*
CO_2	*Carbon dioxide*
E	*Young's modulus*
EOW	*Electrolysed oxidising water*
$FADH_2$	*Flavin adenine dinucleotide-reduced form*
HPDC	*High pressure carbon dioxide*
LDPE	*Low-density polyethylene*
MAP	*Modified atmosphere packaging*
MaxF	*Burst strength*
MaxD	*Flexibility*
MaxW	*Maximum work of compression*
MinTS	*Minimum tool separation*
NaCl	*Sodium chloride*
NADH	*Nicotinamide adenine dinucleotide-reduced form*
NEW	*Neutral electrolysed water*
O_2	*Oxygen*
OPP	*Orientated polypropylene*
OTR	*Oxygen transmission rate*
PE	*Polyethylene*
RGB	*Red, Green, and Blue colour space*
RSD	*Relative standard deviation*

RSM	*Response surface methodology*
RTE	*Ready-to-eat*
TCA	*Tricarboxylic acid*
WVTR	*Water vapour transmission rate*

CHAPTER 1

INTRODUCTION

1.1 The importance of vegetables

Vegetables play an important role in contributing vitamins, minerals, and dietary fibre in human diets. They are well known for their benefits in a healthy lifestyle where the nutritional values contained within the vegetables can protect against chronic degenerative ailments (Leather 1995; Hartley et al. 2013; Dinnella et al. 2014) e.g. vitamin K, lutein, β-carotene, α-tocopherol, folate, nitrate, and kaempferol found in green leafy vegetables offer numerous health benefits including reduced risk of heart disease, obesity, high blood pressure and mental decline (Enloe 2018; Morris et al. 2018). Folate which is found abundantly in spinach also plays a role in red blood cell production and prevention of neural tube defects in pregnancy (Furness et al. 2013) whereas, β-carotene and lutein have shown to prevent eye disorders such as cataracts and muscular degeneration (Semba & Dagnelie 2003; Bone et al. 2007). Sulforaphane which is found in cruciferous vegetables such as broccoli, Brussels sprouts and cabbages may improve bacterial gut flora and decrease the risk of cancer and heart disease (Tian et al. 2018).

Cox et al. (1996) reported that in the nineties, many food health campaigns have been launched to promote the intake of vegetables and fruits where 400g of vegetables and fruits were suggested as a daily intake by the World Health

Organisation (WHO 1990). However, despite these public health campaigns besides clinical evidence, there was still a lack of vegetable and fruit intake in many countries where consumption remained below the recommended daily intake. This was due to the lack of will power to change to a better healthy diet and economic constraints among lower income groups (Marshall et al. 1994; Anderson et al. 1998). It has been reported that among the low income groups in the United Kingdom, there was low intake of vitamin C and carotene which was associated with low intake of “expensive” vegetables and fruits; this possibly resulted in a higher mortality rate (Leather 1995). On the other hand, within the higher income groups, constraints came from the consumers’ social environment where more women got less time for cooking due to working outside home, lack of interest in cooking and increase in the consumption of take-out foods (Lambert 2001; Ragaert et al. 2004). This shows that convenience plays the major motivation for the consumer in buying the product. It has been reported that health attribute was not as important to be the major motivation in buying the minimally processed vegetables despite consumers’ awareness on the health benefits (Ragaert et al. 2004). Due to hectic and busy lifestyles, consumers prefer products that are easy to prepare with convenient packaging. Time pressure is one of the main factors that lead to consumers preferring convenience products (Verlegh & Candel 1999; Candel 2001).

Nowadays, ready-to-eat (RTE) vegetables are gaining more attention from consumers and the food industry (Ragaert et al. 2004). Today’s societies are more conscious towards healthier diet which leads to the explosive demand of packed leafy greens and salads (Gilbert 2000). The growing of interest towards RTE products is due to the practicality while at the same time they are good source of antioxidants and phytochemicals associated with some disease prevention (Ames et al. 1993). The packed RTE vegetables are very easy to prepare, time saving, lower shipping cost, and store well (Odumeru et al. 2002). Due to their convenience, nutrition and fresh-like properties, they become one of the most important food components in food industry. Wide variety of minimally processed vegetables have been developed to meet consumers' demands for quick, convenient, and healthy products (Ahvenainen 1996; Ragaert et al. 2004). In a study reported by Ragaert et al. (2004), the demand of the freshly processed vegetables grew approximately 10 – 25% per annum since 1990 in Western Europe, while specifically in Belgium, the minimally processed vegetables formed more than 50% of the total vegetables and fruits at the retail level.

RTE or also known as minimally processed vegetables are defined as fresh vegetables that have undergone minimal processes to increase their functionality while maintaining their fresh-like properties (Ragaert et al. 2004; Dinnella et al. 2014). Examples of the processes are washing, cutting, mixing and packaging. Centralised processing system helps in facilitating products from different farms for the process series. However, these series of processes caused the products to become highly perishable as they created a longer food chain which exposed the products to mechanical damage and growth of microorganisms e.g. *E.coli*, *Salmonella, and Listeria monocytogenes* (Jacxsens et al. 2001; Rico et al. 2007; Oliveira et al. 2010; Anderson et al. 2014). It also helped in any possible distribution of the potential pathogens to more people in more geographically disperse areas (De Roever 1998). It is such a challenge for the food industry to maintain and improve the quality and shelf life of the bagged leafy vegetables as the qualities of the vegetables are gradually changing throughout postharvest storage due to the physiological activities involved in tissue senescence (Rico et al. 2007). Therefore, there is a need to sustain the qualities of the RTE vegetables in order to improve the shelf life of the products during postharvest storage.

1.2 Current practices in fresh food industry

Food industries have invented so many methods and treatments in order to maintain and improve the shelf life of the RTE vegetables. However, uncontrolled or excessive treatments caused accidental damage which then leads to quality and shelf life reduction of the products. Up to date, the industry has been monitoring the quality of the fresh product varieties before they are ready to be marketed. Quality inspection has been done extensively in order to provide the best product for the consumers. The industry usually goes for the best variety for sale. List of criteria was made to ensure the product that passes the quality inspection is good enough for the consumer. However, the criteria on choosing the best type was made before the product undergoes the processing steps. Question arises as does the product chosen as best variety before processing still remains the best type after processing? The best variety is not only the one that exhibits good qualities before processing but also the one that is best in resisting stress even after undergone the processes. There is still lack of understanding on the effect of processing toward quality and shelf life between different varieties. In the industry, there is still lack of understanding between these interactions as the industry did not do this collectively; rather they assess the quality individually. The industry needs to understand and able to correlate the impact of mechanical injury that caused during processing as well as which types of varieties is the best in resisting the stress. In handling quality management, it is important to have understanding on the raw material, the performance of the raw material during processing and how they connected to each other. Selection of raw material, effect of processing, and also storage treatment need to be studied as a whole rather than individual assessment as what the industry treat them. My work is including compression as one of the impact caused during processing and how different types of varieties response to it.

Apart from that, the industry also only monitors quality of original fresh leaves, while they don't do shelf life study on the compressed leaves. Although quality inspection has been conducted in choosing the best state of product, situation that happens inside the packed product especially the fresh one is out of the industry coverage. Fresh product that already undergone series of processing was packed and goes straight to the retailers and then consumers. It is important to study the effect of compressed leaves towards quality and shelf life. Logically speaking, when comparing between fresh and damage fresh products, people tend to expect that the fresher the products, the longer the shelf lives. However, that was just from the logical thinking. Do mechanically injured leaves proven to have shorter shelf life than intact leaves? In the literature, there is still lacks of data to describe this situation. Thus having the understanding on the effects of processing which this study focused on compression, and developing a method to assess different degree of injury and their effects towards quality and shelf life of the product would be beneficial for the fresh produce industry. Precaution can be made to minimise damage during processing thus improve the quality and shelf life of the fresh product. Having this knowledge also could help to educate consumer as for them to make better decision in buying the product.

1.3 Research objectives

The aim of this thesis was to study the effect of processing on quality and shelf life of ready-to-eat (RTE) spinach. This thesis focused on compression as one of the possible situations that occurs during handling of the RTE spinach from the retailers to the consumers. This thesis comprised of four research objectives:

1. To study the effects of compression towards mechanical property, microstructural property and quality of RTE spinach.
2. To develop a method to assess different degrees of mechanical leaf injuries and together with force and degrees of compressions, how they affect quality and shelf life of RTE spinach during postharvest storage.
3. To investigate consumers' evaluations on the visibility of leaf damage and how it affects their perceptions in buying the RTE spinach.
4. To study the effects of the mechanical leaf damage and type of packaging film towards respiration rate and quality changes of the RTE spinach during postharvest storage.

1.4 Thesis overview

This thesis covers the effect of processing towards quality and shelf life of RTE leafy vegetables. Chapter 1 covers the importance of the RTE leafy vegetables. Research objectives are also included in this chapter. In Chapter 2, all background studies related to this project are described. The processing and handling of fruits and vegetables are explained extensively and their effects towards quality changes of the products during postharvest are described. Besides that, background of sensory test and respiration rate that are conducted in this study are also described. Chapter 3 combined all the methodologies carried out in this study. From the main sample preparation, to uniaxial compression method, assessing degree of injury, measuring quality changes, measuring mechanical property and evaluating microstructural property, all are included in this chapter. Sensory test and respiration rate protocols are also described. Chapter 4 is about compression as the representation of processing that cause mechanical damage to the RTE products and how it affects the mechanical and microstructural property, and quality of the RTE leafy vegetables. Chapter 5 describes the effect of degree of mechanical leaf injury and force of compression towards quality and shelf life of the RTE leafy vegetables. The responses from the mechanically damaged leaves were compared to non-damaged leaves based on distribution of deteriorations, kinetic decay, and fresh weight loss. Chapter 6 covers sensory evaluations by consumers towards visibility of leaf damages and perceptions in buying the product. Ranking test was conducted to give rank to the visibility of leaf the damage and Acceptance test was conducted to study consumer’s acceptance in buying the product. The final chapter is Chapter 7, where the respiration rates of the RTE leafy vegetables stored at different packaging films were discussed. The respiration rates between mechanically injured and intact leaves were also compared. Uncompetitive Michaelis-Menten and exponential models were used to study the respiration rate mechanism. The effects of the packaging film towards quality changes of the product during storage were also included. Finally, Chapter 8 is the conclusion and recommendations for future study.

CHAPTER 2

LITERATURE REVIEW

2.1 Vegetables

Vegetables are derived from various parts of the plant (Figure 2.1). They are generally grouped into three main categories: fruit, seeds and pods; bulbs, roots and tubers; flowers, buds, stems and leaves (Wills et al. 2007). Grouping or classification of the plant parts is necessary as the plant products have varied responses after harvest. It is beneficial to group together products with similar environmental requirements after harvest for quality preservation or those that are vulnerable to chilling or other types of injury (Kays 1991).

The main focus of this thesis is on leafy green vegetables, represented by RTE spinach (*Spinacia oleracea*). Spinach is a green leafy vegetable that is rich in healthy compounds such as vitamin A and C, flavonoids, iron, calcium, and dietary fibre (Conte et al. 2008). Spinach contains high antioxidant capacity which regular consumption may be of interest for disease prevention. It ranked third in total antioxidant capacity after garlic and kale (Cao et al. 1996). Spinach originated from central and southwestern Asia and it started to be introduced in the UK in the 14th century. In recent years, the demand for RTE spinach has been increasing, thus the production increased as well. In 2016, according to analyst from IRI UK, the RTE spinach placed second top most wanted green vegetables in the UK supermarkets

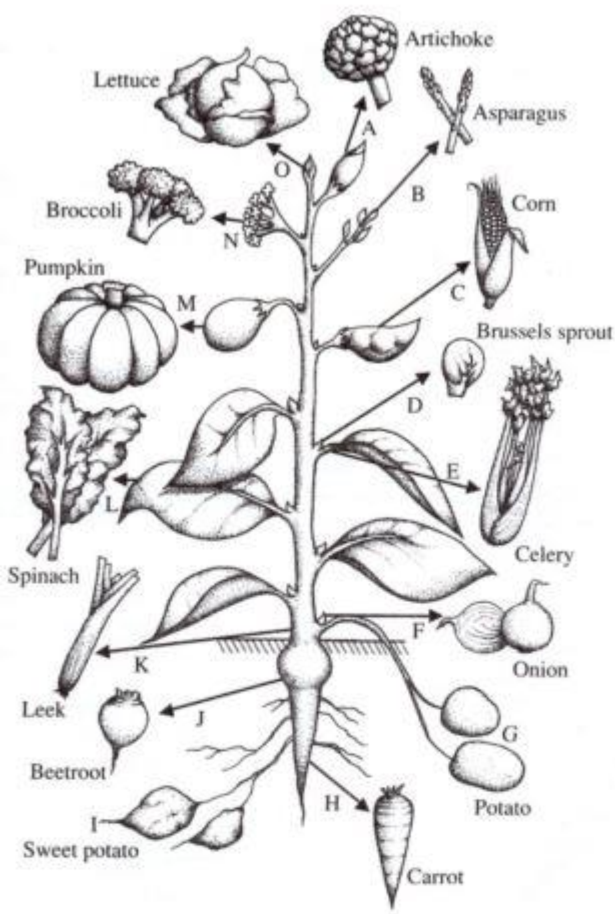


Figure 2.1: Derivation of vegetables from different parts of the plant. The letters indicate the principal origins of representative vegetables as follows: (A) flower bud, (B) stem sprout, (C) seeds, (D) axillary bud, (E) petiole, (F) bulb (underground bud), (G) stem tuber, (H) swollen root, (I) swollen root tuber, (J) swollen hypocotyls, (K) swollen leaf base, (L) leaf blade, (M) fruit, (N) swollen inflorescence, (O) main bud. Image from (Wills et al. 2007).

with 21% increment of demand, after avocados with sales increment of 31% (IRI 2016). People use spinach for cooking, juicing and it can also be eaten right away as a salad. Spinach is also very good in promoting healthy skin, giving aids in digestion, nourishing eyes, building bones, improving nerve function and it also good as antioxidants and anti-inflammatory (Khaliq 2014).

As spinach consists around 90% of water content, it has high respiration and water loss rates which made it susceptible to tissue decay (Hodges & Forney 2003), nutrients loss (Pandrangi & Laborde 2004; Bergquist et al. 2006), and microbial growth (Conte et al. 2008) which then lead to shelf life reduction.

The minimal processes of spinach usually involved washing and cutting procedures and they have a shelf life of 5-6 days (Cocetta et al. 2014). Although the processes are mild, the fresh-cut spinach lost its physical and nutritional qualities as the tissues are damaged. The cutting procedure speed up the senescence process of the spinach (Cocetta et al. 2014). Senescence is due to the deterioration of cellular membranes and mainly associated to excessive reactive oxygen species (ROS) (Hodges et al. 2004). ROS is significant in maintaining abiotic stress response and physiological process in plants. However, during oxidative stress, when ROS level increase dramatically, it can lead to significant damage to the cell structures (Cocetta et al. 2014). Disintegration of cellular membranes, repression of enzyme activities, and loss of pigments were always demonstrated as shelf life and nutritional quality reduction of spinach (Hodges et al. 2004).

2.2 The plant cell

Most postharvest quality changes are due to changes at the cellular level, thus this section provides brief explanation of plant cell and its constituent organelles (Figure 2.2).

Plant cells vary widely in size, function, organisation, and response after harvest. Interior to the cell wall is the plasma membrane or plasmalemma that divides the interior of the cell and its contents from the cell's external environment.

The plasmalemma keeps the vegetables turgid by maintaining pressure inside the cell. It is this turgidity that keeps the vegetables crisp and firm (Wood et al. 2005). Plasmodesmata is a series of channels that allows exchange of various substances

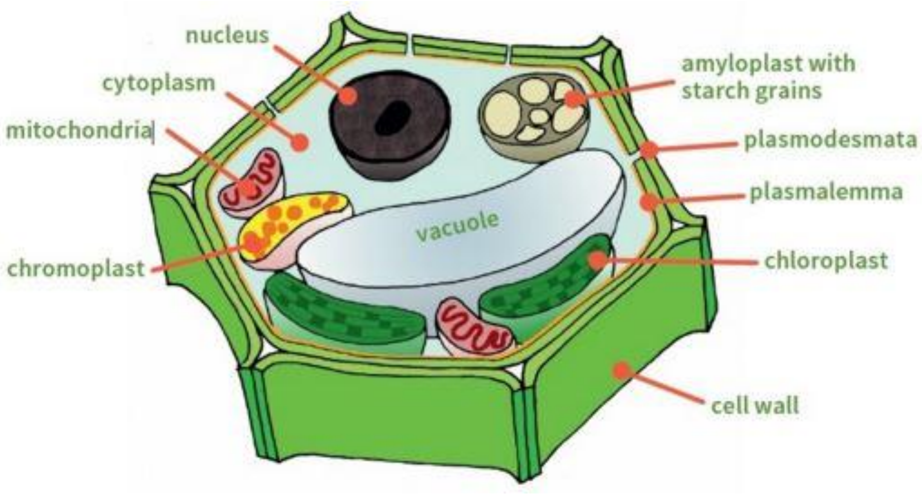


Figure 2.2: Plant cell and its constituent organelles (Marques n.d.).

between the cell. Inside the cell, there is a fluid matrix of proteins, solutes and macromolecules known as cytoplasm. Important processes and reactions that occur within cytoplasm include cells energy transfer, protein synthesis and breakdown of storage reserves of carbohydrate by glycolysis (Kays 1991; Wills et al. 2007). Within the cytoplasm, there are also several important organelles which include the nucleus that control the activity of the cell, mitochondria that acts as the powerplant of the cell by breaking down products from sugar into energy, chromoplast which developed mainly from chloroplast once chlorophyll is degraded and it gives colour to fruits and vegetables, chloroplast that is responsible for photosynthesis, vacuole the reservoir of liquid containing sugars, acids and other materials, and amyloplasts that contain starch grains (Wills et al. 2007).

Breaking or crushing the cells allows the compounds inside the cell to mix. For example, the oxidising enzymes in the cytoplasm mix with phenols held in the vacuole thus turning them brown. This browning is what we see in bruises and on cut surfaces. If the cell walls break due to mechanical or chilling injury, then the cell lost its turgidity and caused the release of the cell's contents, resulting in tissue softening (Marques n.d.). An understanding of the structure and function of the cells provides basis for a thorough understanding of quality changes occur in the product during postharvest.

2.3 Leaf structure

The main function of leaves is to acquire carbon dioxide for photosynthesis. The leaves also help to regulate the plant's temperature by controlling the transpiration process (Kays 1991). However, these functions no longer operative after harvest. Therefore, leaves depend on recycled carbon found within the cells for their maintenance of life. Still, most leaves do not able to store the carbon for a long term which then caused lack of energy reserves within the leaves leading to shelf life reduction (Kays 1991).

A cross section of leaf is shown in Figure 2.3. The exterior surface of leaves is covered with waxy cuticle that helps to decrease water loss where water vapour and gaseous must pass through the opening of stomata between the guard cells. The opening of lenticel also allows gaseous exchange inside and outside of the plant tissue. The epidermis lies beneath the cuticle acts as a border between inner cells and the surrounding. Spongy mesophyll lies directly beneath the epidermis and this is the important site for supplying the living plant with energy during photosynthesis. During transpiration, water evaporates from the cell walls of the mesophyll into the intercellular space that is directly connected to the outside air during the opening of stomata (Salisbury & Ross 1992). The veins (xylem and phloem) are the vascular tissue located in the spongy layer. They help to transport fluid and nutrients within the plant.

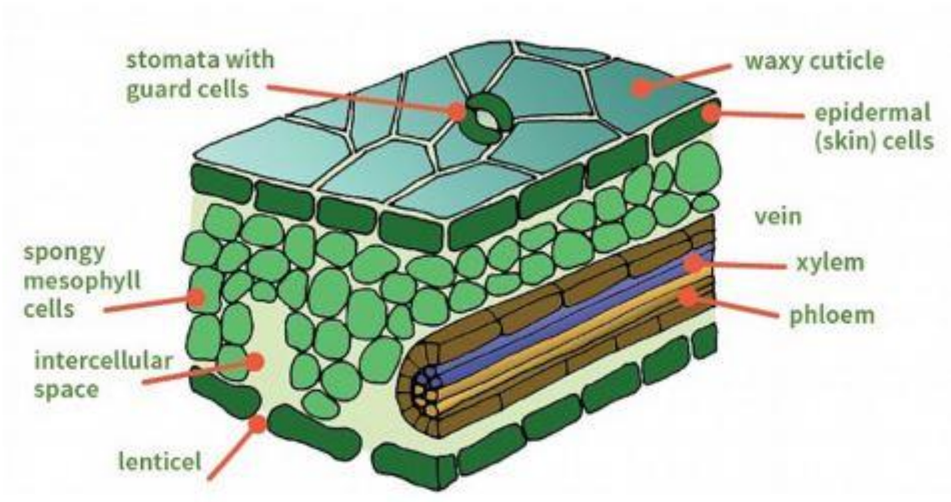


Figure 2.3: Cross section of a leaf (Marques n.d.).

2.4 Shelf life of leafy vegetables

The shelf life of a product is defined as the time duration that a product can maintain a specific quality level under specified storage conditions. The shelf life of a product must exceed the distribution time from the retailer to the consumer in order to give reasonable period of usage and home storage (Piagentini & Güemes 2002). Giménez et al. (2012) defined shelf life as the storage time where the product has reach above its predetermined deterioration level which is not saleable.

Since leafy vegetables are living organisms, there are transformations on the mechanical and chemical properties of the vegetables during storage because of the respiration, transpiration, and loss of moisture content to surrounding after harvested (Jung et al. 2012). These result in unfavourable qualities of the products such as browning, yellowing, shrinking leaves, releasing bad odours and bad taste (Niemira & Fan 2006; Neal et al. 2010). The shelf life of the fresh cut vegetables was significantly limited by appearance changes and odour (Medina et al. 2012; Dinnella et al. 2014).

2.5 Postharvest handling and processing

The main purpose of postharvest technology is to develop methods by which deterioration of fresh produce can be restricted as much as possible starting from the postharvest period until the end use of the product, thus maximum market value for the product can be achieved. However, the postharvest technology involves series of handling and processing which uncontrolled and mishandling could lead to quality degradation of the product. As for RTE products, harvesting, washing, bleaching, packaging and transporting, are series of basic actions before the products are ready for the market (Figure 2.4). The RTE products also known as minimally processed or fresh-cut. It includes minimal processes such as peeling, cutting or shredding in order to provide convenient product for consumers. However, they are highly perishable due to loss of epidermis (tissue outer protective layer) on large part of

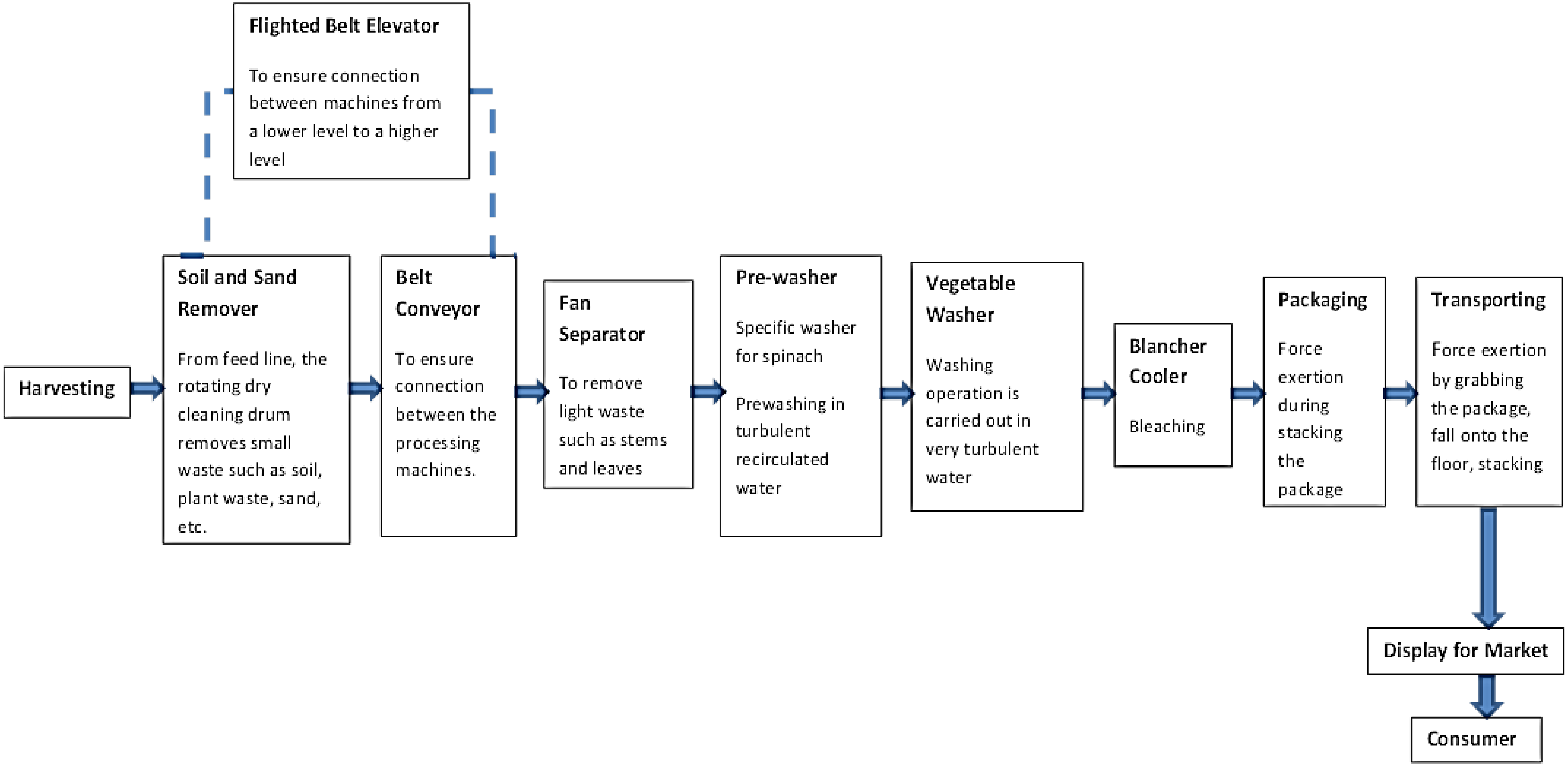


Figure 2.4: Basic processing line for spinach in the industry. Processing steps adapted from Femia, France (Femia n.d.).

their surface area (Watada et al. 1996). Fresh-cut vegetables are susceptible to decay, tissue softening, discolouration, and development of off-odours (Zagory & Kader 1988; Bolin & Huxsoll 1991). The ruptured cells due to grating and peeling caused the release of the oxidising enzymes (Ahvenainen 1996; Almualla et al. 2010). Large number of cut surfaces as well as high humidity are the perfect conditions for the growth of microorganisms (Ahvenainen 1996). Thus, selecting proper maturity of products for processing, controlling defects and disorders while maintaining relative humidity, temperature, and atmosphere are important to produce high quality fresh-cut products (Watada & Qi 1999). Constant temperature and proper gas composition maintained throughout the storage helped to extend the shelf life of spinach (Neal et al. 2010). Optimum atmosphere efficiently delayed the quality deterioration of the produce during storage (Watada et al. 1996). The fresh-cuts need to be stored at lower temperature than that recommended for intact products. Usually, 0°C is the preferable temperature for most fresh cut products, however, many are prepared, shipped and stored at 5°C and sometimes up to 10°C which then fasten the deterioration process (Watada et al. 1996). As for spinach, commercially, they are packed in polypropylene bags and stored close to 0°C, however, most of the times they are kept in the range of 4-10°C (Bergquist et al. 2006). In a study reported by (Fan et al. 2014), storing spinach at 7.5°C with high relative humidity (> 90%) can improve the shelf life of the product.

Several studies have been done to determine the effect of washing towards improving the shelf life of the RTE leafy vegetables. Warm chlorine water was found effective to improve overall appearance while reducing psychrotrophic microorganisms that might cause deterioration of RTE packed lettuce (Odumeru et al. 2003). Several workers have studied the effects of different sanitising agents towards microbial loads, nutritional contents, and sensory quality of fresh-cut escarole, lettuce, rocket leaves, and baby spinach (Martínez-Sánchez et al. 2006; Allende et al. 2008; V. M. Gómez-López et al. 2013). The highest concentration of vitamin C in baby spinach was found after washed with sodium hypochlorite and EOW, while product washed with peroxyacetic acid and EOW + NaCl showed significantly lower values of vitamin C. The increased amount of oxidants produced in EOW + NaCl and the oxidant action of peroxyacetic acid decreased the content of vitamin C after washing, although this effect became less relevant during storage. In fact, after 6 days of storage, baby spinach washed with chlorine-based sanitisers showed lower vitamin C content than the non-chlorine based sanitisers (V. M. Gómez-López et al. 2013). Previous studies have also reported the degradation of vitamin C in rocket leaves washed with peroxyacetic acid was lower than with other commercial sanitisers such as acidified sodium chlorite and lactic acid (Martínez-Sánchez et al. 2006). Combination of washing with chlorine dioxide and packaged under MAP helped to reduce the microbial loads thus improve the safety of spinach during consumption time (Lee & Baek 2008).

Studies have also been done to determine factors such as temperature and conductivity of the wash water as well as the packaging method that affecting the microbial counts for lettuce and spinach during processing lines and postharvest storage (Bolin & Huxsoll 1991; Barrera et al. 2012). Cocetta et al. (2014) stated that temperature and cutting methods greatly affected the reduction of ascorbic acid (AsA) of fresh-cut baby spinach leaves. Similar trend of AsA reduction were also reported on various fresh-cut fruits and vegetables as reported in the study stored at different temperatures (Lee & Kader 2000; Fernando Reyes et al. 2006; Gil et al. 2006).

Other factor such as low relative humidity could minimise *Salmonella* in baby spinach (Gómez-López et al. 2013). Inappropriate use of pesticide could lead to the increment of microbial loads thus affecting shelf life and safety of vegetables (Ng et al. 2005). Microbial predictive models have been developed in order to predict the total microbial count reduction at various conditions. *E. coli* O157:H7 growth prediction models of lettuce based on the effect of MAP and storage temperatures after treated with chlorine water (Posada-Izquierdo et al. 2013) and neutral electrolysed water (NEW) may be efficiently relevant to be incorporated in the quantitative risk assessment studies (Posada-Izquierdo et al. 2014). Pirovani et al. (2001) stated that Response Surface Methodology (RSM) would be sufficient to predict microbial log count reduction as well as chlorine concentration in wash water of fresh-cut spinach.

Modified atmosphere packaging (MAP) also has been widely used in the food industry to control the atmosphere of the packed foods. MAP is widely applied in the RTE or fresh vegetables industry which it helps in preserving and prolonging the food produces. Maintaining low CO_2 concentration below 5% in the packaging bag preserves and improves the shelf life of fresh-cut butter head lettuce (Varoquaux et al. 1996). Exposing fresh-cut Romaine lettuce under light promotes photosynthesis which resulting in the increment of O_2 concentration thus stimulate browning and loss of quality (Martinez-Sanchez et al. 2011). Maintaining optimum atmosphere during handling process of fresh-cut kale, and mixed load of snap beans and strawberries could be effectively achieved by combining the controlled atmosphere/MA system (Brecht et al. 2003). Jo et al. (2014) tested an active flush system of the sensor-controlled MA and its effectiveness in maintaining the atmosphere inside the container compared to a perforated package, thus preserving the quality of the vegetables. MA container with time-controlled gas-diffusion tube was capable of producing an optimal MA which agrees with the estimation from the design thus lead to better control of spinach quality (Kwon et al. 2013). Other work has been done by (Rodriguez-Hidalgo et al. 2010) which helps to preserve the quality and shelf life of minimally processed baby spinach planted in floating trays where N_2O-enriched atmospheres was used as an alternative to passive MAP.

In addition to that, up to recent postharvest technologies, treatment with low to moderate UV-C radiation is an effective alternative to sanitisation with chlorine water to preserve the quality of minimally processed spinach leaves (Artes-Hernandez et al. 2009). Combinations of UV-C, and super O_2 enriched packaging could be effective to preserve and improve the quality of Tatsoi baby leaves (Tomas-Callejas et al. 2012) and fresh-cut kalian-hybrid broccoli with pre-treatment with electrolysed water (Martinez-Hernandez et al. 2013) while minimising water usage. Apart from that, gamma radiation has also been widely used in the vegetables handling process to ensure the quality and safety of the products. Rezende et al. (2014) stated that the quality of spinach leaves could be preserved and improved by exposing the leaves under appropriate gamma irradiation. Combination of low irradiation with MAP has also been tested towards RTE baby spinach at different temperatures (Gomes et al. 2011b) and fresh cut romaine lettuce where it caused ten percent loss in firmness while maintaining visual appearance, colour, generation of off-flavour (Prakash et al. 2000). Gamma irradiation has also been practiced with other foods such as spices (Kirkin et al. 2014), grated carrots (Lacroix & Lafortune 2004), and traditional African cereal (Duodu et al. 1999) as an alternative treatment to optimise their qualities and shelf lives. Other method such as ozone treatments were found effective to reduce the microbial loads of spinach, parsley, and lettuce

(Karaca & Velioglu 2014), however, significant colour and quality changes after treatment requires more research (Klockow & Keener 2009). Garcia et al. (2003) combined chlorine washing and ozone treatment which helps to improve the shelf life and quality of lettuce salads.

Other novel techniques that lead to favourable results to the products' qualities were also introduced such as treatment with high pressure carbon dioxide (HPDC) to preserve fresh-cut carrot slices (Bi et al. 2011), fumigation of sodium hydrosulfide (NaHS) to reduced leaf yellowing of water spinach (Hu et al. 2015), e-beam irradiation to reduce bacterial counts and to prolong the shelf life of fresh spinach (Neal et al. 2010), advantage use of nano-mist as an alternative to conventional mist humidification during spinach-storage atmosphere (Saenmuang, Al-Haq, Makino, et al. 2012), effective combination of spraying microencapsulated antimicrobials with e-beam irradiation to deactivate microorganisms on spinach (Gomes et al. 2011a), as well as beneficial use of bio-activity of whey fermented extract towards organic grown lettuce as the other option of sanitiser which gives good contribution in the organic fresh vegetables industry (Santos et al. 2015).

All the processing and handlings need to be conducted carefully to prevent and minimise any possible damage to the products. Physical stress during processing affects physiology and biochemistry of the fresh-cut products which in returns affect the quality and shelf life of the products (Watada et al. 1996). Due to the series of processes, the products are opened to such an ideal medium for growth of microorganisms. Although they are stored in optimum temperature, that low temperature seems not to inhibit the development of psychrotrophic microorganisms, specifically saprophytic fluorescent pseudomonads together with other pathogens such as *Listeria monocytogenes,* and *Aeromonas hydrophila* and foodborne pathogenic bacteria such as *L.monocytogenes* and *Salmonella* spp. where all these microorganisms are responsible for the contamination and spoilage of the fresh products (Babic et al. 1996). Excessive treatments cause breakage or softening of the cell walls and on the pectins which provide the structural rigidity to plant tissue (Wood et al. 2005). Change in textural characteristics is due to the spoilage microorganisms breaking down the pectins which lead to softening (Neal et al. 2010). Quality degradation of fresh-cut product is due to the mechanical damage, moisture loss, tissue metabolism and microbial contamination (Piagentini et al. 2002). The mechanical damage will elevate the rate of decay of the products thus affect the sensorial and nutritional qualities of the products, which might lead the product to be inappropriate for the consumers' consumptions (Tang et al. 2011).

Processing steps give both advantages and disadvantages impacts towards products' qualities during postharvest storage. Regardless of the extensive works covered on the postharvest handling and processing of vegetables, further research is still needed to differentiate different degrees of mechanical injuries resulted from the processing and how they affect the quality and shelf life of RTE spinach.

In this thesis, the processing focused on compression and it effects towards degree of mechanical leaf injury. The effects of degree of compression and degree of mechanical leaf injury towards quality and shelf life of the RTE spinach are also discussed.

2.5.1 Uniaxial compression

Uniaxial compression test has been widely used to determine mechanical properties of fruits and vegetables. To create the degree of compression, peeled apple was compressed by 20% of its height (Billy et al. 2008), tomato was compressed by 3% of its diameter (Hu et al. 2005), and cooked potatoes were compressed at 75% of its height (Thybo & Nielsen 2000). Similar study has also been early reported on apple, carrot, frankfurter, cream cheese and pretzels, where the extension cycle control of the Instron was adjusted to give compressions of 50%, 60%, 70%, 80%, 90%, and 93%, corresponding to clearances between the plates at maximum compression (Bourne & Comstock 1981). Force-deformation testing has also been done to study the mechanical properties of melon (Emadi et al. 2009) and lentil-based snack with compression of 100N load cell (Lazou et al. 2011).

Failures on papaya and pineapple have been reported by (Peleg et al. 1976) under compression, where the authors described the actual failure was caused by the shear stresses where the samples sliding 45° along the plane to the compressive load. Tri-axial compression test on cylinder apple flesh caused the sample to slip 45° from the loading direction; however, most of the cells on either inside or outside or on the side of the fracture line remained undamaged (Holt & Schoorl 1982). Diehl et al. (1980) reported on the breakage of apple flesh and shearing of potato flesh under uniaxial compression. Mohsenin (1977) described the cracking of potatoes as well as splitting of tomatoes where it expended the fruit diameter due to the stresses. The author also reported the cracking in cabbage, watermelon, and apple under certain loading conditions.

Apart from that, heat-treated beet and turnip roots were compressed under a constant force of 30N in a single-cycle compression-decompression test using the Universal Testing Machine, UTM to evaluate texture properties (Taherian & Ramaswamy 2008). By performing this test, several mechanical parameters of the samples could be obtained, e.g. firmness, stress-to strain ration (Young's modulus) as a measure of the sample stiffness, and the percentage of recoverability of input energy (percentage ration of the area under the force-to-deformation curve during relaxation in relation to the total area) as a measure of sample springiness.

Uniaxial single-cycle loading/unloading compression method was chosen as the type of compression used throughout this study. The purpose of the compression was to induce damage to the leaves in the laboratory relative to damage that may occur during processing and handling in the industry. Before ready to be marketed, the RTE spinach bags undergone series of actions. Stacking the bags, piling the trays, grabbing the bags and throwing the bags are examples of actions that caused the spinach leaves inside the bag to be accidentally compressed, causing the leaves inside to be mechanically broken and damaged. The external forces exerted on the plant caused mechanical injuries which then lead to changes of texture, colour, flavour, physiological, biochemical, and chemical composition of the commodities (Wills et al. 2007). As the spinach leaves were tested in bulk amount and random arrangement in the acrylic chamber, having a single complete cycle of loading/unloading compression would be most suitable to represent the bulk sample as a whole. The compressions have a standard degree of compression which means all the compression started at constant starting position and constant sample height.

Throughout this thesis, Universal Testing Machine Z030 (Zwick/Roell, Germany) was used as a compression method to induce damage to the leaves (Figure 2.5). Compressions were conducted within certain range of forces. The compressed samples were loose spinach leaves; however, they were compressed in bulk (fixed weight) as an imitation of packed spinach sold in the market.

From the compression, work done, W is expressed by,

$$W = Fx \tag{1}$$

where F is the force applied, N; and *x* is the original distance travelled, mm which also means the starting position of the upper platon before compression, L_0.

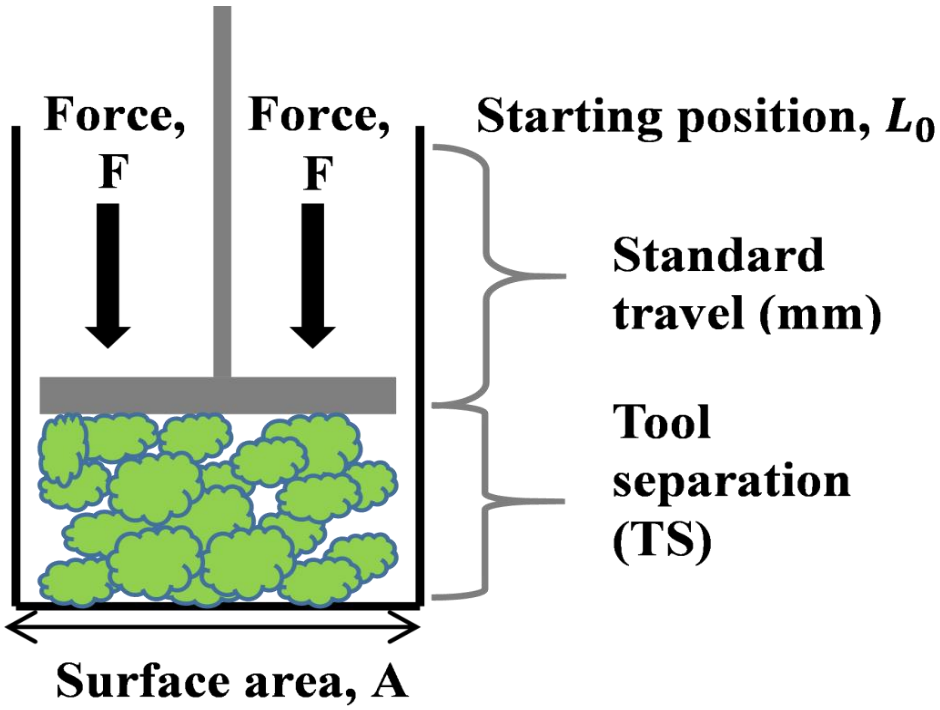


Figure 2.5: Illustration of the bulk spinach inside the bed chamber under the compression test using Universal Testing Machine (Zwick/Roell UK).

Bed volume inside the chamber, V is expressed by,

$$V = A \times TS \tag{2}$$

where A is the cross sectional area of the sample inside the chamber, mm^2; and TS is the tool separation, mm. Tool separation is the distance between the upper platon and the surface of the sample after compression which also means the change of distance after compression, ∆L.

The value of work, W (J) was generated from TestExpert II software. Thus, maximum work, MaxW (J) was the highest energy required to complete one loading/unloading compression. Force-displacement curves were also generated from the TestExpert II software. From the curve, area under the curve, AUC was calculated. The AUC was calculated by integrating the polynomial equation of the curve. The curve fits third order of polynomial equation:

$$y = Ax^3 + Bx^2 + Cx + D \tag{3}$$

The equation was then integrated from the value of maximum force, a to the minimum force, :

$$\int_b^a Ax^3 + Bx^2 + Cx + D \tag{4}$$

After the integration, the equation became:

$$y = [Ax^4 + Bx^3 + Cx^2 + Dx]_{x=a} - [Ax^4 + Bx^3 + Cx^2 + Dx]_{x=b} \tag{5}$$

Here, the minimum force, b was assumed to be 0 N as it was the value of the force at the starting position of the compression. The area under the curve was the sum of each integrated unknown in equation 5. As the polynomial equation was obtained from the force-displacement curve, it has the unit of N.mm. To convert the unit N.mm to J, the value was divided by 1000.

2.6 Mechanical injury

Holt & Schoorl (1982) classified mechanical failure of vegetables and fruits in three categories which are cracking, slipping, and bruising. Cracking is when the tissue got teared apart due to tensile stresses. On the other hand, slipping is when two pieces of samples slide relative to each other due to shear stresses, whereas the third group of failure which is bruising is associated with the extensive damage of the tissue due to cell bursting. The property of these failures varies depending on the loading conditions, and ripening state. For example, the failure of potato flesh may be due to breaking, slipping or bruising depending on the loading conditions (Holt & Schoorl 1982).

A common failure in fruits and vegetables is bruising. Bruising is defined as the damaged on the plant tissues caused by external forces which lead to changes in physical and chemical attributes of the product (Mohsenin 1977). The ruptured plant cells released the cell contents to the intercellular air space. Due to this, the enzymes are oxidised and lead to discolouration of the plant tissues. Several studies have reported on the tissue browning of pears, cherries, apples, grapes, peaches, apricots and bananas (Mohsenin 1977), papaya, mango, watermelon, and pineapple (Peleg et al. 1976), due to the cellular oxidation. Holt & Schoorl (1982) stated that for bulk sample, the bruising could also be associated with shear failure.

Gonzalez et al. (1988) studied the effect of leaf injury towards Ethylenethiourea (ETU) residues where the injuries were induced by crushing the spinach leaves manually. The author reported that the leaf injury provides more areas for the fungicide residues to be trapped in the leaves. The mechanically injured leaves had significantly higher ETU residues level than the non-injured leaves (Gonzalez et al. 1988).

Mechanical injuries are irreversible plastic deformations that lead to organ breaking which then lead to cellular and tissue ruptures within the plant (Luengo et al. 2008). Mohsenin (1977) stated that it is important to understand the mechanical strength of food through the understanding of the failure mode to reduce mechanical damage and energy consumption during mechanical processing. The applied stress and material are the two factors that need to be considered in making the prediction of failure (Holt & Schoorl 1982).

Regardless of the established works developed on classifying the modes of failures into cracking, slipping, and bruising, further study is still needed to evaluate the degree of injury experienced by the fresh produce regardless of the modes of failures. The study on modes of failures focused on classifying the fresh product into category that caused the failure whereas the study on different degree of injury is focusing on evaluating and classifying the degree of injury experienced by the fresh products that may come from cracking, slipping, and bruising. In this thesis, different degrees of injuries were evaluated focusing on mechanical injuries due to compression and their effects towards quality and shelf life of spinach were investigated.

2.7 Quality changes of leafy vegetables during postharvest

2.7.1 Mechanical properties of leafy vegetables

2.7.1.1 Young's modulus

Young's modulus, E is an intrinsic property of a material. Elastic and plastic deformation components of a material are assumed to follow these two fundamental parameters: an elastic modulus and a "resistance to plastic deformation". In hard and compact material such as metal, the elastic deformation is minimal due to the relatively small resistance to plastic deformation thus hardness can be measured by the resistance of the plastic deformation. In most other materials including mineralized tissues, both elastic and plastic deformations contribute equally to the total deformation, thus the hardness of the material is dependent on the elastic modulus, E. (Oyen 2006).

Young's modulus or also known as elastic modulus is the ratio of stress, σ over strain, ε for elastic deformation.

$$E = \frac{stress}{strain} \tag{6}$$

Hooke's law stated that stress is directly proportional to strain in the elastic region, $\sigma = E\varepsilon$ (Figure 2.6). The plastic deformation is due to the movement of atomic dislocations within the structure of a material. Basically, E is the measure of the strength between the atoms. Hardness is measured as the ability of the material to resist the deformation during surface indentation. The higher the value of the elastic modulus, the harder the material is (Instruments n.d.).

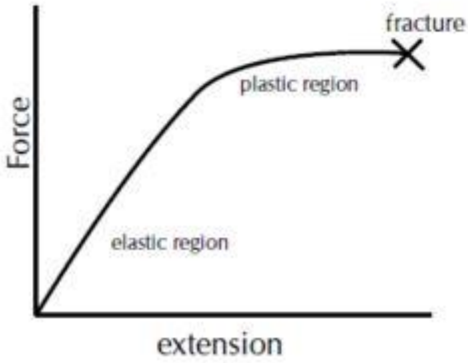


Figure 2.6: Hook's law graph (Williams 2011).

Stress is defined as,

$$stress = \frac{F}{A} \tag{7}$$

where F is the force exerted on a sample under compression, N; and A is the cross sectional area of the sample inside the chamber through which the force is exerted, mm^2. Strain is defined as,

$$strain = \frac{\Delta L}{L_0} \tag{8}$$

Where ΔL is the change in length after compression, mm; and L_0 is the starting position of the platon before compression, which indicates the original height of the sample inside the chamber before the compression, mm. The mechanical property of spinach is measured by measuring the Young's modulus, E,

$$E = \frac{FL_0}{A\Delta L} \tag{9}$$

By substituting equation 1 and 2 into equation 9,

$$E = \frac{W}{V} \tag{10}$$

Therefore, E is the measurement of the work done or the energy stored per unit volume of the sample inside the chamber under compression. The unit for V in this case was mm^3. It was then divided by 1000 and the unit became cm^3. The unit for W is J. 1 J/cm^3 is equivalent to 1 MPa. Therefore, the unit of E throughout this study would be in MPa.

2.7.1.2 Texture

Texture is one of the main factors in quality evaluation of fruits and vegetables offered in the market (Billy et al. 2008; Desmet et al. 2002; Stoneham et al. 2000; Rizvi & Tong 1997; Van Buren 1979; De Roeck et al. 2010; Barrett et al. 2010).

In this study, Penetration test was chosen as the method to measure texture changes of spinach throughout storage. Studies have reported that for both monocotyledon and eudicotyledons leaves; their mechanical properties, which connected to their anatomical characteristics, lead to differentiation in choosing the instrument to be utilised (Aranwela et al. 1999). Due to parallel alignment of veins along the vertical axis of the leaves, tensile test is ideal to determine the mechanical properties of monocots. On the other hand, due to its palmate venation and pinnate patterns, penetration test is ideal to study the eudicot mechanical properties (Aranwela et al. 1999). Penetration test is commonly used to analyse the mechanical properties of vegetables (Shiu et al. 2015). Through penetration test, the burst strength of the leaves comes from shear strength and compressive forces that are spread along the leaves orientation. The technique uses a spherical probe that will spread force homogeneously across a focus area and cause failure to the products (Bourne & Comstock 1981). The penetration test generate force-displacement curves where the mechanical properties such as strength, toughness, stiffness and displacement can be derived and analysed (Gutiérrez-Rodríguez et al. 2013).

Leafy salads like spinach have tender and soft texture which can easily lead to damage. It also has a very complex natural system that caused the measurement of its mechanical properties to be challenging. This resulted in lacking of engineering mechanical testing on it (Tang et al. 2011). Although there are researches that have been done to study the mechanical properties of spinach (More et al. 2014; Tang et al. 2011; Gutiérrez-Rodríguez et al. 2013; Tudela, Marín, Garrido, et al. 2013), there is still insufficient information in the literature to determine the effect of mechanical injury towards textural property of the spinach.

2.7.2 Browning and decay

Appearance is the main deciding factors when consumers are selecting fruits and vegetables (Kader 2002; Ferrante et al. 2004). Consumers ‘buy with their eyes’ to associate quality attributes to their desires in making decision to buy a product.

The physiological changes contribute to the shelf life reduction of the product (Odumeru et al. 2003). Samples with browning higher than 2% are usually rejected by consumers (Varoquaux et al. 1996).

One of the convenient methods to evaluate the appearance of fresh produce is by capturing the image with a digital camera. The computer based image software could be set to specify a consistent evaluation of the produce. This prevents any bias and inconsistencies of visual assessment by humans. It permits classification and quantification of the original existence of the sample regardless of the shape, size and texture (Studman 2001). Papadakis et al. (2000) stated that this technique is versatile and inexpensive which with additional development of the image analysis, it could be used in the food industry as a trustable quantitative tool in monitoring the quality and shelf life of the RTE products (Zhou et al. 2004). Besides that, (Pedreschi et al. 2005) has also suggested that the quick and easy measurement of image analysis should be used to determine browning rather than the meticulous chromatographic methods. Percentage of browning measured by image analysis showed better indication of lettuce quality compared to the values of colour changes measured by colorimeter as it closely associated to human visual evaluations (Zhou et al. 2004). From the digital camera, RGB images are produced. RGB which represents red, green, and blue colour space describes colour ranges by combining different amount of red, green, and blue with possible values ranging from 0 to 255 (Luzuriaga et al. 1997; Zhou et al. 2004). Tests have confirmed that image analysis performed on the RGB images allowed good segmentation, generating images that showed high difference between values of the leaf and the background (Lunadei et al. 2012).

Kruse et al. (2014) developed a method for identifying and quantifying leaf surface injury. However, the study only focused on the study of pixel classification and the leaf injury was referring to plant diseases and nutrient deficiency. There is still lack of study in the literature regarding identifying and quantifying mechanical leaf injury due to processing and handling and how it affect quality and shelf life of RTE spinach during postharvest storage.

2.7.3 Colour changes

The main colour changes in spinach leaves are due to the green colour degradation. It has been reported that spinach stored at 4°C demonstrated decay on the surface colour which turned from green to yellow (Lunadei et al. 2012). Yamauchi & Watada (1991) stated that changes in colour were correlated with the senescence of the harvested leaves. Polyphenol oxidase is an important enzyme that is responsible for the browning of the minimally processed vegetables, whereas lipooxidase is another important enzyme that catalyse peroxidation which caused the formation of bad-odour ketones and aldehydes (Ahvenainen 1996; Watada & Qi 1999).

Various studies have reported the use of colorimeters to study colour changes of spinach (Artes-Hernandez et al. 2009; Klockow & Keener 2009; Saenmuang, Al-Haq, Makino, et al. 2012; V. M. Gómez-López et al. 2013; Gómez-López et al. 2013; Glowacz et al. 2015), beans (Karathanos et al. 2006), and spices (Kirkin et al. 2014) during postharvest storage. However, they can only analyse small sections of the product, therefore repetition of measurement is required in different areas of the sample. Besides that this method also favours objects with uniform surfaces whereas irregular surfaces need to be processed or blended in order to get the uniformity. This only gives the average colour values and do not represent the original existence of the sample (Abbott 1999). Thus developing a colour analysis technique would be useful to predict the shelf life as well as to understand consumers acceptance towards the fresh produce (Jolliffe & Lin 1997). The technique was developed for overcoming colorimeter not able to capture whole sample equally due to limitations such as aperture size, ambience lighting, and sample background (Mery & Pedreschi 2005). This technique also would be useful for measuring colour changes on food product with irregular surface and size (Valous et al. 2009; Pedreschi et al. 2006; Du & Sun 2005; Mendoza et al. 2006; Kruse et al. 2014; Zheng et al. 2006; Yam & Papadakis 2004).

In this study, the colour analysis technique is developed. The results from this technique were then compared to results obtained from colorimeter.

2.7.4 Fresh weight loss

Fresh weight loss is another important quality attributes lead to quality loss of leafy vegetables. Serrano et al. (2006) reported that high rate of weight loss was the major problem associated with the quality of broccoli during storage. Decrement of surface wetness and juiciness over storage days indicated the water loss of the spinach samples which caused shrivelling and wilting (Neal et al. 2010). Excessive shrinkage is caused by many factors such as delay before storage, harvest during hot time and store the hot harvested-product in the cool storage, high temperature, low relative humidity due to insufficient insulation, excessive air circulation and packing the products into cartons or dry wooden boxes (Wills et al. 2007).

There has been increased in the weight loss as the exposure to the air increased (Jung et al. 2012). There is study reported on having high initial dry matter content and ascorbic acid lead to better visual quality of baby spinach during storage (Bergquist et al. 2006). It has been reported that 5% of weight loss was considered as acceptable by consumers which interpreted as “Relatively fresh/Fresh”. Percentage of weight loss can be used as a physical measurement to evaluate the freshness of spinach as well as to predict the perception of freshness by consumers (Jung et al. 2012).

2.8 Sensory evaluations

Sensory evaluation is one of the major methods that contribute to the understanding of a particular food product based on human perceptions. The deterioration of the product sensory property can be early detected by consumers before the nutritional and microbial decays occurred (Dinnella et al. 2014).

Various studies have been reported on the sensory evaluations of fresh products. Jung et al. (2012) studied consumers’ perceptions on freshness of spinach stored at different storage conditions based on freshness scales and interviews. Sensory test based on freshness ratings was also done to study consumers’ perceptions on the freshness of strawberry, apple and carrot (Péneau et al. 2007). Freshness is the main quality attribute for consumers in choosing vegetables n fruits (Ragaert et al. 2004; Jung et al. 2012). However, freshness degradation of RTE mixed salads (radicchio, endive, rocket, watercress and lettuce) during storage gave little relevance towards the hedonic scores (Dinnella et al. 2014). On the other hand, the hedonic scale significantly showed differences between RTE lettuce treated with different chlorinated water (Odumeru et al. 2003). Survey was also conducted to study consumers' perceptions on apple freshness (Péneau et al. 2006). Training and ballot development sessions have been conducted to determine texture (roughness, hardness, slimy, surface wetness), basic taste (bitter, sweet, sour), aromatics (nutty, earthy, green, decay), mouthfeel attributes (burn, astringent) and colour of spinach (Neal et al. 2010; Medina et al. 2012; Kou et al. 2014; Dermesonluoglu et al. 2015), arugula and mustard greens (Zhao et al. 2007), cauliflower (Cervera et al. 2007), and fresh-cut Romaine lettuce (Martinez-Sanchez et al. 2011).

There are many factors affecting consumers' perceptions and acceptances towards the fresh products. Besides convenience, the purchase intention was also affected by the delicious taste of the product. This showed that consumer's past experience plays important role for them to repeat the purchase of the product (Gardial et al. 1994; Bech et al. 2001; Ragaert et al. 2004). The weight of the packed product and the surface area of the package are also the pre-determine factor to obtain certain appeal to the consumers in the market (Kim et al. 2004a). In contrast, brand and organic farming were less important factors for consumers in selecting the RTE salad (Cardello & Schutz 2003).

2.9 Respiration rate of fresh produce

Respiration rate is an important parameter in postharvest quality. In general, the deterioration rate of the fresh produce is proportional to its respiration rate (Yamauchi & Watada 1991). Respiration rate can be used as a shelf life prediction for the fresh produce; however, the physiology of the fresh-cut products is changed too drastically to make the prediction (Watada et al. 1996).

Respiration is a catabolic reaction in which sugars, fatty acids, amino acids, and organic acids in the plant cell are oxidised to produce carbon dioxide, water and energy. The overall reaction for the breakdown of sugars is:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686\ kcal$$

Respiration in living organisms involves three major pathways of chemical reactions which are glycolysis, Kreb's cycle (tricarboxylic acid, TCA) and oxidative phosphorylation (electron transport system) (Figure 2.7) (Kader & Saltveit 2003; Aworh 2014). Glycolysis is a series of reactions that converts each glucose molecule to two molecules pyruvate, two molecules ATP and two molecules of NADH. During aerobic condition, pyruvate is completely oxidised to carbon dioxide and water in the Kreb's cycle. The glycolysis and the Kreb's cycle are linked by the formation of acetyl coenzyme A from the oxidative decarboxylation of pyruvate. The third pathway, oxidative phosphorylation involves the electrons transfer from NADH or $FADH_2$ to oxygen by electron carriers and produces ATP. In living tissue, the complete glucose oxidation yields 36 ATP with 32 ATP produced by oxidative phosphorylation (Aworh 2014).

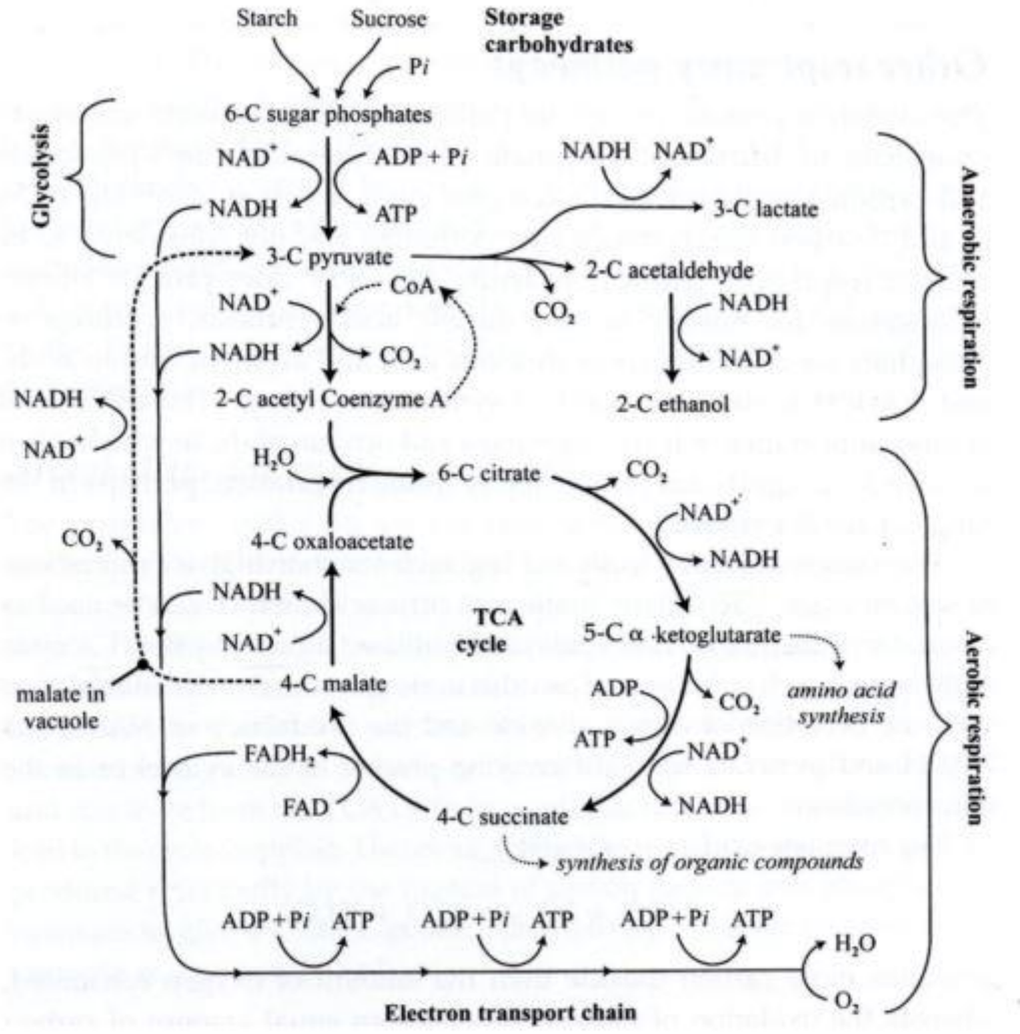


Figure 2.7: Simplified diagram of the biochemistry of aerobic and anaerobic respiration in plants. **(Wills et al. 2007)**

In anaerobic respiration, pyruvate is converted into lactate or acetaldehyde and ethanol. In total, the anaerobic respiration gives two moles of ATP and 21 kcal heat energy from each molecule glucose (Kader & Saltveit 2003).

2.9.1 Measuring respiration rate of fresh produce

Respiration rate is an overall measure of the metabolic activity. It involves cellular respiration, gas exchange process and the respiration of microorganisms as well as any other plant physiological processes that involve O_2 and CO_2 (photosynthesis, oxidation reactions, synthesis of plant hormones) (Løkke 2012).

Respiration rate is measured as the amount of O_2 consumed or CO_2 produced per weight unit per time unit. It is often measured in either a closed or flowing system. In the closed system, the product is kept in a tightly closed container (Figure 2.8) and changes in O_2 and CO_2 concentrations are measured over a period of time. The initial gas composition is usually the atmospheric (Jacxsens et al. 2000; Escalona et al. 2006) or the container can also be flushed with certain gas concentrations (Escalona et al. 2006; Torrieri et al. 2009). The respiration rate is calculated from the difference between two measurements. This system is easy and quick to set up. Disadvantage of this system is that the gas composition cannot be controlled during the measurement period (Løkke 2012).

In the flowing system, the container was flushed with gas continuously and the gas composition was measured at the inlet and outlet. The respiration rate was calculated from the difference between inlet and outlet. During the experiment, the gas composition can be varied and be any mixable combination of e.g. O_2, CO_2, and N_2 (Løkke 2012). However, it should be ensured that the gas flow is constant and the inlet gas should be humidified (Kader & Saltveit 2003). Thus, this system is more complicated to setup than the closed system.

In this thesis, closed system was chosen as the method of measuring the respiration rate as it is convenient to be set up yet efficiently works for various studies on measuring respiration rates of fresh product such as cut broccoli (Hagger et al. 1992), litchi (Mangaraj & Goswami 2011b), spinach (Saenmuang et al. 2012), fresh-cut papaya (Rahman et al. 2013), and cherry tomatoes (Sousa et al. 2017).

Figure 2.8: Closed system containing spinach samples.

The O_2 respiration rate in the closed system can be calculated by using equation:

$$[O_2]\ respiration\ rate = \frac{\left([O_2]^{initial\ time} - [O_2]^{final\ time}\right) \times free\ volume}{100 \times mass\ of\ produce\ \times (final\ time - initial\ time)} \quad (11)$$

$$[CO_2]\ respiration\ rate = \frac{\left([CO_2]^{initial\ time} - [CO_2]^{final\ time}\right) \times free\ volume}{100 \times mass\ of\ produce\ \times (final\ time - initial\ time)} \quad (12)$$

where $[O_2]$ and $[CO_2]$ are concentrations of O_2 and CO_2 respectively.

2.9.2 Modelling respiration rate of fresh produce

The enzyme kinetics, Michaelis-Menten model has been extensively applied in many studies in giving good fits to experimental data on measuring the respiration rate of many fresh produces such as chicory, apple, and tomato (Hertog et al. 1998), apples (Andrich et al. 1991; Andrich et al. 1998; Torrieri et al. 2009), shredded galega kale (Fonseca et al. 2002), guava (Mangaraj & Goswami 2011a), cut chicory, asparagus, broccoli, muungbean sprouts (Peppelenbos & van't Leven 1996), and cut broccoli (Hagger et al. 1992). This decision was made based on the assumption that in plant tissue, solubility and diffusivity of O_2 and CO_2 regulates reactions catalysed by enzymes.

In this study, uncompetitive inhibition was selected to study the effect of CO_2 on the respiration rate of the product. This selection was made by considering CO_2 that acts as a respiration inhibitor. During uncompetitive inhibition, the inhibitor (CO_2) does not bind with the enzyme but binds reversely with the enzyme-substrate complex, thus reduce product formation rate. The increase of O_2 concentration at high CO_2 concentrations has no effect on the consumption rate of O_2. This means that maximum respiration rate is not affected at high CO_2 concentration.

The uncompetitive Michaelis-Menten model equation is:

$$R_{O_2} = \frac{V_{m(O2)} \times [O_2]}{\left\{\{K_{m(O2)} + [O_2]\} \times \left\{1 + \frac{[CO_2]}{K_{i(O2)}}\right\}\right\}} \tag{13}$$

$$R_{CO_2} = \frac{V_{m(CO2)} \times [O_2]}{\left\{\{K_{m(CO2)} + [O_2]\} \times \left\{1 + \frac{[CO_2]}{K_{i(CO2)}}\right\}\right\}} \tag{14}$$

where RO_2 is the respiration rate, mL[O2] $kg^{-1}h^{-1}$; RCO_2 is the respiration rate, mL[CO2] $kg^{-1}h^{-1}$; $[O_2]$ and $[CO_2]$ are the gas concentrations for O_2 and CO_2 respectively in percentage; $V_{m(O2)}$ and $V_{m(CO2)}$ are the maximum respiration rate for O_2 consumption and CO_2 production respectively; $K_{m(O2)}$ and $K_{m(CO2)}$ are the Michaelis-Menten constants for O_2 consumption, and CO_2 production, % O_2, respectively; and $K_{i(O2)}$ and $K_{i(CO2)}$ are the inhibition constants for O_2 consumption, CO_2 production, % CO_2 respectively.

Apart from that, exponential model was also used for the model. It gave good fit with tomato (Cameron et al. 1989), cut iceberg lettuce (Smyth et al. 1998) and litchi (Mangaraj & Goswami 2011b). The exponential model equation is:

$$R_{O_2} = a.\exp(b[O_2]) \tag{15}$$

$$R_{CO_2} = a.\exp(b[CO_2]) \tag{16}$$

where a and b are arbitrary constants.

The parameters of the model equations were solve directly by non-linear regression analysis.

2.9.3 Quality changes of fresh produce stored at different films

Cause of failure is different depending on the packaging film used. The packaging film used affected the failure attribute associated to the shelf life of minimally processed cabbage (Pirovani et al. 1997). The respiration of the product itself and the oxygen transfer rate of the film (Lucera et al. 2011; Allende et al. 2004), as well as the weight of the packaged product, the respiration surface area, and storage temperature (Kim et al. 2004a) strongly affected the headspace gas composition of the fresh vegetables inside the system. The end of shelf life for minimally processed leek was associated with the texture as well as the type of packaging films and storage conditions (Ayala et al. 2009).

Perforations of the bags lead to dehydration and the loss of moisture to the surrounding (Pan & Sasanatayart 2016). Thus alternative packaging systems have been developed such as laser micro-perforated films, microporous membranes (Zagory 1997) and perforation-mediated MAP (Fonseca et al. 2000). The number and size of the micro-perforations affected the rate of gas exchange between the system and external environment. The micro-perforated film was found to be efficient in maintaining the sensory quality, and reducing weight loss and wilting of the produce in longer term. During commercial, the shelf life of baby spinach may not be prolonged over 7 days at 7°C unless having use of micro-perforated film with high CO_2 permeability (Tudela, Marín, Garrido, et al. 2013).

The salad savoy packaged in the highest oxygen transmission rate (OTR) film developed higher discolouration than those packaged in the lower OTR film (Kim et al. 2004a). 6000 OTR PE film caused 0.55 % whereas 3200 OTR PE film produced 0.39% weight loss of bok choy after 25 days of storage (Pan & Sasanatayart 2016).

Fresh-cut products are highly prone to weight loss due to the lack skin/cuticle and exposure of the internal tissue. However, relative humidity is very high in film bags thus dehydration is not a problem (Watada & Qi 1999). A problem associated with the packaging films is the condensation of the moisture inside the package which disturbs the viewing of the products and the droplets opens a site for possible microbial growth. Another problem is that, insufficient transmission of O_2 or CO_2 in-out of the package can cause injury to the product (Watada & Qi 1999). Therefore, selecting appropriate film with proper gas transmission while at the same time maintaining the proper RH is important (Watada & Qi 1999).

CHAPTER 3

EXPERIMENTAL PROCEDURES

3.1 Sample preparation

Spinach (*Spinacia oleracea*) varieties that were used throughout the project were Teen (S_1), Organic (S_2), and Salad (S_3) spinach sourced by UK wholesaler and Baby (S_4) and Young (S_5) spinach purchased from a local retailer in Birmingham, UK. During transportation, spinach leaves were stored in cool bags with aid of cool packs. Sorting of the leaves was conducted to include only good spinach leaves for the testing. Good spinach leaves referred to leaves that have no obvious breakage or damage, no signs of disease and no discolouration (yellowing or browning). Samples were stored in a 5 ± 1°C walk-in fridge.

Table 3.1: Types of spinach used in this project.

Types of spinach	Digital images
Teen spinach (S_1)	
Organic spinach (S_2)	
Salad spinach (S_3)	
Baby spinach (S_4)	
Young spinach (S_5)	

3.2 Leaves deformation by uniaxial compression

Compression tests were performed using a mechanical tester, Universal Testing Machine Z030 (Zwick/Roell, Germany) (Figure 3.1). Samples S_1, S_2, S_3, S_4, and S_5 were placed inside an acrylic chamber (110mm×150mm) and undergone uniaxial compression by upper platon. For every condition tested, there were three to five replications of compressions. Maximum position the upper platon can compress the samples inside the chamber at the pre-set compression force was noted as minimum tool separation, which was the minimum distance between the upper platon and the bottom of the chamber after the compression. Example of the sample's position before and after compression inside the chamber is shown in Figure 3.2.

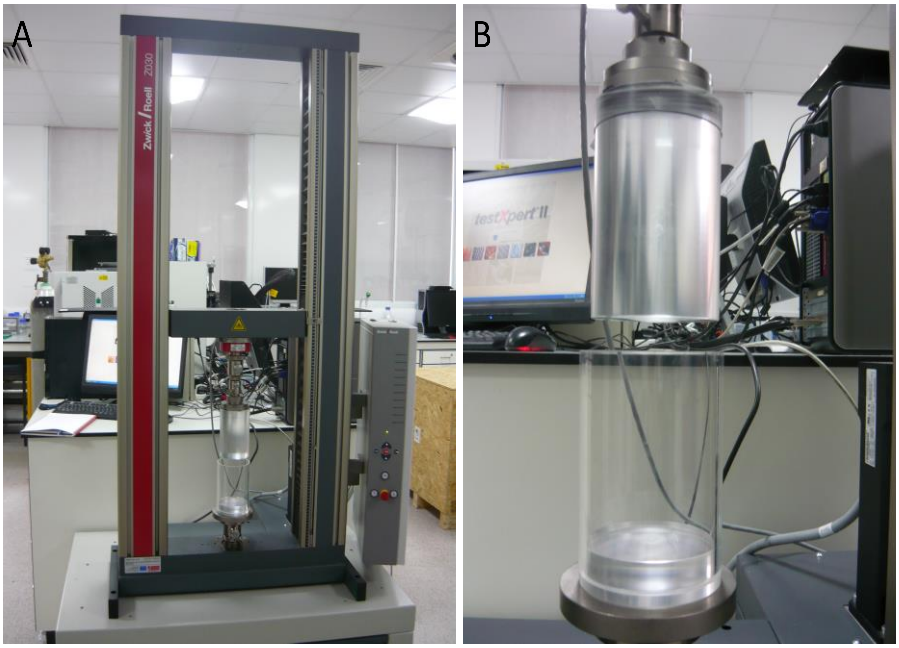


Figure 3.1: A) Universal testing machine Z030 mechanical tester (Zwick/Roell, UK) and B) the acryllic chamber and the upper platon.

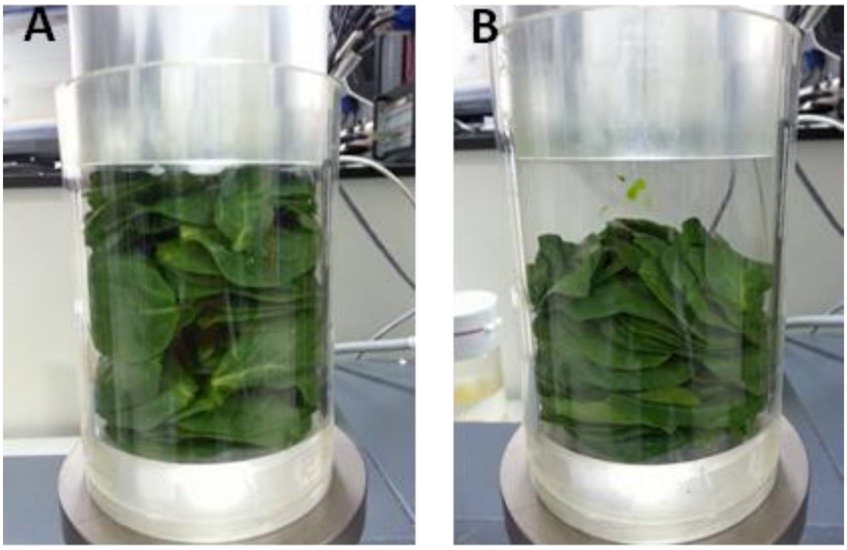


Figure 3.2: The position of the spinach leaves inside the chamber A) before compression; and B) after 100N compression.

3.3 Texture analysis by penetration test

The mechanical property of individual spinach leaf for sample S_4 and S_5 was measured by performing penetration test. A 55 mm length stainless steel with 5 mm spherical probe penetrated through a section of a single leaf using a texture analyser (T.A-XT, Stable Microsystem) (Figure 3.3). In order to hold the leaves in flat position during the test, they were clamped in between a Perspex film support rig with an aluminium circular top plate with existing holes of an area 0.95 cm^2 (Figure 3.4). The settings of the texture analyser are tabulated in Table 3.2 by following (More et al. 2014).

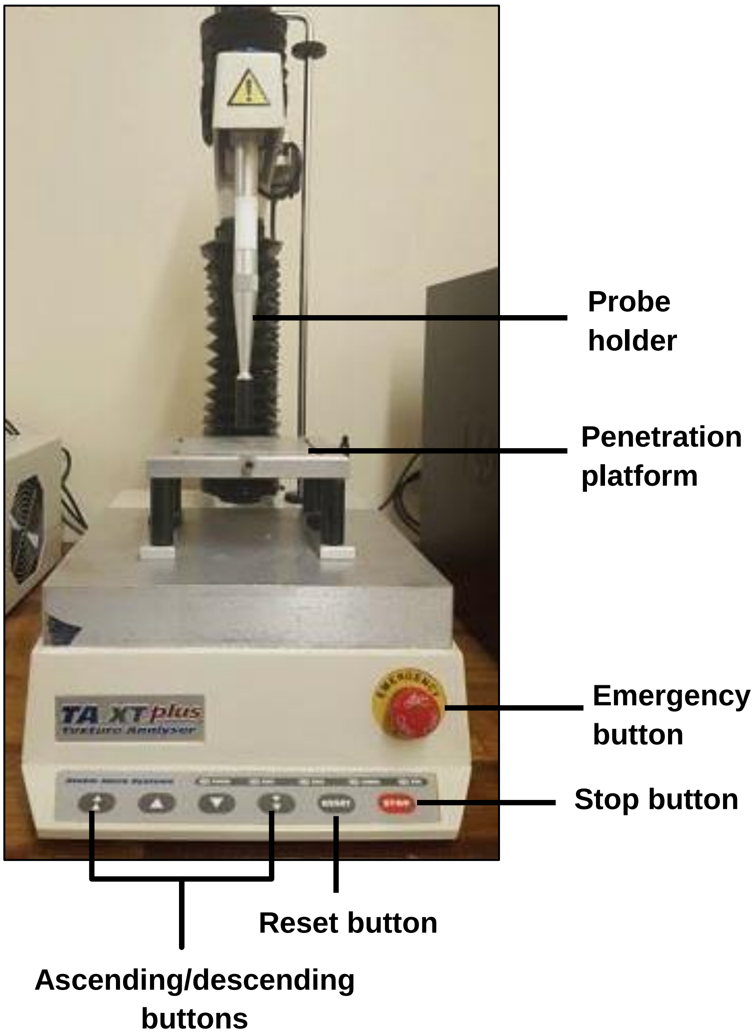


Figure 3.3: TA-XT texture analyser (Patel & Owen 2016).

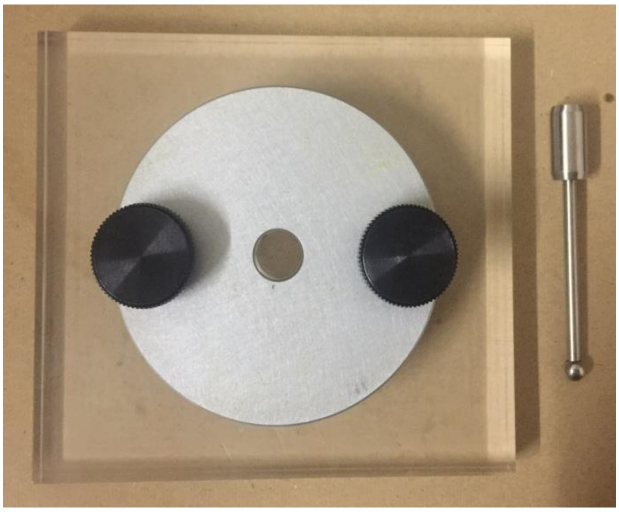


Figure 3.4: Penetration rig and probe.

Table 3.2: Texture analyser settings for penetration test .

Options	**T.A Settings**
Library	Return to Start
Test Mode	Compression
Pre-Test Speed	2.0 mm/s
Test Speed	1.0 mm/s
Post-test Speed	10.0 mm/s
Distance	10 mm
Trigger Type	Auto Force
Force	0.049 N
Probe Selection	P/5S (5 mm Spherical Probe)
Parameters	Undefined
Data Acquisition	PPS: 500; Typical Test Time: 1000s

Force-displacement curves were generated by software, Exponent. From the curves, burst strength (peak force, MaxF) and flexibility (displacement of MaxF) were determined. As suggested by (Aranwela et al. 1999), the point for the penetration was made consistent to minimise variation between the samples . The test was conducted on the flat surface of the leaf avoiding major veins as they could contribute to the variation of the results due to the different texture of the veins and the lamina parts. Tests were conducted by penetrating either on the left or right ad-axial sides of the leaves relative to the main vertical vein (Figure 3.5). For reproducibility, 10-20 replications of spinach leaves were tested for each category.

Figure 3.5: Ad-axial side of the spinach leaf where the penetration took place.

3.4 Microscopy imaging techniques

3.4.1 Light microscopy

The microstructure of the leaves was observed under a light microscope, DM RBE (Leica, Germany). First, the study focused on analysing the microstructure of different types of spinach samples S_1, S_2, and S_3. Second, the study focused on analysing the microstructure of spinach sample S_4 compressed at different forces. There were three replicates for each study. About 2 cm of the leaves were finely cut using a sharp disposable scalpel and placed on a glass slide protected with a thin cover glass. Images were viewed under 10 x 1.0 x 10 objective lens magnification. Cell structure and size of the spinach leaves were analysed and measured using image analysis software, Leica QWin.

3.4.2 Microtome

Spinach sample S_4 was cut of approximately 3 mm x 5 mm from the centre of the leaf lamina near the midrib and parallel to it. The tissues were fixed for 2.5h at 4°C in 0.1M sodium phosphate buffered (pH 7.2) mixture of 2.5% glutaraldehyde and 4% paraformaldehyde (Morales et al. 2001) . Tissues were rinsed with distilled water for five times to get rid of the phosphate ions. Tissues were then post-fixed with 1% osmium tetraoxide for 2h (Tudela, Marín, Garrido, et al. 2013). Tissues were rinsed with distilled water for five times again to get rid of the excessed osmium tetraoxide. Tissues were then dehydrated with 70%, 80%, 90%,and 100% ethanol concentrations consecutively with ten minutes each treatment (Spurr 1969). In prior to embed in wax, tissues were immersed in HistoClear (clearing agent) for one hour. Tissues were then incubated in warm wax for 24 h. Once wax has infiltrated the sample, the sample was carefully positioned and surrounded with additional wax using a steel mould to form a block (Rolls n.d.). Next, tissue cassette was attached to the wax block containing the tissues and ready for sectioning using microtome, RM2235 (Leica, Germany). The thickness of the sectioning was 5 µm. The sections are usually on the order of 4-10 µm thick for use with light microscopy. Tissue slices were first placed into a warmed water bath (48°C) and then lifted out of the water onto the glass slide and allowed to dry. After thorough drying (usually takes overnight), the samples on slides are ready for staining with 0.5% toluidine blue for five minutes. The micro-cut tissues were then observed under the light microscope, DM RBE (Leica, Germany).

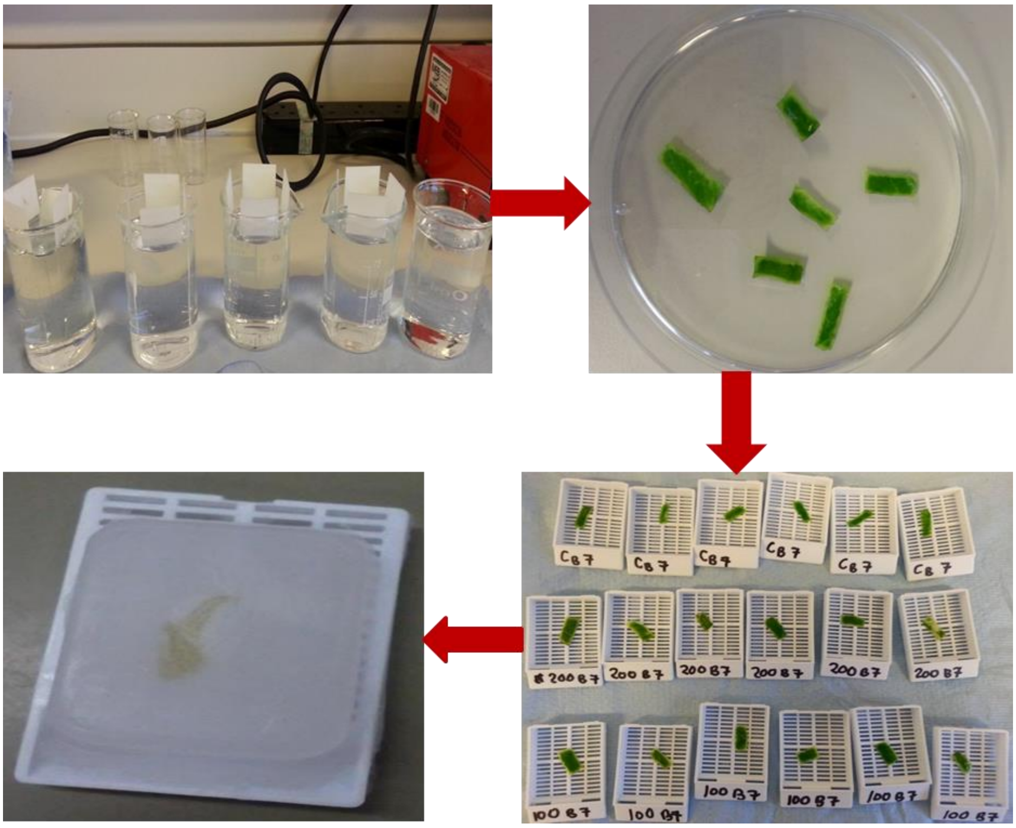


Figure 3.6: Series of tissue fixing. First image showed tissue dehydration within series of ethanol concentrations. Second image showed tissues immersed in clearing agent, HistoClear. The third image showed leaves tissues were positioned inside the cassettes before surrounded with additional wax to form the block. The last image is a ready wax block with the tissue embedded inside the wax.

3.5 Assessment of leaf injury by Population test

Population test is a method developed to evaluate physical appearance of the sample. Population test was developed with the aim to evaluate different degrees of mechanical leaf injuries and how they affect the quality attributes of leafy vegetables. Spinach sample S_4 were damaged by compression as described in Section 3.2. The compressed leaves were categorised into four different degrees of injuries as shown in Table 3.3 and Table 3.4. Uncompressed leaves (Control) were also prepared. For each leaf category, 24 replicates of leaves were used for sampling. The categorised leaves were packed inside packaging bag; biaxially oriented polypropylene (BOPP) film with dimension of 230 mm x 135 mm (ASP Flexibles, UK). Bags were heat sealed using a heat sealer (Kite Packaging, UK). All samples were stored at 5 ± 1°C for 14 days.

Table 3.3: Characterisation of the leaf injuries.

Degrees of leaf injuries	**Description**	**Average size/length/area**
Undamaged	No damage after compression	7.39 cm × 5.52 cm ($l \times w$)
Minor teared	Minor breakage after compression	0.92 cm
Halfway teared	Major breakage after compression but not teared completely	3.05 cm
Complete teared	Major breakage after compression that has damage part completely separated from other part of the leaf	1.67 cm^2

Table 3.4: Front and back side images of spinach sample S_4 for Control, Undamaged, Minor teared, Halfway teared, and Complete teared.

Leaf category	Control	Undamaged	Minor teared	Halfway teared	Complete teared
Front side					
Back side					

3.5.1 Acquisition of the leaf image

The compressed spinach leaves of sample S_4 were arranged on a rectangle white tray inside a black box with dimension of 65 cm x 40 cm x 40 cm. Two Pro Series LED video light, LE-130A (Mcoplus, China) were attached inside the top side of the black box and 9V of direct current supplies were used (Figure 3.7). The images were captured using digital camera with standard setting (Table 3.5). The images of Control spinach of sample S_4 were also acquired using similar method.

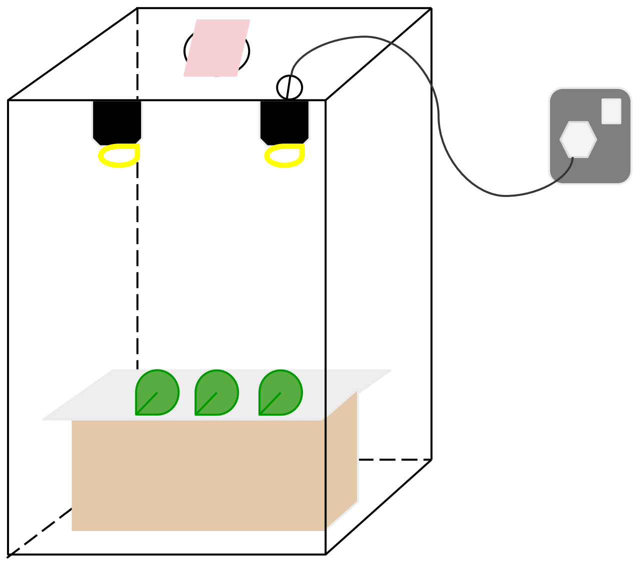


Figure 3.7: Spinach leaves arranged on a white tray inside the black box.

Table 3.5: Camera settings.

Variable	Value
Megapixels	8 MP
Focal distance	29mm
Aperture Av	f/2.2
Shutter speed	1/180s
ISO	32
Temperature	4900K
Flash	Off

3.5.2 Image analysis

Leaves' deteriorations were evaluated by analysing the digital images (RGB) using image processing program, ImageJ. A total of 24 replicates of spinach sample S_4 for each degree of leaf injury were analysed. The black/brown colours of the decays were detected by the ImageJ by adjusting the 'Hue, Saturation, and Brightness' in the 'Threshold colour' box. The detected areas were covered in red shade and these areas of decays were measured automatically by the software (Figure 3.8).

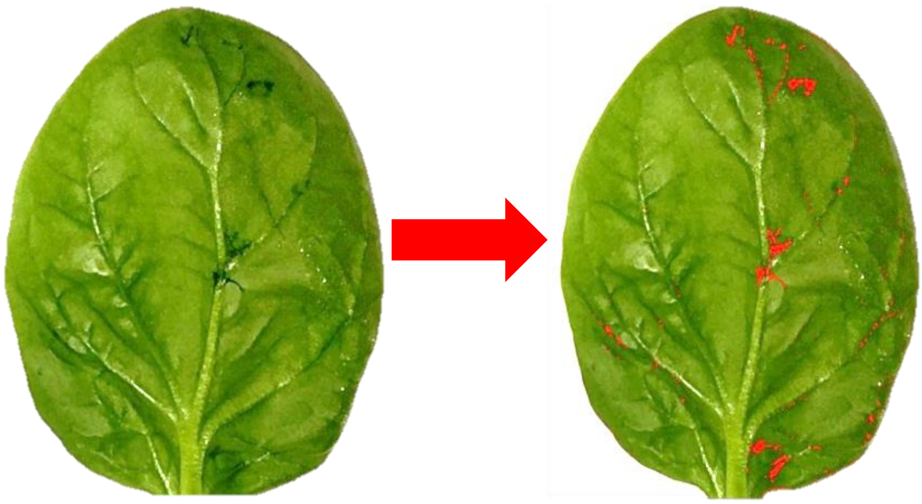


Figure 3.8: ImageJ detecting the deteriorations on the back side of the leaf. The red shaded regions were the regions of deteriorations detected by ImageJ.

3.5.3 Contour colour boxes

Contour colour boxes were also generated through a coding developed in Matlab (R2016b) (Figure 3.9). From this method, the degrees of leaf injuries are presented with the coloured boxes according to the percentage of deteriorations throughout storage. The colour of the boxes started from blue which represents 0% of decay to red which represents 100% of decays.

```
image(tabletwo);
colormap jet(100)
h=colorbar;
set(h,'ylim',[0 100])
for ii=1:3
    for jj=1:5
        if tabletwo(ii,jj)<30 || tabletwo(ii,jj)>70

text(jj,ii,num2str(round(tabletwo(ii,jj))),'FontSize',14,'Color','w')
        else

text(jj,ii,num2str(round(tabletwo(ii,jj))),'FontSize',14,'Color','k')
        end
    end
end

set(gca,'xtick', [1,2,3,4,5])
set(gca,'xticklabel',{'Day 1','Day 4','Day 8','Day 11','Day 14'})

set(gca,'ytick', [1,2,3])
set(gca,'yticklabel',{'Control','100N','200N'})
set(gca,'FontSize',16)
```

Figure 3.9: Matlab coding for contour colour boxes.

3.6 Fresh weight loss

Packed spinach samples S_4 were weighted before and after storage using an electronic weighing scale, BE610 (Beetle, UK). Water lost from the product to the environment during storage was measured by measuring fresh weight loss. The percentage of fresh weight loss of spinach was calculated based on equation:

$$W_{loss} = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100 \quad (17)$$

where W_{loss} is the weight loss (%), $W_{initial}$ is the weight of the spinach bag before storage (g) and W_{final} is the weight of the spinach bag after storage (g).

3.7 Moisture content

Moisture content of spinach sample S_4 was measured using electronic moisture balance, MA37 (Sartorius, Germany) using gentle drying (80°C). 200N-compressed leaves and control leaves were used as the samples. The samples were placed on a small aluminium tray for the testing (Figure 3.10). The time taken for each leaf sample to dry completely ranged between 30-40 mins.

Figure 3.10: The spinach leaves were placed on a small aluminium tray to measure the moisture content.

3.8 Sensory evaluation

Sensory test was conducted by a total of 80 non-trained assessors. Since storage days involved in this study, the bagged spinach were prepared in a reverse day method. Packaging of the spinach bags began 14 days before the test date. Bags were prepared on the 14^{th}, 11^{th}, 8^{th}, 4^{th}, and a day before the test date. Spinach leaves of sample S_4 were packed inside BOPP film with dimension of 230 mm x 135 mm (ASP Flexibles, UK). Bags were heat sealed using heat sealer (Kite Packaging, UK). The packed bags were stored at temperature 5 ± 1°C for 14 days.

The spinach bags were coded with random three digit codes generated by online digit code number generator (University of Oregon, n.d.). The packed spinach were provided on white paper plate and placed at individual table during the test. Ranking test was used to evaluate consumers' perceptions in evaluating the visibility of deteriorations of the product. Assessors were asked to rank the visibility of deterioration on the leaves' surfaces (5 = most visible; 1 = least visible). 'Allowing ties' or 'no preference' option was not allowed as to retain more statistical power (Kemp et al. 2009). Statistically, both parametric and non-parametric tests were combined to analyse the data. Fisher LSRD and t-test ($p<0.05$) were performed to find significance difference for the visibility of leaves deteriorations between degrees of leaf injuries throughout storage days. Parameter $t_{\alpha/2}$ was taken from table for Student's t distribution (see O Mahoney 1986).

To determine consumers' acceptances in buying the product, acceptance test was carried out. Assessors were asked to rate their level of liking in buying the bagged spinach using five levels of hedonic score (5 = I certainly would buy; 4 = I might buy; 3 = I might buy/I might not buy; 2 = I might not buy; 1 = I certainly would not buy). Assessors were allowed to hold and turn the spinach bags as to make it as natural as consumers usually behave in the supermarket. The assessors were allowed to make evaluations based on visual appearance of the samples. Friedman ANOVA-Wilcoxon with post-hoc Bonferroni test ($p<0.017$) was conducted to find the significance difference on consumer's acceptance in buying the product. Statistical software IBM SPSS (version 24) was used to conduct the FRIEDMAN ANOVA-Wilcoxon test.

For both tests, assessors were allowed to give opinions and criticism regarding the products on the questionnaire used for the sensory evaluation. A set of questionnaire is available in Appendix A.4.

3.9 Measuring respiration rate of spinach using closed system

Spinach sample S_4 was used to study the respiration rate of spinach using a closed system. Glass jars (1L) containing air as initial gas atmosphere were used as the closed system. The jars were tightly sealed with micro-perforated BOPP film with different oxygen transmission rate (OTR) and water vapour transmission rate (WVTR) (Table 3.6). Oxygen and carbon dioxide concentrations were measured using gas analyser, Checkmate II (Dansensor, Denmark). Prior to taking gas measurement, a 15 mm resealable rubber septum was attached on top surface of each jar to prevent gas leakage into/outside the system (Figure 3.11). The gas sample was taken using a 0.88 mm stainless steel needle every 1 hour for the first 6 hours of storage, every 6 hours for the next 42 hours and finally every 24 hours for the next 12 days. For each film tested, three jars of replications were prepared. The jars were then stored at temperature 5 ± 1°C for 14 days.

Table 3.6: Types of packaging films.

Type of films	Material	Film gauge (µm)	OTR (cc/m^2/day)	WVTR (g/m^2/day)
Film 1	BOPP	60	400	3.0
Film 2	BOPP	35	1200	5.5
Film 3	BOPP	35	1100	0.9

Figure 3.11: Spinach leaves inside the closed system.

3.10 Measuring colour changes of spinach

There are two parts of colour measurements in this study. The first part is measuring the colour changes using colorimeter, CR-410 (Konica Minolta, Japan) and the second part is developing a colour analysis technique. In this study, the colour analysis technique was developed by combining several steps as reported by (León et al. 2006) and (Pedreschi et al. 2006). The system composed of (i) acquire the image of the sample in a digital format (Section 3.5.1), (ii) a computer to store the images, (iii) Matlab coding that converts the RGB values of the sample into L*, a*, b* units (Figure 3.12), (iv) series of colour charts to calibrate the digital colour system (Figure 3.13) where L*, a*, b* values of each colour chart was measured using colorimeter. Additionally, a RGB digital image was also taken from each colour chart and the RGB values were converted to L*, a*, b* using the image analysis coding integrated in the Matlab, (v) Calibration curves (L*, a*, b* values from colorimeter vs. L*, a*, b* values from camera) where the values were obtained from the colour charts, (vi) Linear equation models obtained from the calibration curves for the conversion of L*, a*, b* values from camera to L*, a*, b* values of colorimeter. *L** represents changes of *Lightness, a** represents changes of *Red* and *Greenness,* whereas *b** represents changes of *Yellowness* and *Blueness.*

```
% Read RGB image into the workspace.
rgb = imread('min1.jpg');
%%
% Convert the RGB image to the L*a*b* color space.
lab = rgb2lab(rgb);
%%

% Display the L* component of the L*a*b* image.
figure(1);
imshow(lab(:,:,1),[0 100]);
figure(2);
imshow(lab(:,:,2),[-110 110]);
figure(3)
imshow(lab(:,:,3),[-110 110]);



L = lab(:,:,1);
a = lab(:,:,2);
b = lab(:,:,3);

disp( [mean2(L(rgb(:,:,1)~=255)),
mean2(a(rgb(:,:,2)~=255)),mean2(b(rgb(:,:,3)~=255))] );
```

Figure 3.12: Matlab coding for conversion of RGB values to L*a*b* values.

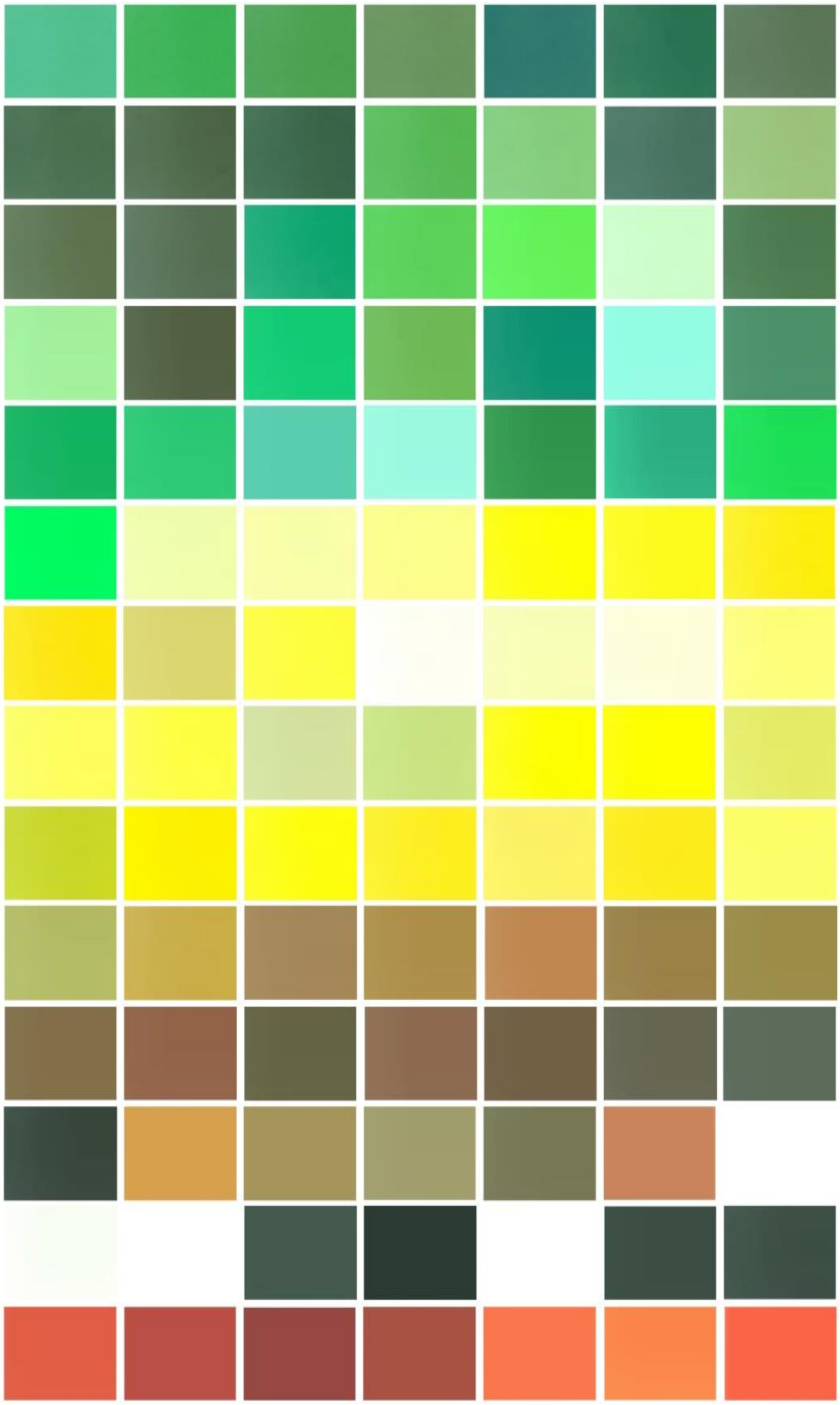


Figure 3.13: Colour charts of 98 colours.

CHAPTER 4

COMPRESSION AND ITS EFFECT TOWARDS MECHANICAL PROPERTIES AND QUALITIES OF LEAFY VEGETABLES

4.1 Introduction

There have been extensive works reported on the mechanical properties of fruits and leafy vegetables; however, there is still lack of information to determine the mechanical behaviour of the leafy vegetables in bulk, as it would be the representative of the RTE leafy vegetables retailed in packaging bags. Most mechanical properties reported in the literature were investigated based on individual sample by performing mechanical tests such as tensile test, compression test, penetration test, cutting, tearing, puncturing and shearing (Ávila et al. 2007; Golmohammadi 2013; Toole et al. 2001; Desmet, Van Linden, et al. 2004; Desmet, Lammertyn, et al. 2004; Vincent 1982; Vincent 1991; Toole et al. 2000; Lucas et al. 1991; Hiller & Jeronimidis 1996; Newman et al. 2005; Read & Sanson 2003; More et al. 2014; Nabil et al. 2012; Tang et al. 2011; Wang et al. 2010; Al-Sulaiman 2000; Aranwela et al. 1999).

Compression-induced injury is frequently encountered during handling, packaging, transporting, and storage (Kays 1991). From grabbing to stacking the bags for display in the market, the RTE leafy vegetables are vulnerable to mechanical damage due to the accidental compression throughout the processes.

Spinach is one of the leafy vegetables that can have high risk to be mechanically broken due to its soft and tender properties. It is helpful to examine the mechanical behaviour changes of the leafy vegetables by applying load in order to understand how the mechanical stress damages the plant tissues (Kays 1991). The mechanical properties of fruits and vegetables can be identified by using compression testing (Sadrnia et al. 2008; Emadi et al. 2005; Bahnasawy et al. 2004; Grotte et al. 2001; Jackman & Stanley 1994; Holt 1970). Therefore, in this chapter, the effect of compression towards mechanical properties of both bulk and individual spinach leaves during storage were investigated. Microstructural property of the leaves was also discussed.

4.2 Materials and methods

In this chapter, the studies were categorised into three sub objectives. The first sub objective was to study the energy of compression which consists of the energy of single loading/unloading compression of bulk spinach (Section 4.3.1.1), the energy of multiple loading/unloading compression of bulk spinach (Section 4.3.1.2), and the energy of multiple loading/unloading compression of stacked spinach (Section 4.3.1.3). For the studies on single and multiple loading/unloading compression of bulk spinach, spinach types S_1, S_2, and S_3 were used and compressed under 100N. The samples were stored for six days. For the study on multiple loading/unloading compression of stacked spinach, spinach type S_4 was used and compressed under 50N, 100N, 200N and the effect of stem towards the energy of compression was also studied.

The second sub objective was to study the Young's modulus, E of bulk spinach and it covers the Young's modulus between different types of spinach (Section 4.3.2.1), Young's modulus of spinach compressed at different forces (Section 4.3.2.2), and Young's modulus of spinach compressed at different weight (Section 4.3.2.3). For the study on Young's modulus between different types of spinach, spinach types S_1, S_2, S_3, and S_4 were used and compressed under 100N. The effect of storage on Young's modulus was also studied with Day 6 as the final measurement day. For the study on Young's modulus of spinach compressed at different forces, spinach type S_4 was used and the leaves were compressed under 50N, 100N, 150N, and 200N. In addition to that, in order to study the effect of compression towards microstructure of the spinach cells, another set of sample S_4 was compressed under 30N, 60N, 90N, 100N, 110N, 140N, 170N, and 200N by following protocols as described in Section 3.4. For the study on Young's modulus of spinach compressed at different weight, spinach type S_4 was also used, with the weight of 50g and 75 g. The leaves were compressed under 100N and 200N and the Young's modulus values between whole and cut spinach were compared.

The third sub objective was to study textural property of an individual leaf by performing Penetration test as described in Section 3.3. Here spinach types S_4 and S_5 were used. The leaves were compressed under 100N, 150N, and 200N and the effect of storage was also studied with Day 12 as the final measurement day.

All the compression works were done using Universal Testing Machine (UTM) as described in details in Chapter 3, Section 3.2. In the beginning, only 100N was used as the compression force as it was the intermediate force that leads the sample to show sensitivity after compressed by UTM. The preliminary study on selecting the forces started from the lowest force possible (30N) and went up to 200N. 200N was the maximum compression force in this study and no further force increment was made as 200N was the maximum force in the study that showed differences in responding towards the variable tested. In Section 4.3.1.3, the stem and no stem spinach were introduced as to study the trend on the presence of stem towards energy of compression. This could give idea to the fresh vegetable industries to determine which type (stem or no stem) is the best in resisting stress thus specific selection of leaves could be proposed for packaging.

4.3 Results and discussion

4.3.1 The energy of compression

4.3.1.1 The energy of single loading/unloading compression

The maximum work (MaxW) is defined as the maximum work generated from the loading/unloading cycle during the compression. It can also be interpreted as the maximum energy required to compress the leaves. Among three types of spinach leaves (Organic, Teen, and Salad), the MaxW required to compress Organic spinach was found to be the most high followed by Teen and Salad spinach (Table 4.1). Besides the MaxW, the area under the curve (AUC) was also calculated. AUC represents the total work required for one complete loading/unloading compression. Again, Organic spinach shown to have the highest AUC compared to Teen and

Salad spinach (Table 4.1). The MaxW and AUC values tabulated in Table 4.1 were the average values of five replications of compressions.

The MaxW was found to be decreasing after storage. After storage, the leaves became softer and tender due to the bruising and decay thus less work was required to compress the leaves. Undesirable loads can be the main reason for bruising (Emadi et al. 2009). Energy absorbed during compression is strongly correlated to the bruise volume and thus is a useful parameter to be evaluated in the handling and packing system of fruits and vegetables (Holt & Schoorl 1977; Holt & Schoorl 1982). The AUC also decreased after storage which showed that the total work generated to compress the leaves was reduced due to texture degradation of the product after storage. This can also be seen from the force-displacement curves shown in Figure 4.1.

Table 4.1: MaxW values after compressions before and after storage. The data represent the mean of 5 replicates ± standard deviation.

	Day 0			Day 6		
	Teen	**Organic**	**Salad**	**Teen**	**Organic**	**Salad**
MaxW (J)	2.4 ± 0.3	3.0 ± 0.7	2.1 ± 0.3	1.7 ± 0.2	2.0 ± 0.3	1.2 ± 0.2
AUC (J)	7.8 ± 2.7	8.0 ± 0.4	7.0 ± 1.2	7.0 ± 3.0	7.4 ± 3.5	6.0 ± 0.2

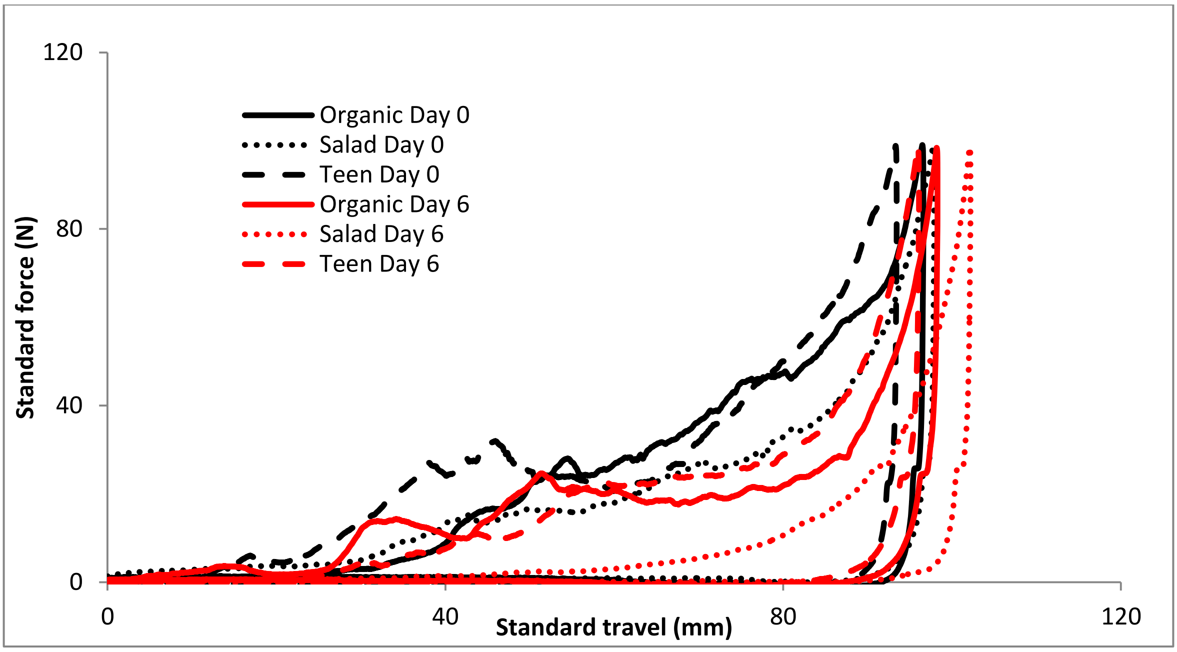


Figure 4.1: The force-displacement curves for Organic, Teen and Salad spinach before and after storage.

Looking from the physical appearance, Organic spinach has round and short shape; Teen spinach has a long-shaped leaf, whereas salad spinach has sharp-to-the-end-of-tip shape (Figure 4.2). From the observation in the laboratory, Organic spinach was found to have the roughest and thickest surface, Teen spinach was found to have medium soft texture and medium-thick leaf whereas Salad spinach was found to have the softest texture and thinnest leaf. Types of cultivar, surface characteristics, stage of maturity, produce size and weight are main factors affecting the mechanical strength of the produce (Kays 1991).

Apart from that, the intercellular bonding and space are also important components of the tissue strength properties (Shiu et al. 2015). The large intercellular space as in spinach leaf provides room for the cells to reorient during compression thus can change the tissue volume significantly (Kays 1991). The intercellular structures of the spinach leaves showed that Organic spinach has

Figure 4.2: Images of Teen, Organic and Salad spinach.

irregular and larger cell sizes. Teen spinach and Salad spinach have round-shaped cells where Teen spinach has larger cell size compared to Salad spinach (Figure 4.3). Regardless of the cells arrangement, the irregular shape of the Organic spinach cells may contribute to the ability of the tissue to resist the mechanical stress exerted during the compression. A molecule's shape plays an important role in determining the physical and mechanical properties of the tissues and the way they interact with each other (Silberberg 2000; Tro 2008).

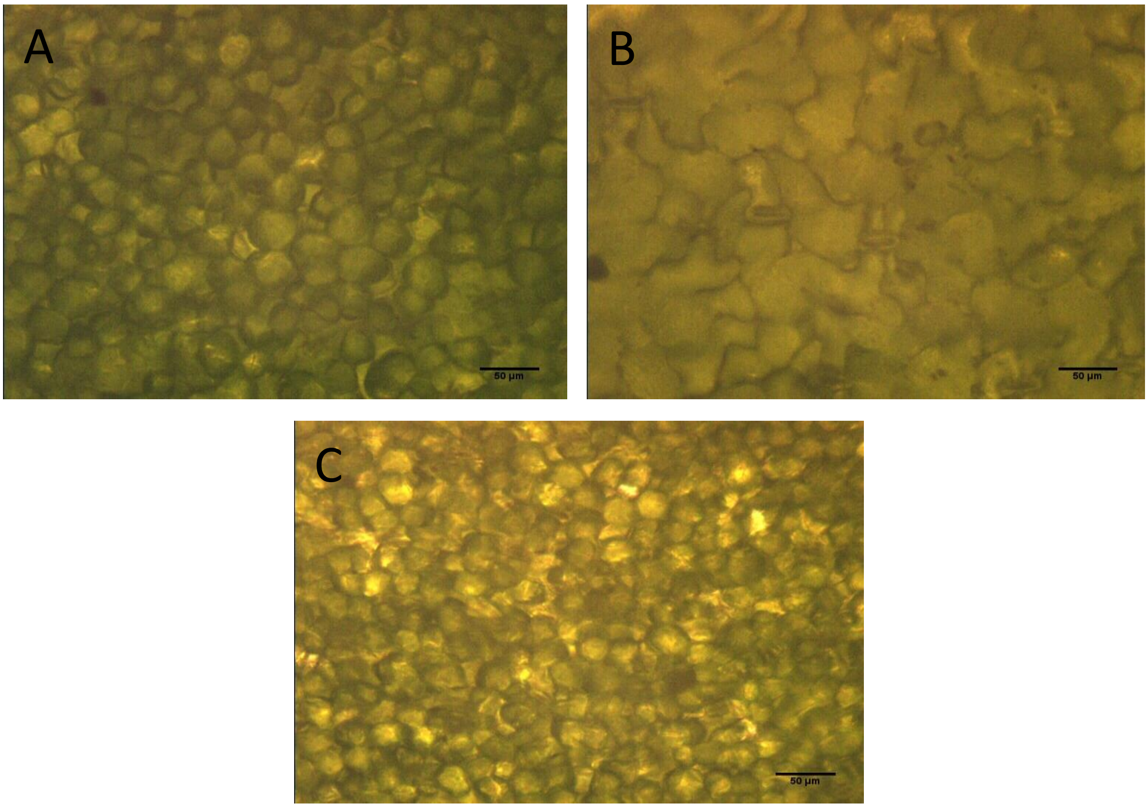


Figure 4.3: The microstructures of 3 different types of spinach leaves; A) Teen, B) Organic, and C) Salad. The average cell sizes were 30.99 μm, 60.75 μm, and 30.10 μm for Teen, Organic, and Salad spinach respectively. – was 50μm.

4.3.1.2 The energy of multiple loading/unloading compressions

Apart from single loading/unloading compression, the bulk spinach was also compressed under multiple consecutive compressions. Previous study on the effect of degree of compression towards texture profile of apple, carrot, frankfurter, pretzels, and cream cheese have been conducted (Bourne & Comstock 1981). The authors controlled the extension cycle to certain range of percentages correspond to the distance between the plates in order to give different degree of compression towards the sample (Bourne & Comstock 1981). However, there was so much variability for each commodity that the method may not be applicable to other compression speeds. It is necessary to standardise the degree of compression and to report the degree of compression used. Therefore, in this study, the consecutive multiple compressions under the pre-set force and speed were conducted to study the behaviour of the work changes required to compress the leaves (Figure 4.4). Having a standard method could be applicable with other commodities. Plus, previous studies on the effect of degree of compressions towards mechanical properties of fruits and vegetables only focused on the individual sample (Shiu et al. 2015; Emadi et al. 2009; Zhu & Melrose 2003; Thybo & Nielsen 2000). Study on the effect of degree of compression towards mechanical properties of bulk sample as what RTE leafy vegetables were usually displayed in the market is still lack in the literature.

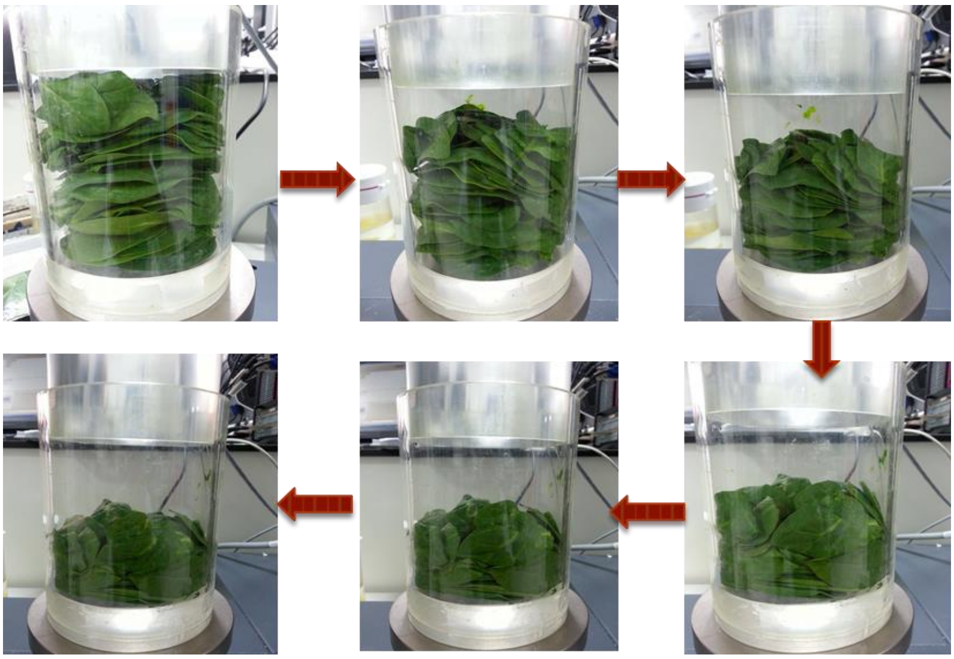


Figure 4.4: Randomly thrown bulk spinach before and after 5 times consecutive compressions of 200N.

In this study, the bulk spinach was compressed five times consecutively to create the standard degree of compression. The standard degree of compression referred to the constant starting position before the compression as well as the height of the bulk sample. The speed of each compression was 0.5 mm/s, thus the time taken for each compression depends on the compression force. The higher the force, the longer the time required for one complete cyclic loading/unloading compression (i.e. the higher the force of compression, the farther the distance travelled by the piston to compress the sample). For example, with constant sample weight, the time required for one complete compression was 135.9 s, 197.6 s, and 214.0 s for sample compressed under 50N, 100N, and 200N respectively.

Four replications were conducted for each variable introduced in this experiment. As the number of compression increased, the MaxW decreased (Figure 4.5 and Table 4.2). Similar trend was observed at day 6. Another thing to look at is the MaxW for all the three spinach types were the same at the fifth compression both before and after storage. This shows that regardless of the spinach types, which, one type might be rougher or tender to the others, after been compressed consecutively, they generated similar MaxW which showed that they have reached the maximum resistance towards stress. This shows that leaf fracture does depend on the degree of compression where higher degree of compression gave more prominent effect (Bourne & Comstock 1981). As the leaves were compressed further, the cell wall that is near to the contact surface reach the elasticity limit (Thybo & Nielsen 2000). The energy is dissipated through the cell breakage or stored by distension of the elastic membrane (Holt & Schoorl 1977). Again, the energy required to compress all types of the spinach leaves after storage was found to be decreasing due to the texture degradation of the leaves after storage.

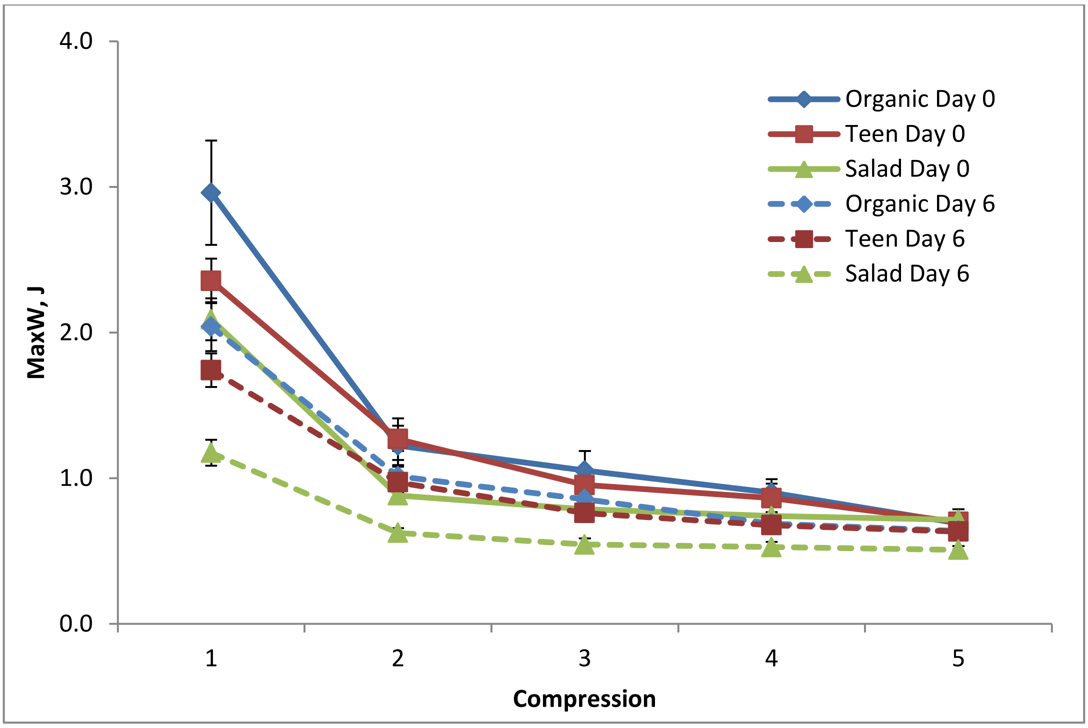


Figure 4.5: MaxW of five consecutive compressions before and after storage for Organic, Teen, and Salad spinach.

Table 4.2: Average MaxW of each five consecutive compressions. The data represent the mean of 4 replicates ± standard deviation.

Compression	MaxW (J)					
	Day 0			Day 6		
	Teen	Organic	Salad	Teen	Organic	Salad
1st	2.4 ± 0.3	3.0 ± 0.7	2.1 ± 0.3	1.7 ± 0.2	2.0 ± 0.3	1.2 ± 0.2
2nd	1.3 ± 0.3	1.2 ± 0.3	0.9 ± 0.0	1.0 ± 0.2	1.0 ± 0.4	0.6 ± 0.1
3rd	1.0 ± 0.2	1.1 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.3	0.5 ± 0.1
4th	0.9 ± 0.2	0.9 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
5th	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.5 ± 0.1

4.3.1.3 The energy of multiple loading/unloading compressions of stacked spinach

The work on the multiple compressions was further conducted by stacking the spinach leaves instead of throwing the bulk spinach into the chamber. The purpose was to study the behaviour of the sample at different arrangement and to investigate the resistance between different parts of the sample towards stress. Here, Baby spinach leaves were used where they were categorised into leaves with stem and leaves without stem. 20 leaves were stacked and carefully placed inside the compression chamber (Figure 4.6). Three replications were conducted for each variable introduced in this experiment.

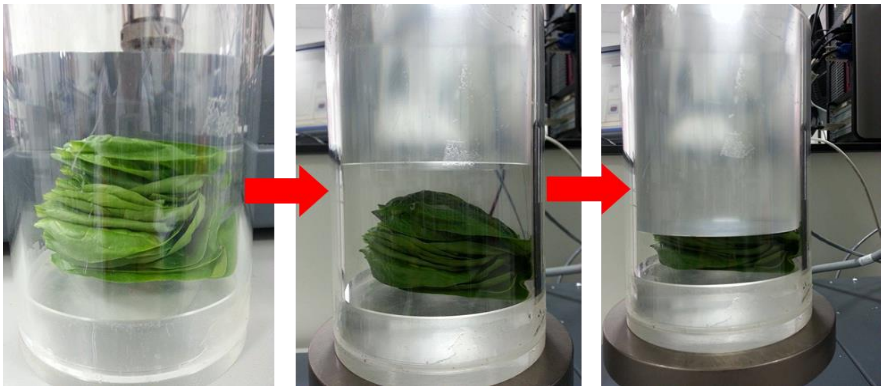


Figure 4.6: Stacked spinach leaves without stem before and after compressions.

Results show that, under 200N compression, leaves with stem required higher energy to compress compared to leaves without stem (Figure 4.7 and Table 4.3). However, for leaves compressed under 50N and 100N, the difference was only noticed on the first compression and showed similar MaxW between leaves with stem and without stem starting from the second compression till the fifth compression. The resistance towards mechanical damage varies between different component parts of a plant (Kays 1991). As the compression force increased, the MaxW increased as well (Bourne & Comstock 1981). The MaxW values reported in this study were lower than the MaxW values reported on the study of multiple compressions on bulk spinach. This is because, the spinach used in this particular objective was Baby spinach which was harvested at early stage which make the leaves small and more tender (Jensen 2017).

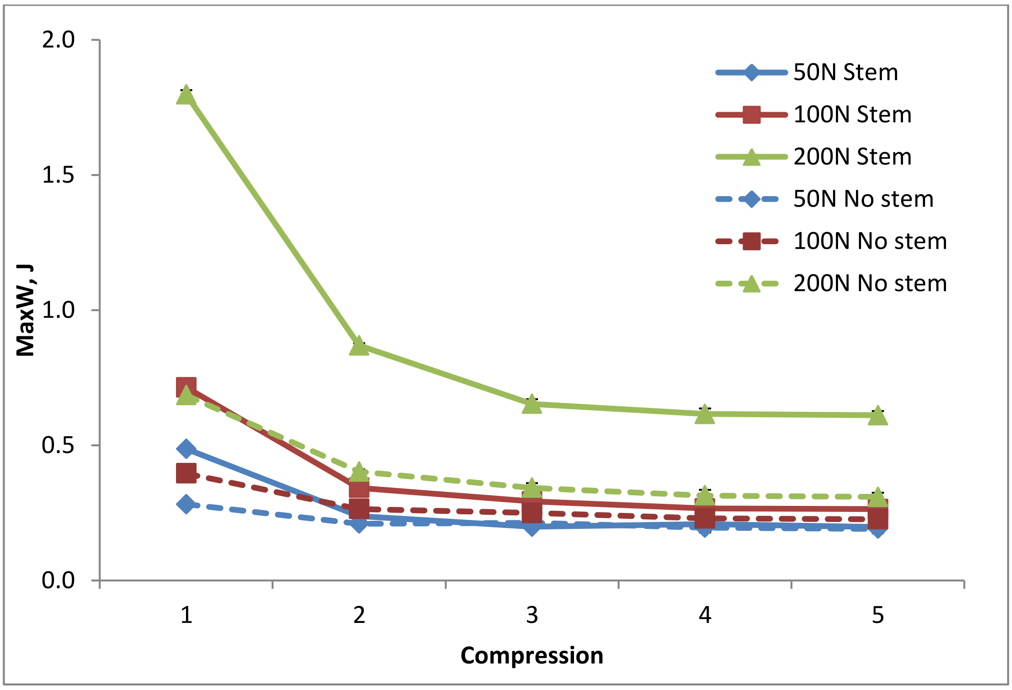


Figure 4.7: MaxW of five consecutive compressions before and after storage for stacked Baby spinach with and without stem.

Table 4.3: Average MaxW of stacked spinach. The data represent the mean of 3 replicates ± standard deviation.

Compression	MaxW (J)					
	Stem			Without stem		
	50N	100N	200N	50N	100N	200N
1st	0.5 ± 0.0	0.7 ± 0.01	1.8 ± 0.03	0.3 ± 0.01	0.4 ± 0.01	0.7 ± 0.03
2nd	0.2 ± 0.01	0.3 ± 0.02	0.9 ± 0.02	0.2 ± 0.01	0.3 ± 0.02	0.4 ± 0.02
3rd	0.2 ± 0.01	0.3 ± 0.03	0.7 ± 0.03	0.2 ± 0.02	0.2 ± 0.03	0.3 ± 0.03
4th	0.2 ± 0.03	0.3 ± 0.03	0.6 ± 0.04	0.2 ± 0.03	0.2 ± 0.03	0.3 ± 0.03
5th	0.2 ± 0.03	0.3 ± 0.01	0.6 ± 0.03	0.2 ± 0.02	0.2 ± 0.01	0.3 ± 0.03

4.3.2 Young's modulus (E) of bulk spinach

4.3.2.1 Young's modulus between different types of spinach

The Young's modulus (E) values for Organic, Teen, Salad, and Baby spinach before and after storage are shown in Table 4.4. Average E values showed that Organic spinach has the highest E value compared to Teen, Salad, and Baby spinach (Table 4.5). Highest E values for Organic spinach showed that organic spinach was the best in resisting stress and damage compared to Teen, Salad, and Baby spinach . After storage, the E values decreased. As the leaves were stored longer, their texture became softer due to the deterioration, thus change of distance travelled by the upper platon, ΔL to compress the leaves increased causing the work to decrease.

Table 4.4: E values of different types of spinach before and after storage.

Replicates	Day 0				Day 6			
	Teen	Organic	Salad	Baby	Teen	Organic	Salad	Baby
1	0.011	0.014	0.013	0.013	0.009	0.010	0.011	0.006
2	0.012	0.013	0.012	0.011	0.01	0.013	0.007	0.004
3	0.012	0.016	0.017	0.008	0.01	0.011	0.008	0.007
4	0.006	0.014	0.014	0.007	0.009	0.007	0.010	0.007
5	0.014	0.019	0.011	0.010	0.008	0.012	0.008	0.006
Average	0.012	0.015	0.013	0.01	0.009	0.011	0.009	0.006

Table 4.5: Average E values for different types of bulk spinach before and after storage.

	Day 0				Day 6			
	Teen	Organic	Salad	Baby	Teen	Organic	Salad	Baby
Average MaxYoung's Modulus (MPa)	0.012	0.015	0.012	0.010	0.009	0.011	0.009	0.006
Standard deviation (MPa)	0.001	0.002	0.001	0.002	0.001	0.002	0.002	0.001
RSD (%)	9.17	16.05	10.94	24.59	9.60	19.58	18.56	16.69

The firmness of the leafy vegetable is influenced by many factors such as plant structure, type of tissue, cell number, as well as the intercellular volume of the plant (Luengo et al. 2008). Other factor that greatly influenced the mechanical properties of the leaves tissues are agronomic treatments, as well as geographic variations and season (Gutiérrez-Rodríguez et al. 2013; Zhang et al. 2017). Reductions in strength and stiffness, and increment in failure strain were due to the lack of plant turgidity. More turgid leaf will be more vulnerable to cracking compared to wilt leaf. Cell turgidity, water content, and the existence of pectins, lignins, hemicellulose and cellulose microfibers within cell wall are important factors that affect plant tissue elasticity and rigidity. This explains the amount of mechanical damage that may occur during processing and handling of plant material (Newman et al. 2005; Falk et al. 1958). However, firmness dependent on turgidity only work for range of compact vegetables and fruits such as potatoes, onions, tomatoes and mangoes. For non-compact vegetables with low density such as leafy vegetables, the turgor solely is not adequate to determine the firmness. Especially for the low apparent density with irregular shape and size such as spinach, direct mathematical conversion of turgor dependent firmness values are not acceptable. The spatial arrangement of the layered tissues within these low apparent density commodities are susceptible to deform which resulted in breakage in the midrib tissue of the leaf when exposed to above certain capacity it can stand (Luengo et al. 2008). As this condition is likely favoured for those looking for crisp and crunchy textures, it is also important to take note on other types of failures experienced by the leaves such as wilting, bruising and browning which also contribute to the reduction of quality and shelf lives of the leafy vegetables.

4.3.2.2 Young's modulus for spinach compressed at different forces

Young's modulus is directly proportional to the force exerted on the sample under compression. Thus, the higher the force, the higher the E values (Table 4.6). This means that the work per unit volume required to compress the bulk spinach inside the chamber increased. The high values of relative standard deviation (RSD) showed that spinach has a very complex system where there are huge possibilities of variations between every single spinach leaf (Table 4.7).

Table 4.6: E values of bulk spinach compressed at different forces.

Replicates	Young's Modulus (Mpa)			
	50N	**100N**	**150N**	**200N**
1	0.008	0.023	0.039	0.071
2	0.007	0.031	0.021	0.041
3	0.011	0.026	0.021	0.074
Average	0.009	0.027	0.027	0.062

Table 4.7: Average E values of bulk spinach compressed at different forces.

	50N	**100N**	**150N**	**200N**
Average MaxYoung's Modulus (MPa)	0.009	0.027	0.027	0.062
Standard deviation (MPa)	0.002	0.004	0.010	0.019
RSD (%)	21.58	15.45	38.32	29.88

The microstructures of the spinach tissues compressed at different forces are shown in Figure 4.8. The range of the cell sizes was from 43.89 µm – 46.97 µm. Cell sizes were measured by taking average of 30 cell sizes for each compression force. The spinach cell sizes did not break although the compression force increased; however, there were cell bursting releasing it contents which caused the blurry images. For example, at 200N, the cell's image was a bit blurred compared to the leaves compressed under 30N. As the compression force increased, there were more cell's bursting among tissue thus caused blurry view when observed under the light microscope. Within the bruise, the cells ruptured and released the sap into the air filled interstitial spaces (Holt & Schoorl 1982).

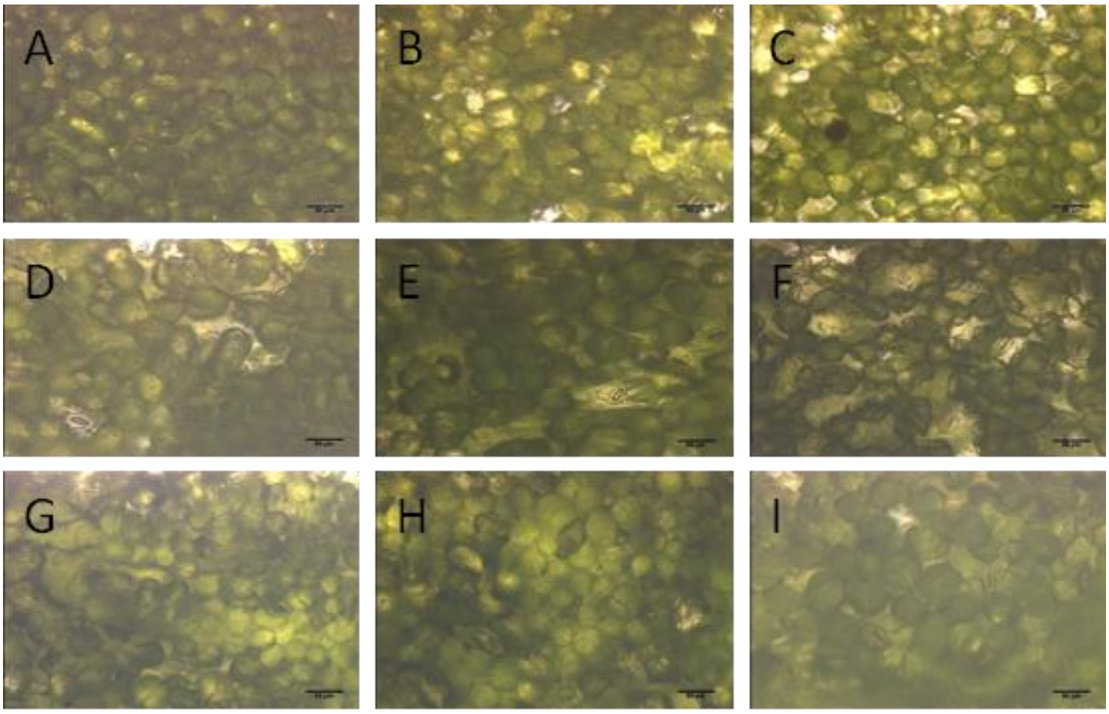


Figure 4.8: The microstructures of spinach sample S_4 for A) Control, and after been compressed with B) 30N, c) 60N, D) 90N, E) 100N, F) 110N, G) 140N, H) 170N, I) 200N. – was 50µm.

There may be cell ruptures when the magnitude of the stress is great enough, however if the stress is relatively low and for a short time, the deformation will be elastic, with the cell recovering its original shape (Kays 1991). Hypothetically, as the compression force increases, the breakage of the cells was assumed to be increasing. As the degree of compression increased, one would expect breakdown of the structural elements of a food and lowered the degree of shape recovery (Bourne & Comstock 1981). However, in this particular study, after the test was run, that was not the case. This is because; the leaves were compressed in bulk. After the compression, not all the leaves inside the chamber were broken. Some partly broken, some completely broken and some remain intact. In some cases, only the leaves at the edges of the chamber were damaged while the leaves in the middle part of the chamber remained intact. Apart from that, the selection of the spinach samples to be put under the light microscope was done in random where the images observed under the light microscope may be those from the unbroken part of the leaves.

Apart from the microstructure of the leaf surface, the cross sections of the leaves were also micro thinly cut by microtome and observed under the light microscope. The images were observed under 10X objective magnification. The 10X objective magnification was chosen as it was able to display the whole cross section of the leaves when observed under the light microscope. The free areas within the cells were measured using image processing software, ImageJ with constant dimension for area of measure. The areas highlighted in red were the free space areas detected by ImageJ (Figure 4.9). Results showed that there was tissue breakage observed from the cross section (Figure 4.9). Before storage, the cross section of the leaves was more connected and there was more volume of tissues presented. After six days of storage, the middle part of the cross section of the leaves was disintegrated and the tissues seemed to lose most of their cell. From the ImageJ, using image calibration of 16 pixels/mm (based on calibration image captured under 10X objective magnification), the average free area of the cells before storage was found to be 108.1 mm^2 whereas after storage, the free area of the cells was found to be 272.4 mm^2. The increased in the free space areas was due to the cells lost their contents due to compression and the effect was more obvious after storage as the deterioration of the leaves increased as the storage day increased.

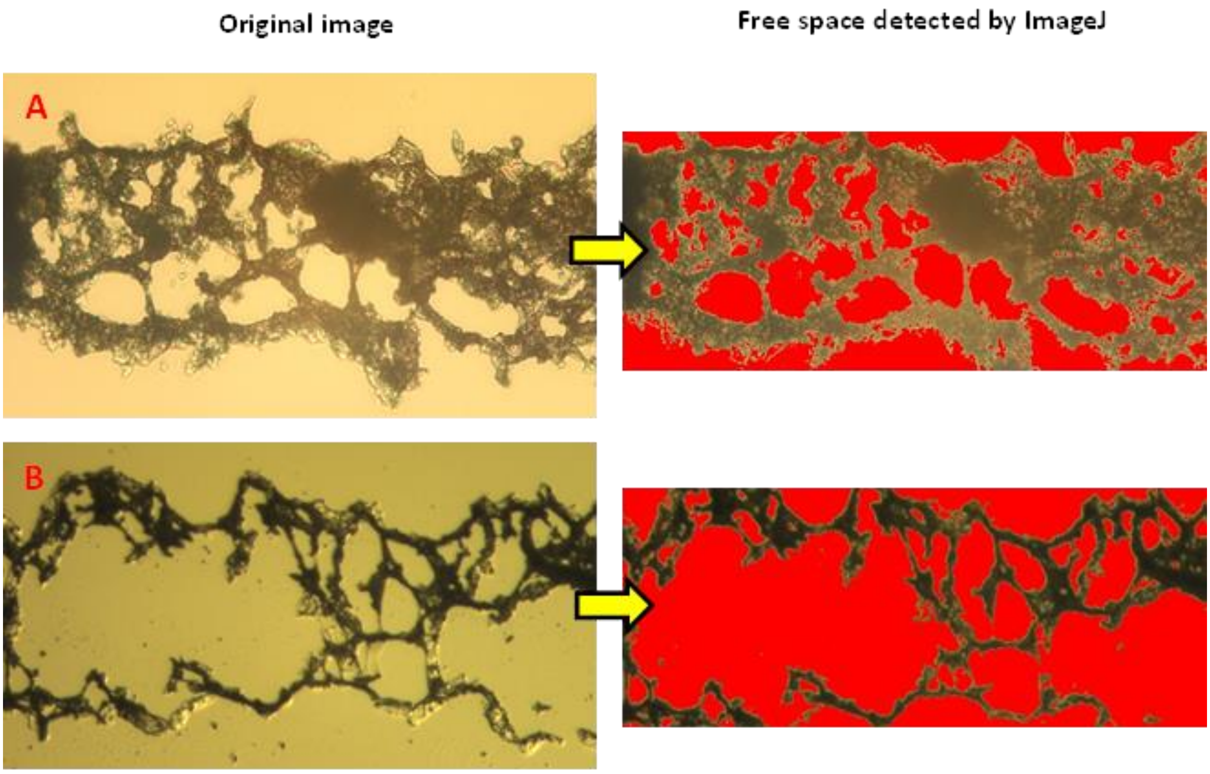


Figure 4.9: Cross section of spinach sample S_4 compressed under 200N observed under light microscope with 10X objective magnification. A) day 0; B) day 7. The areas highlighted in red were the free space areas detected by ImageJ.

Regardless of the microstructural property of the leaves, by looking at the physical appearance, the leaves did show breakage when compressed under the pre-set force (Figure 4.10). Physical appearance plays major role in determining the quality of the product. The quality changes of the produce are depending on both degree of compression as well as the nature of the produce (Bourne & Comstock 1981).

Figure 4.10: Digital images showed mechanical damage of Baby spinach leaves after 200N compression A) Front sides; B) Back sides.

4.3.2.3 Young's modulus for Baby spinach compressed at different weight

Comparing the E values between two different weights, it showed that as the weight of the sample increased, the E value decreased (Table 4.8 and Table 4.9). Mass is directly proportional to the bed volume, V. Recalling Equation (7), E is inversely proportional to the bed volume, V. Thus, increasing the weight of spinach inside the same package size reduced package-free volume and caused faster quality changes in the in-pack atmosphere (Kaur et al. 2011). This lead the spinach to be more vulnerable to damage compared to bag that contain lesser weight of spinach. This is because, as the weight of spinach increased; there will be more contacting and overlapping between the leaves. As spinach leaves are soft and tender, packing necessary weight of spinach will reduce the 'collision' between the leaves in the bag, thus reduce the leaves tendency to be accidentally broken. Pack with excessive weight increases compression and impact bruising (Wills et al. 2007). Production of water vapour inside the package also increased as the weight increased which then led to condensation of water vapour inside the package, and thus quality degradation of the product (Kaur et al. 2011). Poor packaging can lead to compression among the leafy vegetables which then lead to mechanical injuries such as cracking, breaking, abrasion, and excessive impact (Luengo et al. 2008; Kim et al. 2004a; Piagentini & Güemes 2002; Lu 2007).

Comparing E values between whole spinach and cut spinach, it can be clearly seen that cut spinach has lower E values compared to whole spinach while the force and weight were kept constant. Lower E values indicate that the leaves were softer and more susceptible to damage. This is obvious as the leaves were cut before the

Table 4.8: E values of bulk spinach at different weight for both whole and cut Baby spinach.

Replicates	Whole Baby spinach				Cut Baby spinach			
	100N		200N		100N		200N	
	50g	75g	50g	75g	50g	75g	50g	75g
1	0.030	0.019	0.068	0.041	0.033	0.008	0.065	0.026
2	0.034	0.023	0.06	0.043	0.026	0.008	0.067	0.024
3	0.034	0.014	0.065	0.045	0.033	0.011	0.064	0.019
4	0.04	0.02	0.066	0.039	0.029	0.011	0.064	0.019
5	0.037	0.021	0.063	0.048	0.025	0.011	0.070	0.018
Average	0.035	0.019	0.065	0.043	0.029	0.010	0.066	0.021

Table 4.9: Average E values of bulk spinach at different weight for both whole and cut Baby spinach.

	Whole Baby spinach				Cut Baby spinach			
	100N		200N		100N		200N	
	50g	75g	50g	75g	50g	75g	50g	75g
Average MaxYoung's Modulus (MPa)	0.035	0.019	0.066	0.043	0.029	0.010	0.064	0.021
Standard deviation (MPa)	0.003	0.004	0.001	0.004	0.004	0.002	0.000	0.004
RSD (%)	9.91	18.25	2.20	8.45	12.92	17.09	0.24	18.35

compression, it opened the leaves surfaces to the air thus the leaves became soft and deteriorated faster. The work done per unit volume of the leaves inside the chamber decreased. This implies that even with small amount of force exerted to the leaves; they could be easily broken and damaged. The leaf injury provides more areas for the fungicide residues to be trapped in the leaves (Gonzalez et al. 1988), causing contamination and spoilage of the products (Babic et al. 1996), which then lead to softening of the leaf tissue (Neal et al. 2010). Spinach processing line industry need to be very careful in selecting good leaves only before they are being packed to reduce the tendency of the whole product inside the bag to deteriorate faster.

4.3.3 Textural property of individual spinach leaf

In this study, Baby and Young spinach leaves were compressed under 100N, 150N and 200N and the effect of compression force towards spinach texture was evaluated throughout storage by performing Penetration test.

Analyses of Variances were performed using statistical software SPSS, considering both One-Way ANOVA and Two-Ways ANOVA with post-hoc tests, Tukey's and Bonferroni. The statistical analysis began by using Bonferroni to do the multiple comparisons following ANOVA. Although Bonferroni test is easy and widely applicable, it is extremely general and often not powerful. Tukey's test was then chosen to do the multiple comparisons as this method is considered the best for all-possible pairwise comparisons when sample sizes are unequal and very good even with equal samples sizes. Another good way to perform multiple comparisons of means is by using Dunnett’s, however, this test was not conducted in this study as Dunnett’s test only interested in comparing one group ("control") to each of the others, but not comparing the others to each other. However, on the other hand, Tukey’s test compares every mean with every other mean. In this study, the comparison of textural differences between leaves compressed at different forces needs to be investigated, how the texture differs between each group to the other group. Thus, Tukey’s test was chosen to do the multiple comparisons of means following ANOVA.

Results showed that the texture differences were not significant between control leaves (uncompressed leaves) with leaves compressed under 100N, 150N, and 200N for both Baby spinach and Young’s spinach (Figure 4.11 and Figure 4.12). There was not any significant difference before and after storage as well. Previous studies have also reported the insignificant difference of spinach texture before and after storage (Medina et al. 2012; Gómez-López et al. 2013; Tudela et al. 2013). The insignificant difference may be due to the variability of the spinach leaves. Although the protocols were kept standard (i.e. position of the leaf before penetration, position on the leaf to be penetrated, distance before and after penetration, speed of penetration) it was still challenging to standardise the physical measure of the leaves (i.e. leaf size, leaf thickness, and smoothness on the leaf surface). However, by comparing between the two types of spinach, there was significant difference between the burst strength of Baby and Young’s spinach for all compression forces tested at day 0. This shows different varieties response differently once being compressed. Different varieties behaved differently towards the quality parameters tested (Reddy et al. 2014). Nevertheless, the results still showed similar burst strength after storage.

The error bars were large due to the variation of the individual spinach leaf. Even leaves packed in a same bag could have different physical properties, e.g. roughness, wetness, and size. The ambiance factor also may contribute to the effect on the variation of the textural property. The samples were stored in a shared cold room and it was not possible to control the amount of student in/out of the cold room thus lead the temperature to fluctuate. These factors made it challenging to have constant physical properties of the samples on each sampling day. The inconsistencies of the physical properties of the samples before the test lead to the variations of the results although high number of replicates was tested.

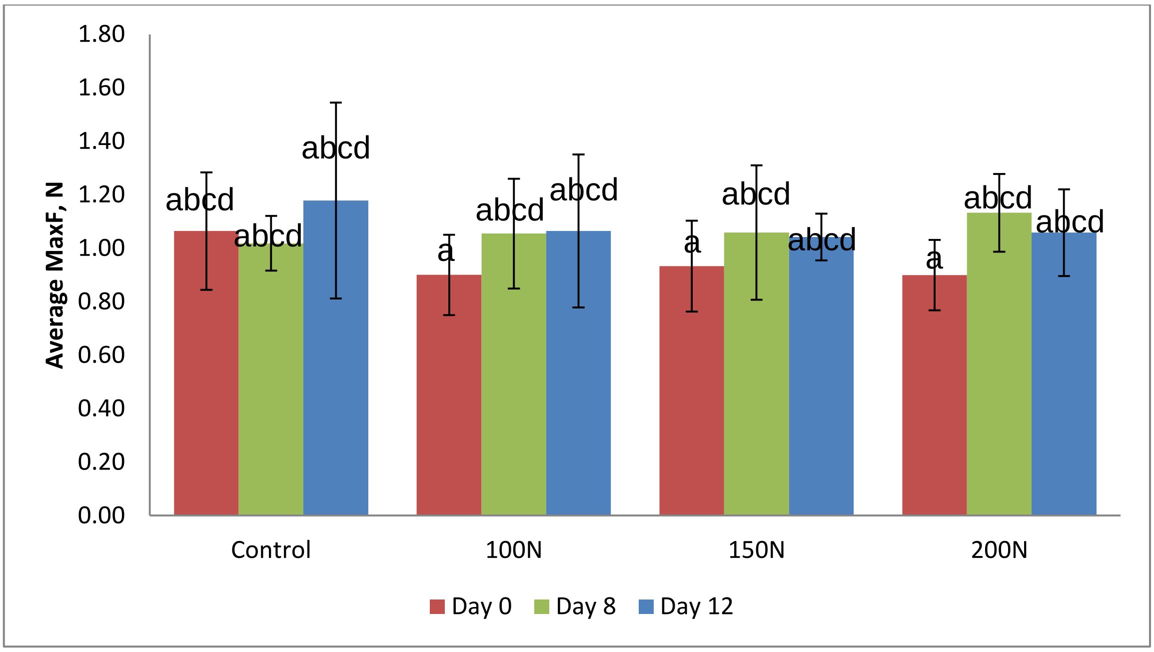


Figure 4.11: Average of Burst strength of Baby spinach between different treatments (Control, 100N, 150N, 200N) throughout storage days. The data represent the mean of 10 replicates ± standard deviation.

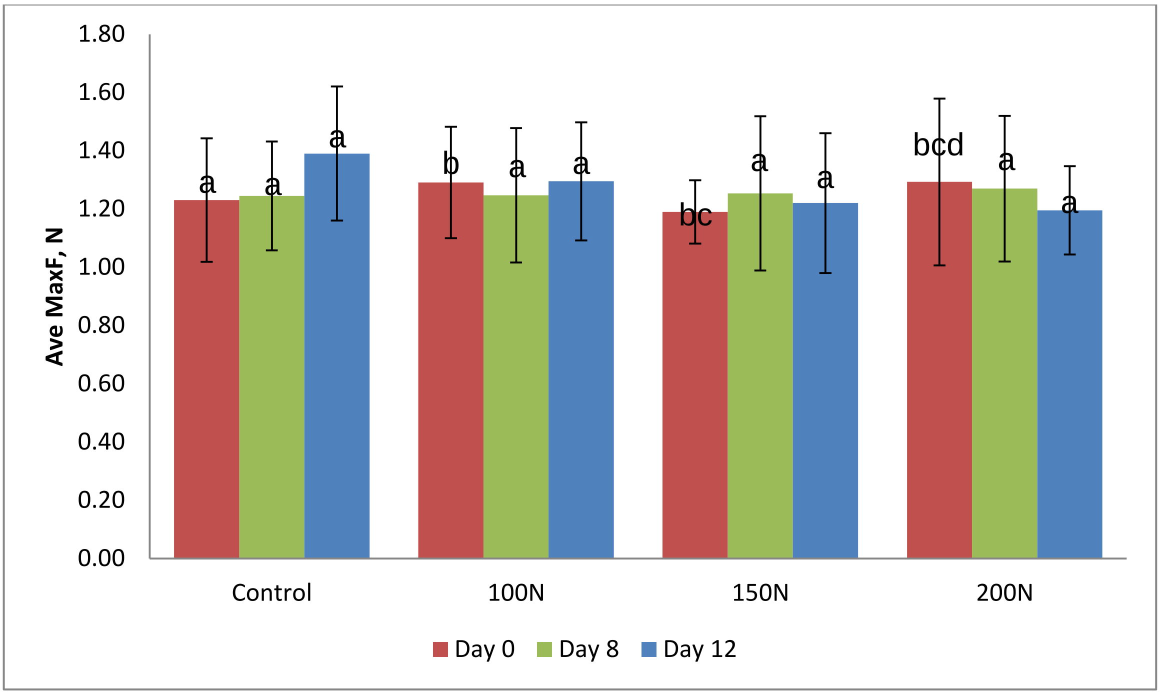


Figure 4.12: Average Burst strength of Young spinach between different treatments (Control, 100N, 150N, 200N) throughout storage days. The data represent the mean of 10 replicates ± standard deviation.

4.4 Conclusion

From the single loading/unloading compression, different types of spinach resulted in different energy of compression. Organic spinach was found to produce the highest energy with maximum work, MaxW required to compress the spinach. Teen spinach followed as second type of spinach that produced the highest MaxW followed by Salad spinach. This can be concluded that Organic spinach was found to be the best in resisting stress and damage compared to Teen, and Salad spinach. The MaxW was found to be decreasing after storage. This is due to the leaves that became softer after storage thus the energy required to compress the leaves decreased. Also, as the storage day increased, the area under the curve, AUC which represents the springiness of the bulk sample decreased as well. This is due to the bulk sample that became wet after storage and got more attached to each other inside the bag.

From the multiple loading/unloading compressions, as the number of compression increased, the MaxW decreased. This trend was found similar between before and after storage. The MaxW values for all spinach types were found to be the same after the 5th compression. This is due to the spinach samples that have reached their maximum resistance towards stress. Again, the MaxW was also found to be decreasing as the storage day increased due to the texture degradation of spinach after storage.

From the energy of stacked spinach, spinach with stem always resulted in higher MaxW compared to spinach without stem. This suggests that leaf with attached stem resist the ambiance stress better than leaf without stem. Suggestion could be made to the fresh vegetable industries during the selection process, to help them choosing leaf criteria that is best in resisting stress.

Results of Young's modulus, E showed that Organic spinach has the highest E values compared to Teen, Salad, and Baby spinach. Again, this suggests that Organic spinach is the best in resisting stress. The E values were found to be decreasing after storage again due to texture degradation.

Compressing the bulk spinach leaves with increasing force (used in this study) did not break the cells wholly; however, there were cells bursting their contents within the intercellular space that caused the blurry image under the microscope. Although the cell breakage was hardly seen under the light microscope, from the physical appearance of the leaves, the mechanical breakage can be clearly seen.

This shows that physical appearance plays major role in affecting consumer's perception while judging the product.

As the weight of spinach increased, the E values decreased. This suggests that by reducing the weight of spinach inside the packaging bag, while putting appropriate weight may reduce the possibility of the leaves inside the bag to be compressed due to crowdedness inside the bag thus lowered the risk for accidental mechanical leaf damage. Besides that, cut spinach also showed lower E values compared to whole spinach. This shows that cut leaves have softer texture compared to whole leaves. As the leaves were cut, it opens the leaves' surfaces for more transpiration and respiration which then lead to deterioration.

The E values from this work were smaller than the E values reported from the literature. This is because, from this work, the E values were measured for spinach in bulk instead of individual leaf as reported by the literatures. Besides that, from the uniaxial compression inside the bed chamber, not all the spinach leaves under the compression got teared or broken completely. As for many works reported by the literature, the values of E were higher because the leaves had been teared completely by tensile test or the individual flesh broke and damaged from the compression test. The E values for baby spinach under tensile test in diagonal, perpendicular, and parallel directions were reported to be 1.084MPa, 0.3914 MPa and 2.137MPa respectively (Tang et al. 2011). English round (ER) and Spanish iceberg (SI) had the range of E values from 1.1 - 2.0 MPa and 2.3 MPa - 4.4 MPa for ER and SI respectively depending on the orientations of the sample position during the test (Toole et al. 2000). Leaves (Calophyllum inophyllum L.) have E values range from 186.4 – 240.2 MPa and 49.8 – 67.5 MPa when tested along and across the veins respectively (Lucas et al. 1991).

From the Penetration test, there was not any textural difference for spinach before and after storage and also between spinach compressed at different forces. However, there was texture difference between detected between Baby and Young spinach at day 0. The variation of the leaves may lead to the insignificant difference of the spinach texture and this can be seen from the large error bars.

This work provides basis to understand the effect of compression towards mechanical and microstructural properties of spinach which can be used to improve the processing and handling of the spinach at the distribution chain and retailer level.

CHAPTER 5

MECHANICAL LEAF INJURY AND ITS EFFECT ON QUALITY AND SHELF LIFE OF LEAFY VEGETABLES

5.1 Introduction

From the farm handling, harvesting, postharvest processing, packaging, and distributing, RTE leafy vegetables are exposed to various loading conditions. These loading conditions cause mechanical failure which then lead to undesirable quality loss of the product. It has been claimed that vegetable types, harvesting season, package types, brand and distributors did have influence on the quality of the vegetables (Wisakowsky et al. 1977; Veit et al. 1996; Watada et al. 1996; García-Gimeno & Zurera-Cosano 1997; Kader 2002; Bergquist et al. 2006; Caponigro et al. 2010; Oliveira et al. 2010; Durand 2013; Tudela et al. 2013;), however, these factors only accounted for less than 25% towards the quality of the product. On the other hand, cultivation environment, processing operations as well as delays of refrigeration had a larger impact on the overall quality of RTE salads (Hodges et al. 2000; Hodges & Forney 2003; Carrasco et al. 2007; Caponigro et al. 2010; Lester et al. 2010; Gutiérrez-Rodríguez et al. 2013; Kou et al. 2014; Garrido et al. 2015). Most RTE products are sold within few weeks after packaging as they have a very short life span.

Processing these minimally processed vegetables helped in reducing the microbial loads of the products. However, the processing promote faster physiological deterioration and biochemical changes which lead to decay, tissue softening, discolouration and development of unpleasant odours (Watada & Qi 1999).

The shelf life of spinach could be defined as the time duration of which the spinach maintains appealing to the consumers. This appearance should be a green leafy vegetable with no browning, no breakage, or wetness present (Jung et al. 2012) .

It is important to understand the mechanical failure of a specific food in order to understand the mechanical strength of the food thus appropriate precaution and work can be designed to reduce the mechanical damage and energy consumption during handling, processing, transporting, and distribution to the consumers (Mohsenin 1977; Holt & Schoorl 1982).

Holt & Schoorl (1982) classified mechanical failures of vegetables and fruits in three categories which are cracking, slipping, and bruising. As important as that study in classifying different modes of failures, it is equally important to evaluate the different degrees of injuries experienced by the product. The degree of injury can also help in giving the information towards the prediction of the failure mode. As appearance is the main deciding factor when consumers are selecting the vegetables and fruits (Kader 2002; Ferrante et al. 2004), developing an image analysis technique to assess different degrees of injuries would be beneficial in giving good information relating to the quality and shelf life of the fresh produce during postharvest storage. Food optical measurement is one of the most successful non-destructive methods in assessing the quality of food products while providing several quality details (Diezma et al. 2013).

Regardless of works reported on evaluating failures for many different vegetables and fruits as described in the literature review, Section 2.5.1 and Section 2.6, there is still lack of research to assess different degrees of mechanical leaf injuries and how they affect the quality and shelf life of RTE spinach during postharvest storage. Therefore, the objective of this chapter is to establish a method that can be used to assess different degrees of leaf injuries and how they affect qualities degradation and shelf life of RTE spinach.

5.2 Materials and method

In this work, 30g spinach sample S_4 were subjected to each 50N, 100N, 150N, and 200N through uniaxial compression using Universal Testing Machine as described in Section 3.2. Classification of the leaves injuries was carried out by following the Population test protocols as described in Section 3.5 where leaves were classified into categories according to the degrees of damage and the RGB image acquisition and analysis were carried out by following methods described in Section 3.5.1 and Section 3.5.2 respectively. Colour contoured boxes were also generated using coding developed in MATLAB (R2016b) (Section 3.5.3).

5.3 Results and discussions

5.3.1 Distribution of deteriorations

Visibility of deteriorations is the main concern during postharvest storage of leafy vegetables (Fan et al. 2014). Generally, consumer assessed the quality of the vegetables from physical appearance rather than measuring the bacterial load in the market. In this section, distributions of deteriorations of packed spinach leaves are discussed.

5.3.1.1 Deterioration of uncompressed (Control) leaves throughout storage

Control leaves were uncompressed leaves which mean the leaves did not have any breakage or deteriorations to begin with. The distribution of deterioration for spinach is presented in histogram bars as shown in Figure 5.1. It is easier and faster to analyse the deterioration for the whole image of each sample by distributing the original quality values in histogram bars (Lunadei et al. 2012). The bars represented the frequency of spinach leaves inside the packaging bag that got more than 1% decays. According to USDA (2006), less than 1% decay on an individual spinach leaf is allowed for the spinach to be sold in the market. The colours of the bars went from blue for day 1 up to red for day 14. 100% of the Control leaves had less than 1% of deteriorations up to 8 days of storage. After 11 days of storage, only 4% of the leaves had more than 1% of the damage whereas after 14 days of storage, 14% of the leaves had more than 1% of damage which was still way lower than half of the total spinach leaves inside the bag. To give the reader a better view, the data was presented in colour contour plot as shown in Figure 5.2.

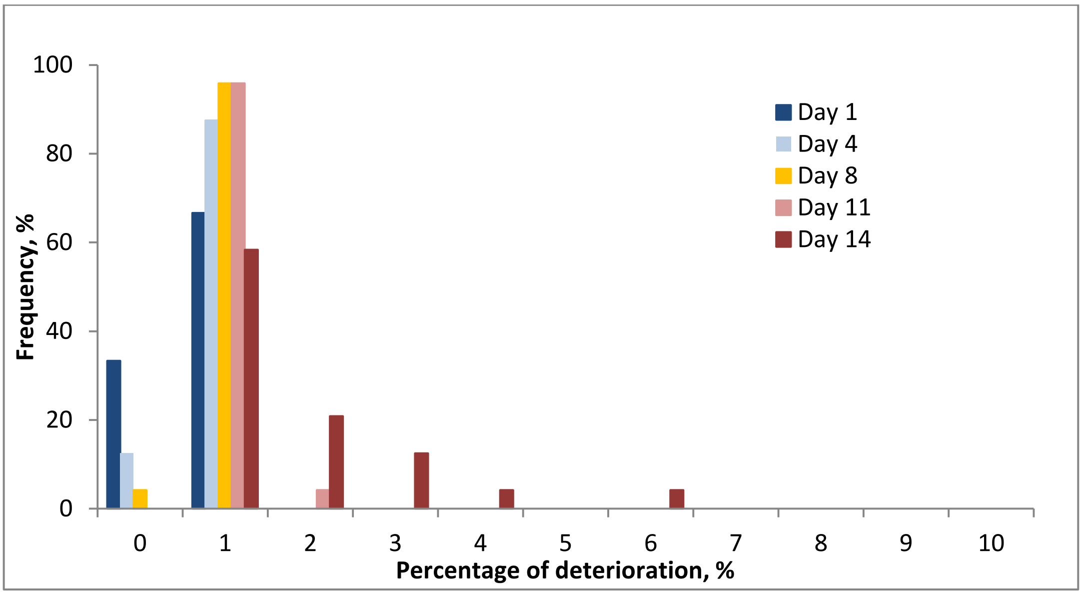


Figure 5.1: Distribution of deterioration of Control Baby spinach.

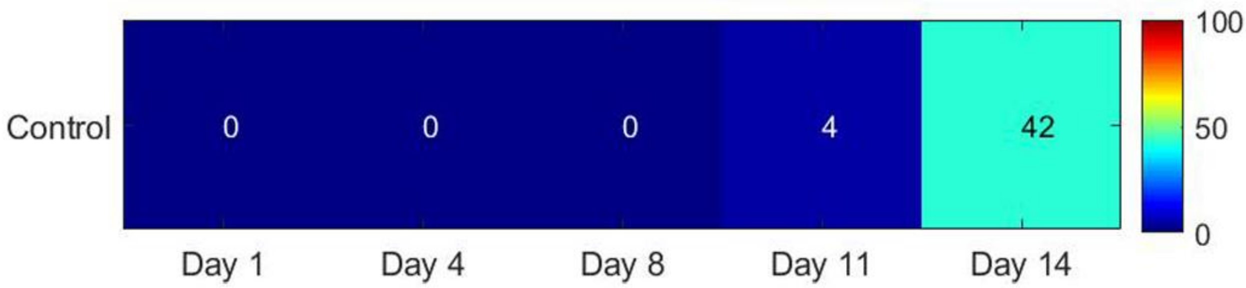


Figure 5.2: Contour colour plot of Control spinach leaves throughout storage days. The number inside each colour box represents the frequency of the leaves that got more than 1% decays.

It is easier to visualise the damage that occurred on the leaf by looking at the image of the leaf itself. Library of images for uncompressed (Control) and 200N compressed spinach leaves (Undamaged, Minor, Halfway, and Complete teared) throughout storage is shown in Table 5.1. For Control leaf, there was not any deterioration observed even after 14 days of storage. On the other hand, for compressed leaves with higher degree of injuries which were Halfway and Complete teared, the deteriorations were obviously visible from the first day of storage. For compressed leaves with lower degree of leaf injury which was Minor teared, the deteriorations started to become visible after day 8 and became more obvious after 14 days of storage. The rate of deterioration of spinach increased after 14 days of storage which agreed with what have been reported by (Hodges & Forney 2003).

Like Control leaf, Undamaged leaf started with no deterioration on the first day of storage, however, it showed more decays after 11 and 14 days of storage compared to Control leaves. This is because, although the deteriorations could not be detected on the first day, remember that Undamaged leaf still undergone the compression process. The compression has caused the leaf to become softer and has higher tendency to decay faster compared to uncompressed leaf (Control) which was fresher on the first day of storage. Here, it shows that although the leaves might look good on the outside, it does not necessarily mean that the leaves have not been 'disturbed' during the handling processes. (Shewfelt 1986) invented the term "latent damage" which was used to describe damage that occurred at one step but not noticeable until a later stage. As physical appearance is the main visual characteristic consumers look at while choosing bagged spinach in the supermarket, it is very important for the consumers to have a good understanding of what can be considered as good quality spinach.

Table 5.1: Library of images of Control and 200N compressed spinach leaves throughout storage.

Storage	Day 1	Day 4	Day 8	Day 11	Day 14
Control					
Undamaged					
Minor teared					
Halfway teared					
Complete teared					

Various works have been conducted to determine the shelf life of spinach. Neal et al. (2010) reported that constant temperature and proper gas composition maintained throughout storage helped to extend the shelf life of spinach. Combination of washing with chlorine dioxide and packed under MAP helped to reduce the microbial loads thus improve the safety of spinach during consumption time (Lee & Baek 2008). Other novel techniques were introduced to study the shelf life of spinach such as fumigation of sodium hydrosulfide (NaHS) to reduced leaf yellowing of water spinach (Hu et al. 2015), use of nano-mist as an alternative to conventional mist humidification during spinach-storage atmosphere (Saenmuang, et al. 2012), combination of spraying microencapsulated antimicrobials with e-beam irradiation to deactivate microorganisms on spinach (Gomes et al. 2011a), e-beam irradiation to reduce bacterial counts and to prolong the shelf life of fresh spinach (Neal et al. 2010), and treatment with low to moderate UV-C radiation which was found effective as an alternative to sanitisation with chlorine water to preserve the quality of minimally processed spinach leaves (Artes-Hernandez et al. 2009).

The shelf life of fresh vegetables is defined as the storage time at which the quality attributes have score below 7 (Pirovani et al. 1997). Spinach leaves were found unacceptable after eight days when stored under 4°C (Pandrangi & Laborde 2004). Piagentini & Güemes (2002) reported that fresh-cut spinach keep at 4°C and 90% HR can be maintained for eight days using LDPE bags and six days using OPP bags. In this study, 14 days was chosen as the final measurement day to make sure the study covers the full range of shelf life of spinach.

5.3.1.2 Deterioration of compressed leaves throughout storage

Spinach leaves are exposed to different degrees of leaf injuries due to range of force exertions throughout the handling processes. The colour contour plots for compressed spinach leaves according to their degrees of leaf injuries as well as the forces of compressions from day 1 till day 14 are shown in Figure 5.3. The number inside each colour box represented the total frequency of leaves that had more than 1% decay. The box's colour started from dark blue for 0% of total leaves that had decay to red colour for 100% of total leaves that had decay. Going down the column, as the degree of leaf injury went higher, the percentage of the leaves that had more than 1% of deteriorations increased. Overall, the boxes were having colour transition from dark blue to dark red. Going across from left to right, as the compression force increased, the percentage of leaves that got more than 1% of deterioration increased as well. Spinach leaves are living organisms thus they continue to respire, transpire, and lose water after harvest (Jung et al. 2012). These physiological activities lead to deterioration, senescence, and weight loss of the product (Aked 2000). In addition to this, the breakage on the spinach leaves due to compression disrupted the protective epidermal layer of the leaf which caused cells to rupture and expose the leaf surface to biochemical reactions. These reactions include enzyme and substrate interactions that lead to tissue browning. Several main enzymes involved in enzymatic browning are oxidative enzymes such as peroxidase, polyphenyl oxidase (PPO), and phenylalanine ammonia lysase (PAL). These enzymes oxidised soluble phenolic compounds and produce orthoquinones, which then undergo polymerisation and produce insoluble brown pigments (Castaner et al. 1999; Delaquis et al. 1999; Zhou et al. 2004). The mechanical damage on the spinach leaves also caused the release of the cell contents which allows ‘free-access’ to the microbes to easily gain nutrients inside the cells, thus promotes positive environment for bacterial reproduction, which lead to shelf life reduction of the product (Delaquis et al. 1999; Zhou et al. 2004).

In addition to that, as the storage day increased, the percentage of deterioration increased as well (Figure 5.3 A-E). This obviously can be seen from the ‘concentration’ of red-coloured boxes for frequency of leaves that had more than 1% decays at day 14 (Figure 5.3E) compared to ‘concentration’ of blue-coloured boxes at day 1 (Figure 5.3A). Meanwhile, the coloured boxes for Complete teared and Halfway teared leaves for all compression forces started to be in orange-red colour after day 4 (Figure 5.3B). After 8 days of storage, only Minor teared leaves compressed under 200N started to change colour to orange-red (Figure 5.3C). This shows that after day 8 with 200N treatment, the Minor teared leaves started to exhibit similar properties as Complete teared and Halfway teared leaves. This data is also plotted and shown in Figure 5.4.

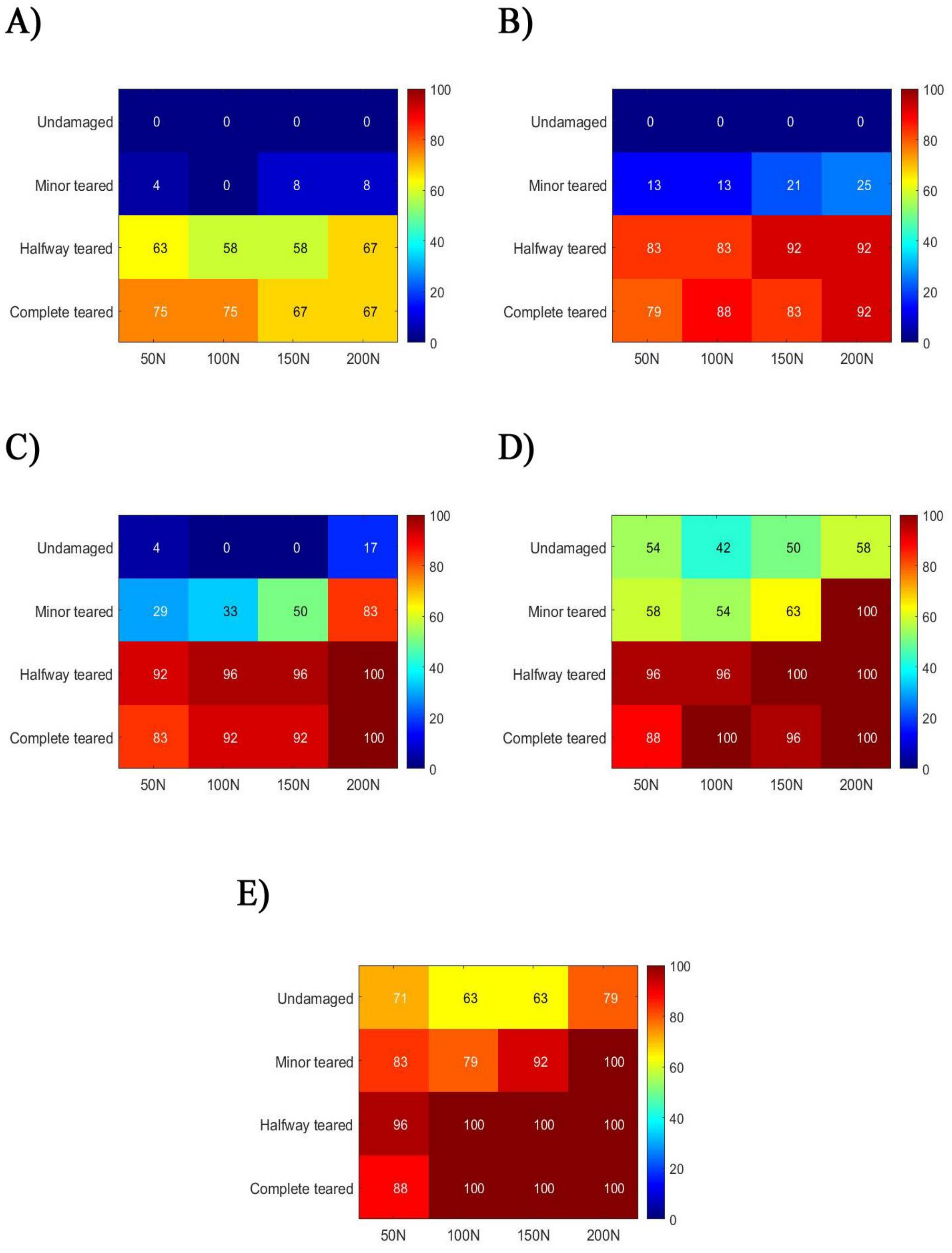


Figure 5.3: : Colour contour plots of different degrees of injuries and force of compressions at A) Day 1; B) Day 4; C) Day 8; D) Day 11; E) Day 14. The number inside each colour box represents the frequency of the leaves that got more than 1% decays.

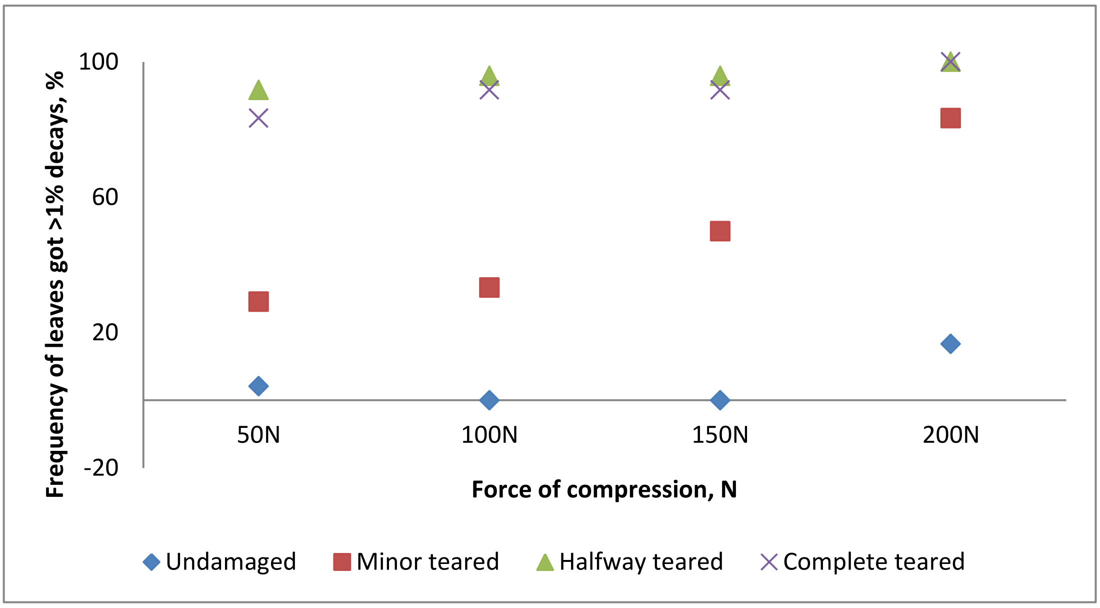


Figure 5.4: Frequency of spinach leaves that got >1% of deterioration after Day 8.

The distributions of deteriorations for spinach leaves compressed under 200N and 100N are presented in Figure 5.5 and Figure 5.6 respectively. For both leaves compressed under 200N and 100N, the distribution of deteriorations showed similar trend as the degree of leaf injury and the storage day increased. For Undamaged leaves, there was not much difference between leaves compressed under 200N and 100N. However, as the degree of injuries went higher which were Halfway and Complete teared, the frequency of the distributions of deteriorations for leaves compressed under 200N shifted more towards the right (increasing the frequency of leaves that had more than 1% of deteriorations) as the storage days increased. For leaves compressed under 200N, 100% of the Halfway and Complete teared leaves had more than 1% of deteriorations after day 8. For leaves compressed under 100N, 100% of Complete teared leaves got more than 1% of deteriorations after day 11, while this case is noticed on Halfway teared leaves after 14 days of storage. It can be concluded that the degree of leaf injury as well as the force of compression did affect the quality and shelf life of the spinach. Both mechanical damage as well as the intensity of the processing injuries accelerate the metabolic processes of the Swiss chard leaves (Roura et al. 2000). Shredded carrots were found to have different respiratory rates than sliced carrots (Izumi et al. 1995) and the rate of ethylene production by cut green bananas was dependent on the thickness of the slices (McGlasson 1969).

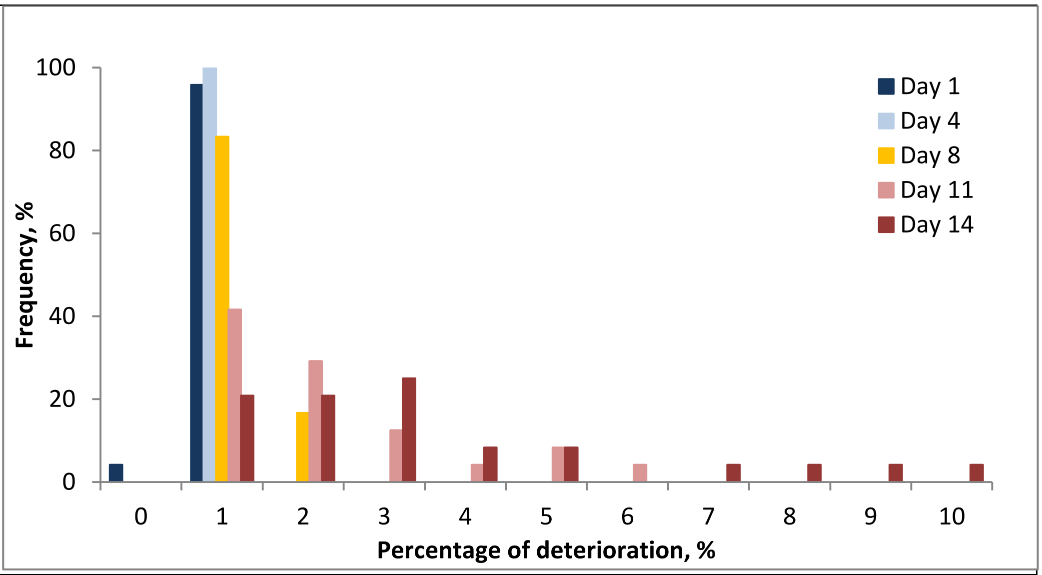
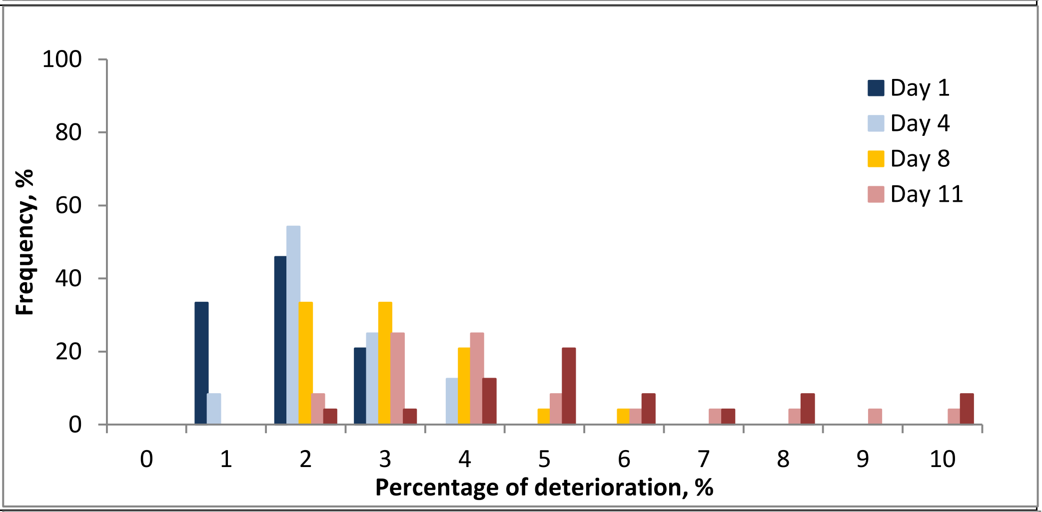
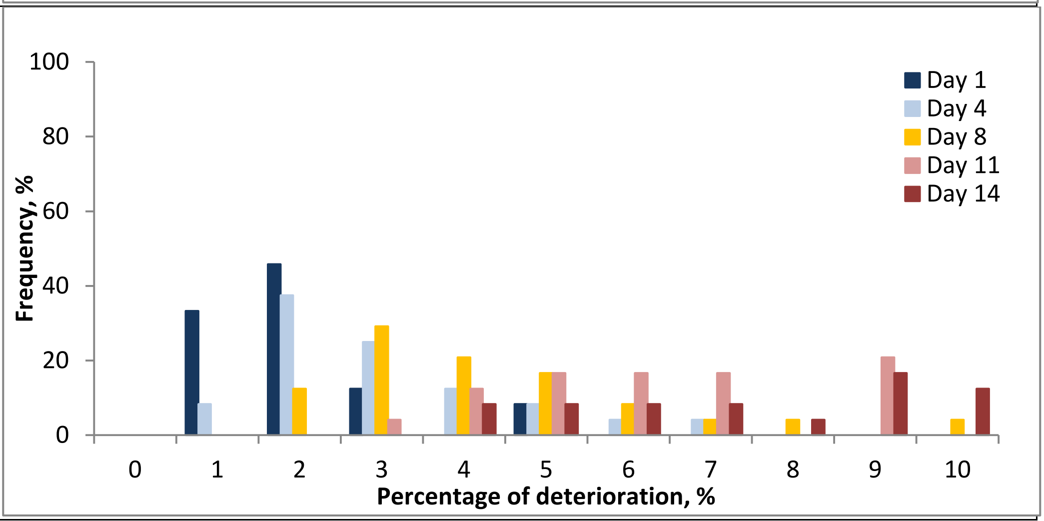
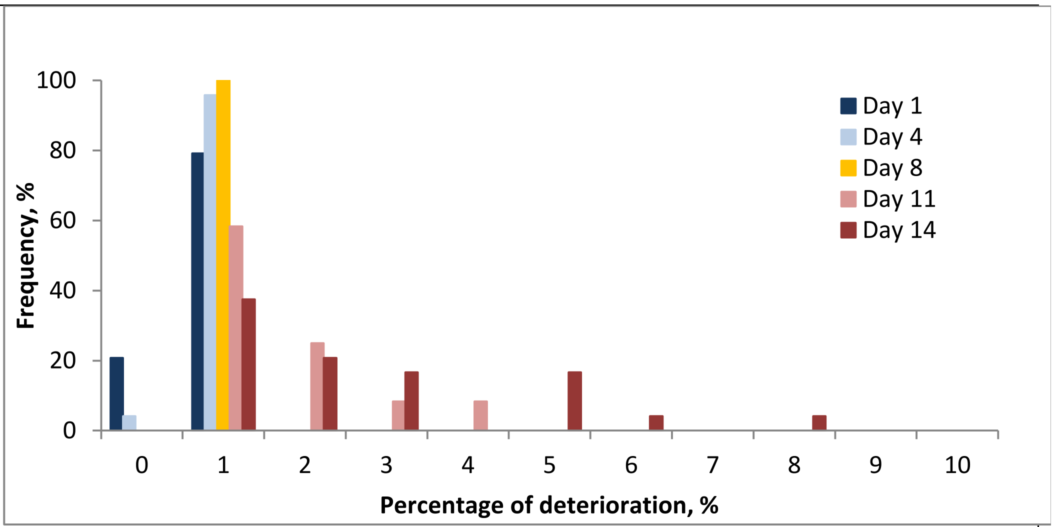
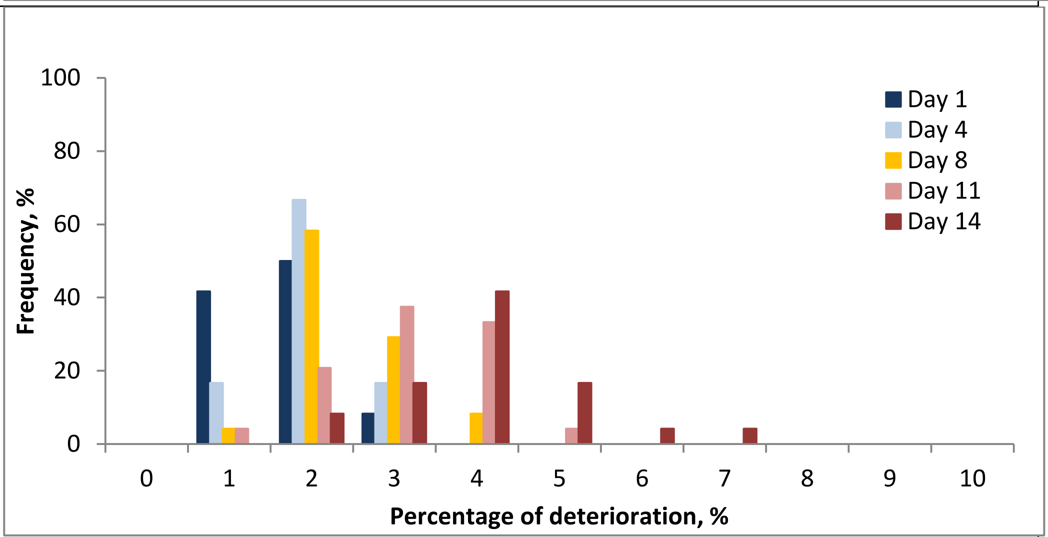
Undamaged	
Halfway teared	
Complete teared	

Figure 5.5: Distribution of deteriorations of spinach leaves compressed under 200N.

Undamaged



Halfway teared



Complete teared

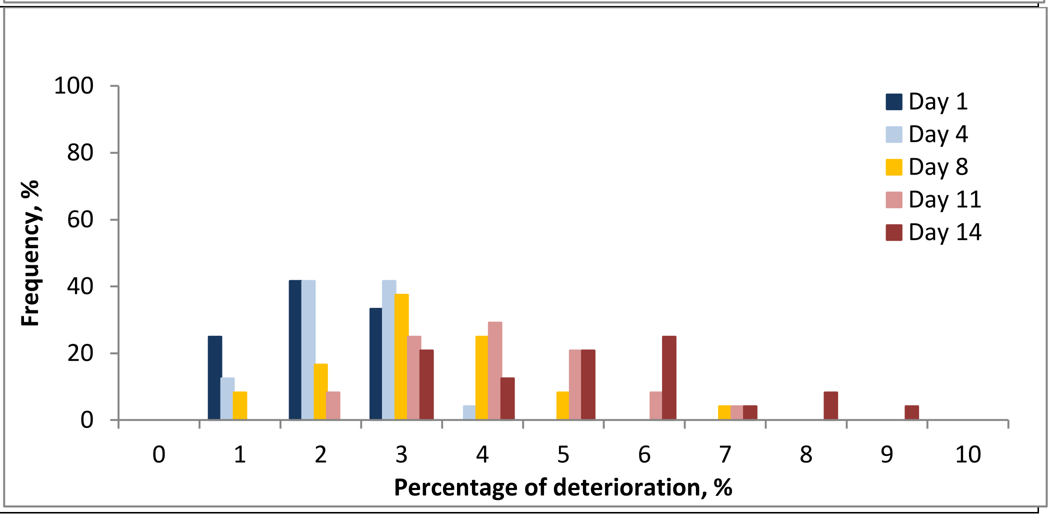


Figure 5.6: Distribution of deteriorations of spinach leaves compressed under 100N.

5.3.2 Comparison of quality and shelf life of Control and compressed leaves throughout storage

5.3.2.1 The frequency of spinach leaves that got more than 1% of total decays throughout storage

The data was further analysed by combining the decays from all degrees of leaf injuries in a same batch of packed spinach. The same batch referred to sample under one complete compression. The Control group was also considered as one single batch. This approach was taken as to study the total deteriorations experienced by the spinach leaves inside the bag as a whole. The frequency of leaves that got more than 1% of total decays for Control and spinach leaves compressed under 50N, 100N, 150N, and 200N throughout storage days is shown in Figure 5.7. Going down the column, starting from Control to the increasing force of compression, the percentage of total decays increased as the compression force increased.

Going right across the row, the percentage of the total decays increased as the storage day increased. For leaves compressed under 200N, the box started to turn orange-red since day 8, whereas for leaves compressed under 50N, 100N, and 150N, the box started to turn orange-red in colour only after 14 days of storage. As for Control leaves, the coloured boxes remained blue to light blue after 14 days of storage which means the leaves could be still consumable/fresh even after 14 days of storage. Intact spinach remained acceptable after 13 days of storage (Artes-Hernandez et al. 2009). The mechanically injured squash showed the worst quality after 15 days of storage affecting physical appearance, chemical parameters and

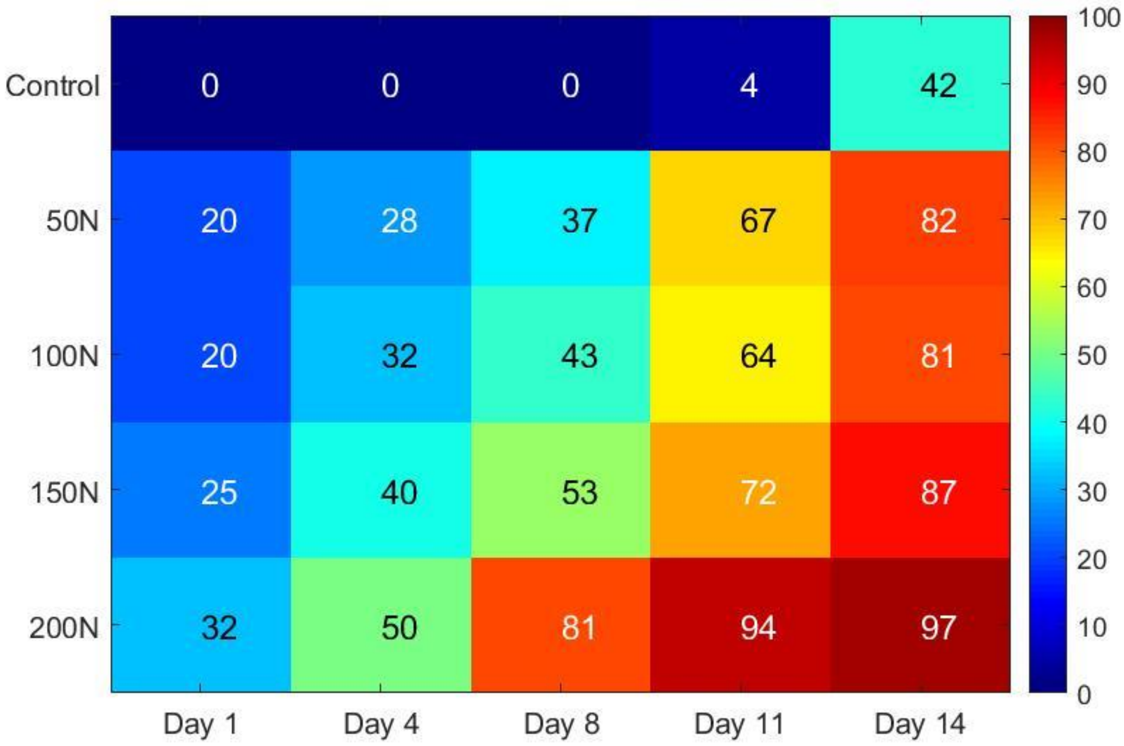


Figure 5.7: The frequency of spinach leaves that got more than 1% of total decays throughout storage for Control and leaves compressed under 50N, 100N, 150N, and 200N.

reduced shelf life (Fernanda et al. 2007). This set of data is important as spinach leaves are sold in packaged/bulk in the market rather than sold individually. Inside the same bag, there were mixed of leaves with different degrees of leaf injuries. As consumers are evaluating the spinach bags randomly, sometimes the leaf injuries might appear visible to them, and sometimes might not. Understanding on the sensory properties of fruits and vegetables played important role in distinguishing consumer perceptions of good quality product (Heenan et al. 2009; Fillion & Kilcast 2000).

5.3.2.2 *Average of leaves deteriorations throughout storage*

Apart from the distributions of deteriorations, the averages of leaves decays were also calculated and analysed. 1-way ANOVA with post-hoc Tukey's test ($p<0.05$) was used to evaluate the significance difference of the leaves decay between treated samples and Control before and after storage using IBM SPSS (version 24). The average changes of leaves decays after storage for Control and compressed leaves is shown in Figure 5.8. In comparison to Control leaves, for 200N, the highest degree of leaf injury which was Complete teared showed significant increment of decays after 11 days of storage whereas Halfway teared leaves showed changes of decays increment after 14 days of storage. There was not any difference in decays changes between Halfway and Complete teared leaves. Lower degree of leaf injuries which were Undamaged and Minor teared leaves did not show any differences with Control leaves before and after storage. Leaves compressed under lower forces (150N and 50N) also showed similar trend as the leaves compressed under 200N. However, please note the difference between the magnitude on the percentage of decays' changes before and after storage. This shows that the amount of force exerted onto the product surface does affect the magnitude of the failure of the product. Again, these showed that the degrees of leaf injuries as well as different force of compressions significantly play important roles in determining and predicting the quality and shelf life of spinach. There was not any difference between Control and leaves compressed under 100N which may be due to the variations of the spinach leaves as well as the rate of metabolic reactions of the particular leaf itself.

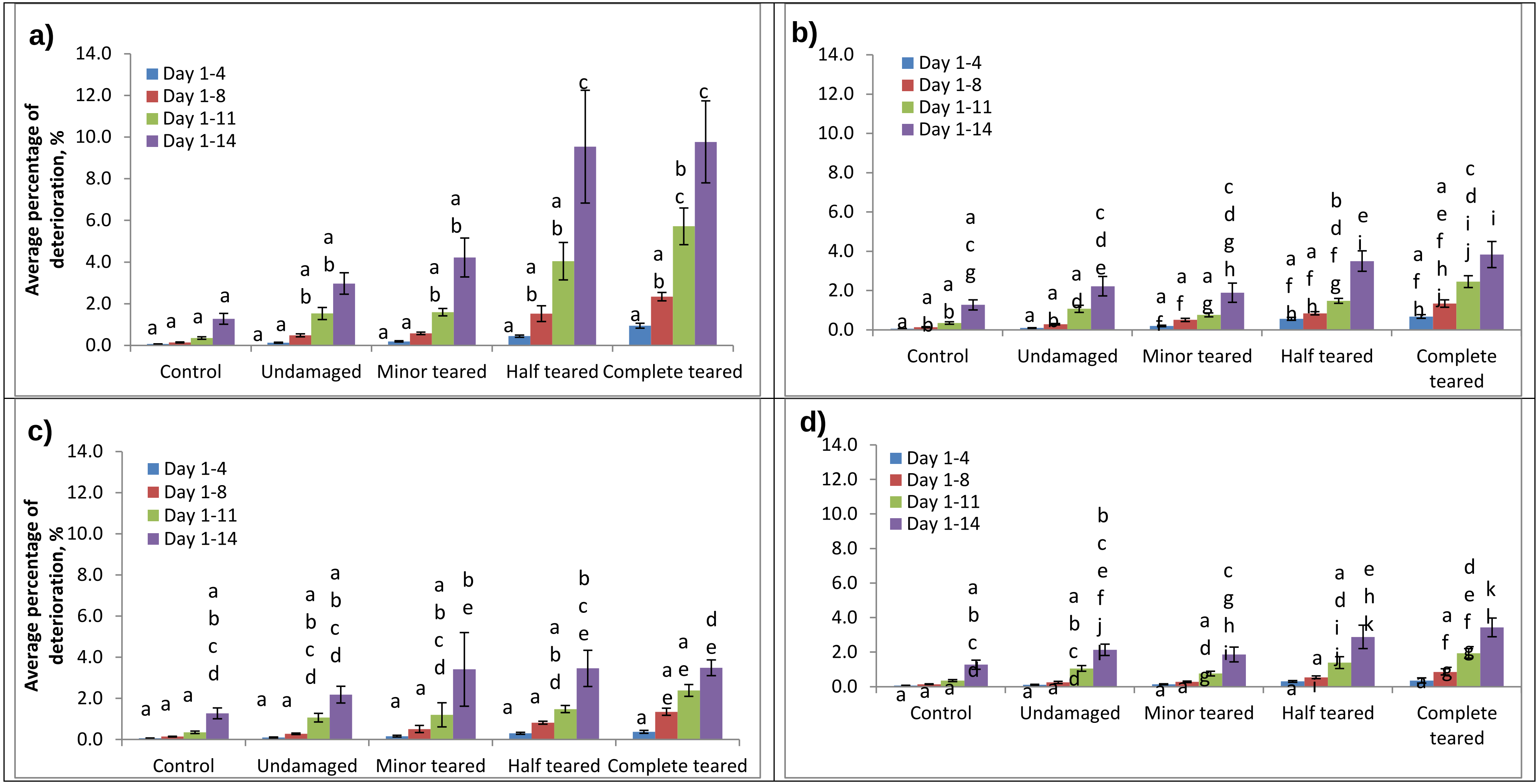


Figure 5.8: Average of leaves deteriorations between Control and different leaf injuries compressed under a) 200N, b) 150N, c) 100N, and d) 50N.

5.3.2.3 Kinetics data of leaf decays

Data on the percentage of leaves deterioration was also modelled using 1st order model. 1st order model has been widely used in modelling quality degradation of fruits and vegetables (Labuza 1979; Lau et al. 2000; Ibarz et al. 2000; Aamir & Ovissipour 2013; Hu et al. 2013; Peng et al. 2014; Dermesonluoglu et al. 2015). The decay of spinach leaves was modelled using first order model:

$$D(t) = D_0 e^{kt} \tag{18}$$

where $D(t)$ is the percentage of spinach decay at time, t; D_0 is the percentage spinach decay on the first day; k is the rate of spinach decay (day^{-1}) ; and t is the storage day.

The decay rates of spinach leaves for Control and leaves compressed under 200N were tabulated in Table 5.2. The values of R^2 fall between the range of 0.867 – 0.993 which showed that the data gave good fit with the 1st order model (Figure 5.9).

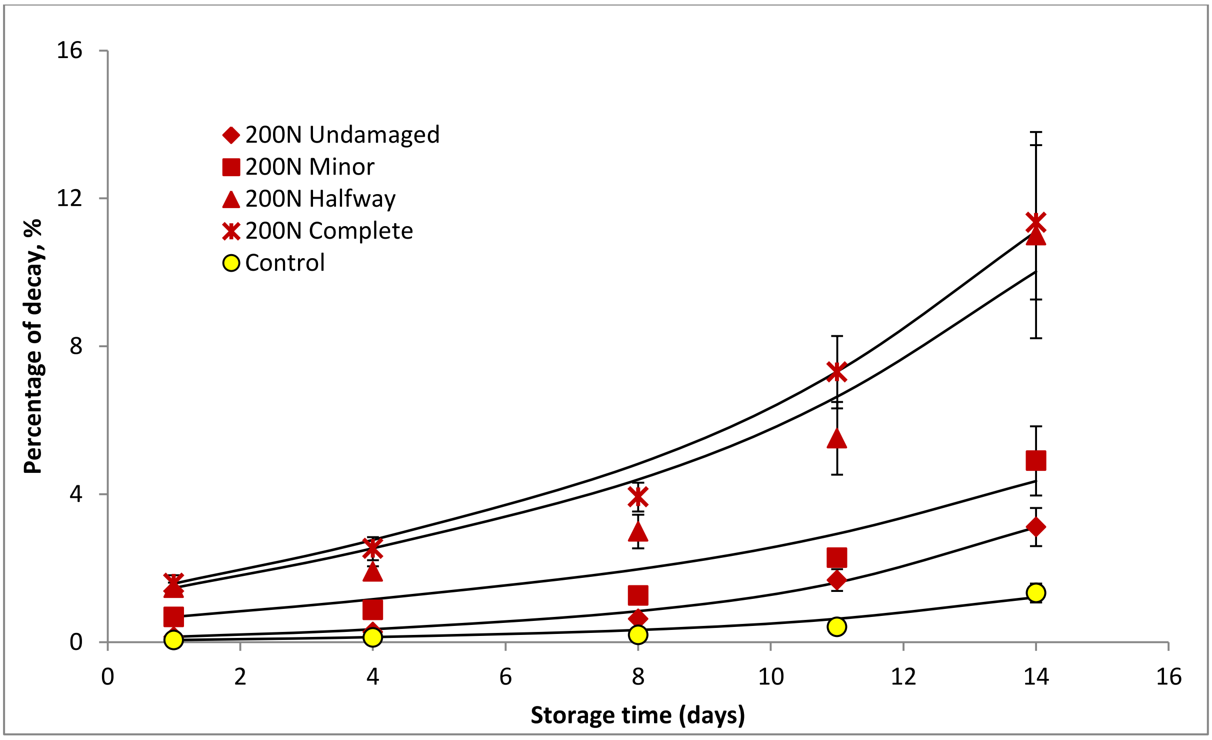


Figure 5.9: 1st order modelled of leaf deterioration under 200N.

Table 5.2: k values of different leaf injuries under 200N, 150N, 100N, and 50N

Leaf category	**k, days^{-1}**			
Control	0.217			
Degree of leaf injury	**50N**	**100N**	**150N**	**200N**
Undamaged	0.224	0.213	0.214	0.218
Minor	0.095	0.130	0.096	0.132
Halfway	0.081	0.090	0.090	0.137
Complete	0.083	0.087	0.090	0.139

Control leaves has similar decay rate as Undamaged leaves and there was difference between them to the other degrees of leaf injuries regardless of the compression forces (Figure 5.10). However, there was not any difference of k values between Minor, Halfway, and Complete teared leaves. Higher k value for Minor

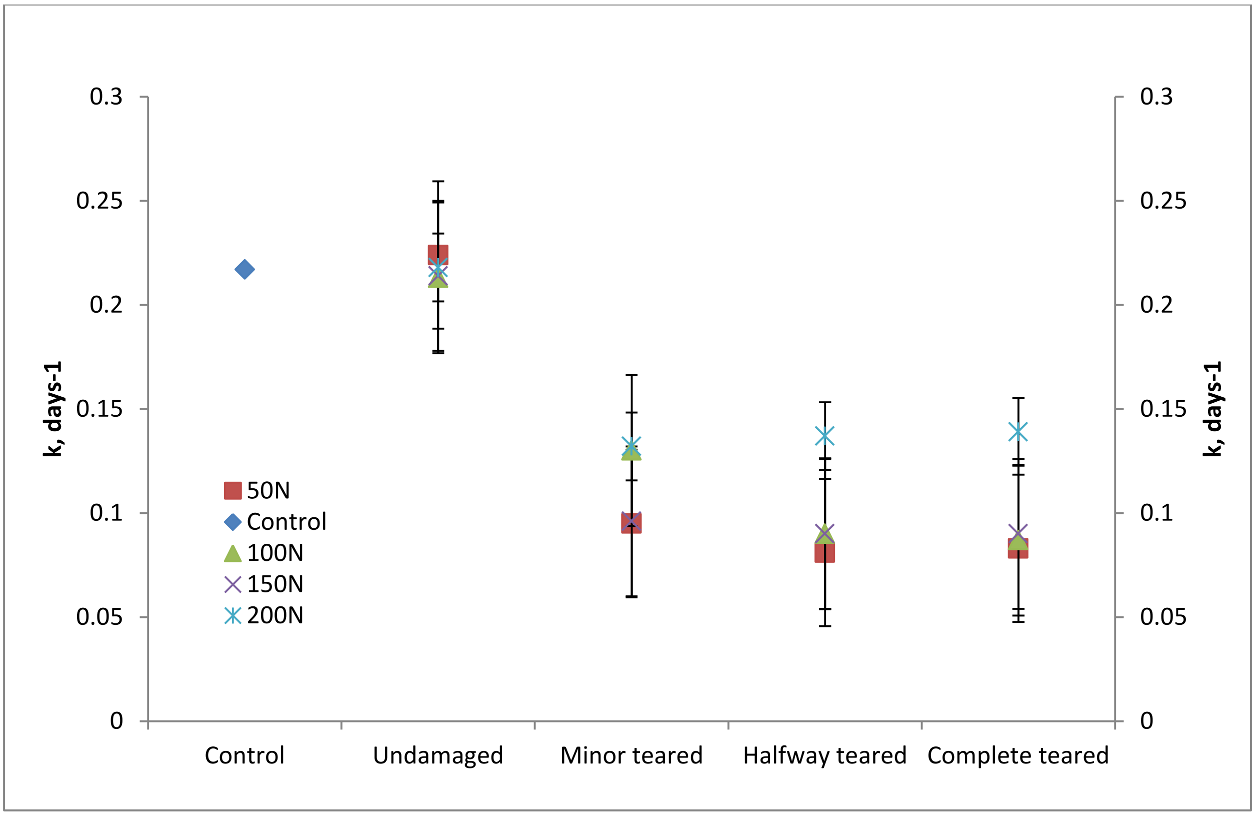


Figure 5.10: Rate of leaves decays at different leaf injuries between different forces of compression.

teared leaves compressed under 100N compared to Halfway teared and Complete teared may be due to the variation of the spinach leaf. Despite of this, for 200N compressed leaves, Halfway and Complete teared leaves showed higher rate of leaves decays compared to leaves compressed under lower forces. From this kinetics data, we can conclude that the degree of leaf injury did not affect the decay rate of the leaves, k; however, different force of compression did affect the k values. As the compression force increased, the degradation rate of the spinach became faster.

The magnitude of the decay's rate was higher for Control leaves compared to the compressed leaves. This is because, Control leaves started with intact and fresher leaves. Thus, throughout the storage, there was more part of the leaves remained ‘to be degraded’; compared to leaves that already damaged on the first day of storage where the leaves were already decayed thus there was not much part of the leaves left ‘to be decayed’ after storage. This property of Control leaves is related to discussion in the next section which is the fresh weight loss. Undamaged leaves also behaved similarly as Control leaves in terms of the decay’s rate.

5.3.2.4 Fresh weight loss

The percentage of fresh weight loss for Control and compressed spinach leaves is shown in Figure 5.11. The Control leaves resulted in highest fresh weight loss compared to leaves compressed with 50N, 100N, 150N, and 200N throughout storage. This is because, Control leaves started as fresh leaves and that is why the fresh weigh lost was high as it contained high moisture content to begin with. As for compressed leaves, they started with low moisture content as some of the water has already lost to the surrounding during and after the compression. Moisture content of the spinach was measured at day 0 to prove that Control leaves have higher moisture content compared to the compressed leaves. There was significant difference in the percentage of moisture content between Control and 200N compressed leaves at day 0 (Figure 5.12). The comparison was made by conducting t-test for equality of mean with $P = 0.016 < 0.05$. The weight loss was associated with the moisture loss through evaporation of the leaf surface. This caused the leaf to soften and became shrivelled and wilted (Schlimme 1995; Pandrangi & Laborde 2004; Villanueva et al. 2005; Ufuk Kasim & Kasim 2012).

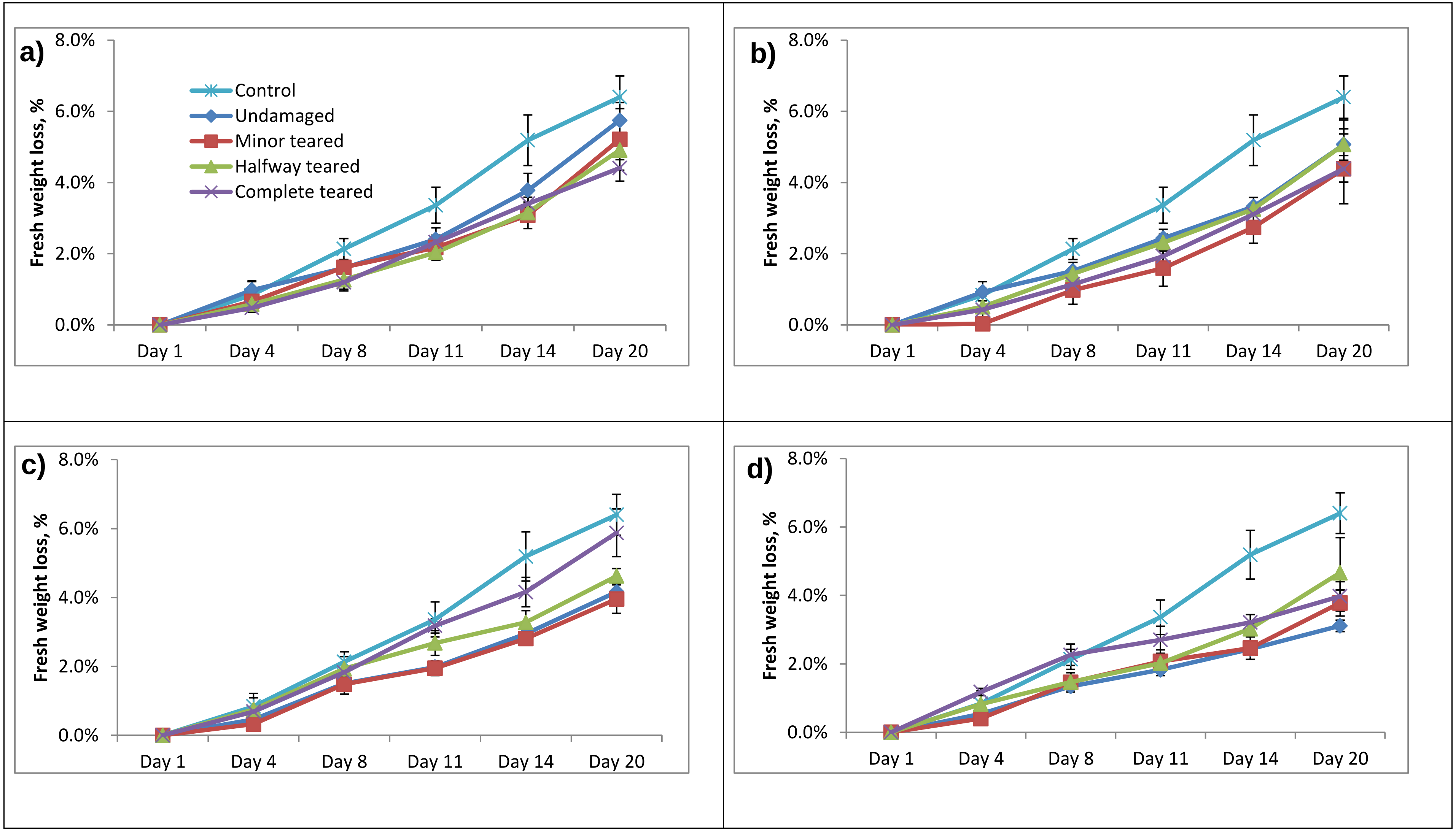


Figure 5.11: Percentage of fresh weight loss for leaves compressed under a) 50N, b) 100N, c) 150N, and d) 200N.

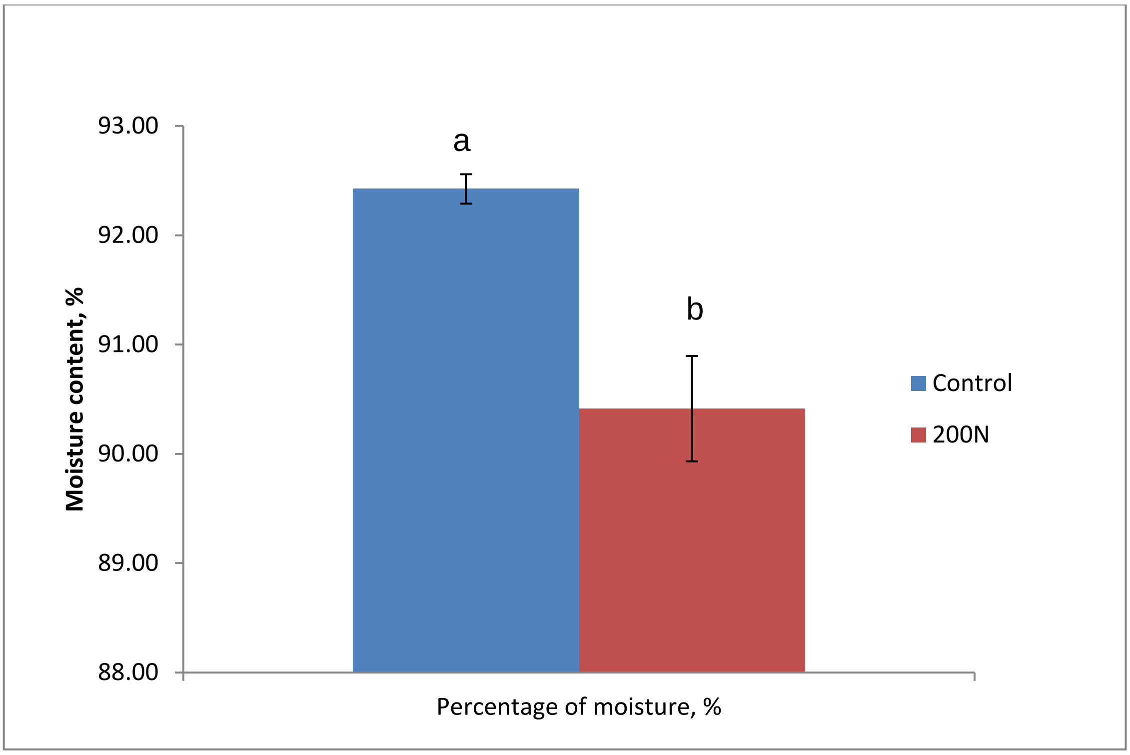


Figure 5.12: Moisture content of spinach leaves at day 0.

Roura et al. (2000) reported that samples with higher damaged area per unit volume caused greater water lost. However, in this study, delay in refrigeration after processing may contribute to the difference in the trend presented. The processed leaves were also exposed to light and changes of room temperature while sample preparation and packaging were taken place. Refrigeration delay, inappropriate light exposure and temperature variation contribute to the quality degradation of the fresh product (Carrasco et al. 2007; Martinez-Sanchez et al. 2011; Liping Kou et al. 2014)

Apart from that, as the storage day increased, the fresh weight loss increased as well. Ageing of vegetation caused the loss of water and chlorophyll reduction (Diezma et al. 2013). In addition to that, the authors also claimed that the decay experienced by the packed leaves is different from the deterioration faced by the leaves during vegetation. The spoilt tissues in the packed leaves were mostly crushed and wet whereas in vegetation, the deterioration was shown by the leaves yellowing, water loss, and pigments degradation.

5.4 Conclusion

Control spinach was found to be in good condition until 14 days of storage. Undamaged spinach showed more decays compared to Control after Day 11 and Day 14. The decays on Minor teared spinach started to become visible after eight days of storage. As for higher degree of leaf injuries which are Halfway teared and Complete teared spinach, the decays were obviously detected since day one. From this Population test, it can be concluded that degree of injury and force of compression affect the quality and shelf life of the spinach.

As the storage day increased, the deterioration of the leaves increased. Force 200N was suggested to be the minimum required to pick up differences of the leaves deteriorations before and after storage. 1st order model was used to model the quality degradation of spinach leaves. Control leaves showed similar decay rate as Undamage leaves whereas there was not any difference of decay rate between Minor, Halfway, and Complete teared leaves. Despite of this, for 200N compressed leaves, Halfway teared and Complete teared spinach showed higher rate of leaves decays compered to leaves with similar degree of leaf injury but compressed under lower forces. From the kinetics data, it can be concluded that the degree of leaf injury did not affect the decay rate of the leaves, k, however, the force of compression did affect the decay rate of spinach. As the force of compression increased, the degradation rate of spinach became faster.

The population test developed throughout this study objective was proven to be beneficial in distinguishing different types of mechanical leaf injuries and their effects towards quality and shelf life of RTE spinach. This method is practical and could be applicable to study other injuries experienced by other fruits and leafy vegetables. Both degree of leaf injury as well as the force of compression have significant impact towards quality and shelf life of the RTE spinach.

The results from this chapter are also supported with sensory data which is discussed in the next chapter. Combining the instrument and the sensory data may provide deeper insights into the properties and sensory attributes of the product (Kemp et al. 2009). This way, the industry could improve and optimise the processing and handling steps in a way to maintain the quality and shelf life of the product while trying to satisfy consumers' perceptions towards the RTE packed spinach.

CHAPTER 6

SENSORY EVALUATIONS BY CONSUMERS TOWARDS VISIBILITY OF LEAF DAMAGES AND PERCEPTIONS IN BUYING THE PRODUCT

6.1 Introduction

The qualities of RTE leafy vegetables depend on ways humans perceive them. These quality attributes include visual appearance (decay, colour, and freshness), texture (firmness, crispiness, turgidity, and toughness), nutrition values (fibre, minerals, vitamins), and safety (free of microbial loads and chemical residues). Due to lack of obvious signs of spoilage, there might be high concentration of microbial loads presented in the produce without consumers' consciousness. Therefore, visual sensory attributes including appearance and freshness level are the main factors consumers look at in making decision to buy the RTE leafy vegetables (Cardello & Schutz 2003; Barrett et al. 2010; Jung et al. 2012; Dinnella et al. 2014). During purchasing decision-making, expiry date also influenced consumers' opinions on the healthiness, nutritional value and freshness level of the fresh products (Ragaert et al. 2004).

Several sensory measurements have been used to evaluate quality attributes of spinach (Piagentini et al. 2002; A. M. Piagentini & Güemes 2002; Ragaert et al. 2004; Artes-Hernandez et al. 2009; Neal et al. 2010; Jung et al. 2012; Medina et al. 2012; Gómez-López et al. 2013; Kwon et al. 2013; Tudela, Marín, Garrido, et al.

2013; Kou et al. 2014; Rezende et al. 2014; Dermesonluoglu et al. 2015; Hu et al. 2015). The quality criteria include degree of browning, fresh appearance, colour changes and other possible sensing criteria (Castaner et al. 1999; Rocha & Morais 2003). The opinion of individuals varies based on their own understanding and experience (Bech et al. 2001; Péneau et al. 2007; Péneau et al. 2006). Products in a same group can also have different freshness perception subjecting to its product classification (Jung et al. 2012), hence it is important to understand consumers' perception on the sensory attribute for a specific food product (Heenan et al. 2009). Despite numerous studies conducted in evaluating sensory attributes of spinach, there is still lack of work to evaluate consumer perception in evaluating the visibility of mechanical damage based on the degree of leaf injury and its effect towards consumer acceptance in buying the product. Therefore, consumer's evaluation on the visibility of the mechanical damage and its effect towards consumer's acceptance in buying the RTE spinach are discussed in this chapter.

6.2 Materials and methods

In this work, 30g of spinach sample S_4 was compressed under 200N through uniaxial compression using Universal Testing machine (Section 3.2) to provide damage to the leaves. Control leaves (uncompressed) were also prepared. Sensory test was conducted as described in details in Section 3.8. The statistics used were also described in Section 3.8.

6.3 Results and discussions

6.3.1 Visibility of leaves deteriorations- Ranking test

For Ranking test, the higher the value, the higher the visibility of deterioration. It goes from 1 (least deterioration) to 5 (most deterioration). Here, there are two reasons of leaves failures which are leaves breakage and decay. Leaf decay includes mushiness and change of leaf colour from green to yellow, brown, or blackish green.

6.3.1.1 The effect of storage day towards visibility of leaves deteriorations

For each of the leaf injury category, there was significant difference in the visibility of deterioration throughout storage (Table 6.1). Undamaged and Minor teared leaves showed significant differences in the visibility of deterioration after day 4. For Halfway and Complete teared, the significant difference was detected after day 8. This is due to the more obvious damage and completely teared leaves which caused difficulty for consumers to differentiate the deteriorations between the two highest degrees of leaf injuries. As for Control leaves, similar trend was detected.

Table 6.1: Scores for Ranking test on the effect of storage days towards visibility of leaves deteriorations.

Leaf injury	RANK SUM				
	Day 1	**Day 4**	**Day 8**	**Day 11**	**Day 14**
Control	82^{a}	65^{a}	152^{b}	163^{b}	138^{b}
Undamaged	86^{a}	55^{b}	143^{c}	181^{d}	135^{c}
Minor	107^{a}	50^{b}	135^{a}	174^{c}	134^{a}
Halfway	59^{a}	69^{a}	144^{b}	181^{c}	147^{b}
Complete	82^{a}	87^{a}	136^{b}	183^{c}	112^{ab}

**similar letter in a same row shows similarity

However, this time consumers were not able to differentiate the intact Control leaves until day 8 as they found the leaves to be in similar state as before the storage.

At day 14, it can be seen that the ranking scores were lower than the scores in day 8 and day 11. This is due to the variation of the leaves. Spinach from different bags behaved differently. Even spinach from the same bag could behave differently as well. The leaves samples were from different batches as the samples were prepared by the reverse-storage-day method before the tests were conducted.

6.3.1.2 The effect of degree of leaf injury towards visibility of leaves deteriorations

For each of the measurement day, there was significant difference in the visibility of deterioration between different degrees of leaf injuries. The deteriorations on Complete teared leaves were significantly visible than all other degrees of leaf injuries up to day 11. On day 14, the visibility of deterioration between Complete and Halfway teared leaves was found to be similar. There was not any significant difference between Minor and Halfway teared leaves on day 1, however, they started to show differences starting from day 4 till day 14. There was not any significant difference between Control and Undamaged leaves throughout storage. It was difficult for the assessors to distinguish the leaves from these two categories as they almost showed a same appearance; Control was uncompressed leaves, while Undamaged was leaves that got no damage resulted from the compression. They began with no breakage and decay before storage thus there was not obvious deteriorations detected throughout the storage. Throughout storage, Minor, Halfway and Complete teared leaves showed significant differences in the visibility of deterioration as compared to Control and Undamaged groups.

The higher the rank sum, the higher the visibility of deterioration. Complete teared leaves got the highest rank sum for each measurement day compared to the other degrees of leaf injuries (Table 6.2). During the sensory test, there were assessors right away rejected bags of spinach leaves that contained Complete teared leaves. Sometimes, broken and complete teared leaves are totally unacceptable. However, they were some assessors responded that broken leaves were still acceptable as long as the leaves did not show any decay, mushiness or wet. This basically turns back to the consumers' preferences. Open-cut on the leaves surface would trigger microorganism growth which could not be seen on bare eyes. As consumers always make shopping decision based on the visual appearance of the products as well as the packaging conditions, it is very important to help to educate the consumers regarding risks that could be faced if the leaves

Table 6.2: Scores for Ranking test on the effect of degree of leaf injuries towards visibility of leaves deteriorations.

Days	RANK SUM				
	Control	Undamaged	Minor	Halfway	Complete
Day 1	66^a	55^a	132^b	148^b	199^c
Day 4	63^a	58^a	119^b	160^c	200^d
Day 8	56^a	66^a	119^b	159^c	200^d
Day 11	53^a	74^a	114^b	159^c	200^d
Day 14	67^a	61^a	114^b	167^c	191^c

**similar letter in a same row shows similarity

safety is disregarded. Halfway teared got the second highest rank sum for all measurement days, while Minor teared got the third highest rank. As expected, Undamaged and Control groups both got approximately similar number of rank sums.

6.3.2 Acceptance level on buying the product- Acceptance test

For Acceptance test, assessors were asked on how they would rate the bagged spinach based on their acceptance in buying the product. In this test, the higher the hedonic score, the higher the assessor's acceptability to buy the product. It goes form I certainly would buy (5) to I certainly would NOT buy (1).

6.3.2.1 The effect of storage day towards buying acceptability

Overall, we could see that as the storage day increased, the rate of buying acceptability decreased (Figure 6.1). This is expected as leafy vegetables are undergoing quality degradation throughout storage.

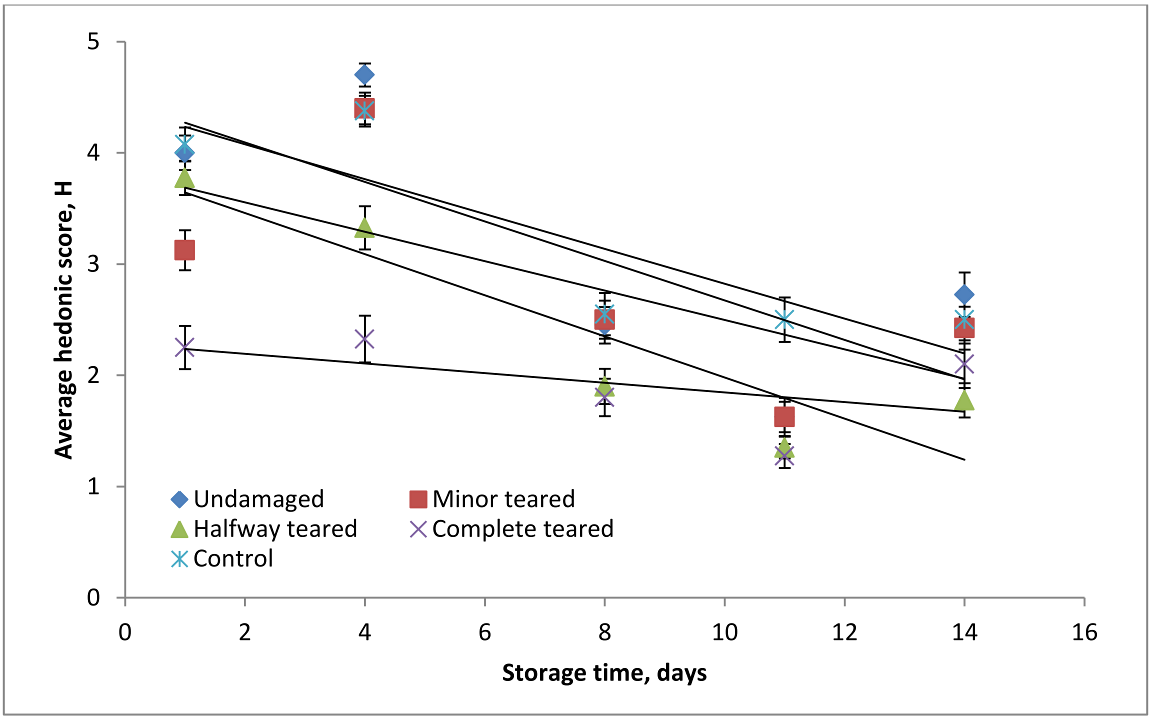


Figure 6.1: Scores for Acceptance test on the effect of storage days towards buying acceptability.

At the beginning of the storage, it was still confusing for the assessors to distinguish different degrees of injuries and make decision whether to buy the products or not. Nevertheless, Complete teared leaves still got the lowest score due to the obvious breakage of the leaves. After day 4, consumers started to be able to differentiate different degrees of leaf injuries, thus affecting their decision in buying the products. Control, Undamaged and Minor teared leaves got higher scores compared to Halfway and Complete teared leaves. After day 11, Minor teared leaves and Undamaged got the same scores as Halfway and Complete teared leaves, and after 14 days of storage, all leaves categories received same scores of buying rate. The storage time was proven to give unfavourable effects towards sensory attributes of spinach (Piagentini et al. 2002; Neal et al. 2010) as well as other leafy vegetable such as romaine lettuce (Prakash et al. 2000). The storage time has an effect towards texture, aroma, tastes and mouthfeels of the spinach. Neal et al. (2010) reported that spinach leaves were found to be less juicy, less rough, and drier surface after 35 days of storage. These effects showed that the water was lost from spinach during storage which correlated to the senescence of the leaves over time. Water loss caused the spinach leaves to be shrivelled and wilted which contributed to the deterioration of spinach. The hardness of the spinach decreased after 11 days of storage whereas the surface roughness decreased after 9 days of storage (Neal et al. 2010). Storage time caused degradation of green colour, turgidity, and built-up off-odour (Dinnella et al. 2014).

There were some inconsistencies in the magnitude of the scores throughout storage because different batch of spinach bags were tested on each measurement days. However, that was not the main focus of the graph as the main focus here is to see the overall decrement of the buying scores as the storage day increased as well as to study the trend on how the consumers ‘grouped’ the acceptance buying rate of different degree of leaf injuries throughout the storage.

6.3.2.2 The effect of degree of leaf injury towards buying acceptability

Going across the row, for each measurement day, it can be concluded that there was significant difference in liking and acceptance level to buy the spinach bags among different degrees of leaf injuries (Table 6.3). The higher the mean values, the higher the acceptance rate to buy the packed spinach. As expected, overall, Control leaves showed the highest acceptance buying rate compared to the other leaf categories. Undamaged leaves followed as the second highest liking, followed by Minor teared and Halfway teared got the third and fourth highest liking score respectively, while Complete teared spinach had the lowest mean of acceptance buying rate. During day 1 and day 4, there was not any significant difference in buying rate between Control and Undamaged. Consumers evaluated these two categories as similar. However, after day 8 and day 11, the buying rate for Undamaged leaves was different and lower compared to Control leaves. Please be reminded that Undamaged leaves were leaves that were found undamaged from the compression, while as for Control leaves, they were totally intact and unprocessed leaves to begin with. As the storage day increased, the Undamaged leaves started to show more deterioration compared to Control leaves due to the invisible deterioration that was hardly detected on Undamaged leaves before day 8. However, on day 14, Control and Undamaged leaves showed similar acceptance buying rate as Control leaves were also started to decay and showed similar deterioration as Undamaged leaves.

Table 6.3: Scores for Acceptance test on the effect of degree of leaf injuries towards buying acceptability.

Days	Mean					Median					Mode				
	Control	Und	Minor	Half	Comp	Control	Und	Minor	Half	Comp	Control	Und	Minor	Half	Comp
1	4.68^{a}	4.80^{a}	3.43^{b}	2.95^{b}	1.25^{c}	5	5	3	3	1	5	5	3	2	1
4	4.85^{a}	4.78^{a}	3.78^{b}	2.60^{c}	1.25^{d}	5	5	4	2	1	5	5	4	2	1
8	4.48^{a}	4.10^{b}	3.05^{c}	1.93^{d}	1.13^{e}	5	4	3	2	1	5	4	3	2	1
11	4.28^{a}	3.80^{b}	2.83^{c}	1.88^{d}	1.05^{e}	5	4	3	2	1	5	4	3	2	1
14	4.35^{a}	4.30^{a}	3.10^{b}	1.93^{c}	1.28^{d}	5	4	3	2	1	5	5	3	2	1

** Read the data row by row. Similar letter in a same row shows similarity

** Und = Undamaged; Comp = Complete

For Minor, Halfway and Complete teared leaves, all of them showed significant differences in buying acceptance rate compared to Control and Undamaged leaves. Between Minor and Halfway teared, there was not any significant difference at day 1, but started to show the difference at day 4 onwards.

From the median and mode, Control leaves maintained to be in the average scores corresponded to ‘I certainly would buy’ from day 1 until day 14 while Undamaged leaves maintained the trend till day 4 only, and then continued to have the average scores equivalent to ‘I might buy’. For Minor teared leaves, in general, consumers were contemplating whether to buy the spinach bags or not as the average scores were corresponded to category ‘I might buy/I might not buy’. As for the Halfway teared leaves, due to the more obvious deteriorations and mushiness on the spinach surfaces, consumers rated them as ‘I might not buy’ category while consumers gave scores equivalent to ‘I certainly would not buy’ to spinach bags contained the Complete teared leaves.

Apart from giving scores on buying acceptability, the assessors were also asked to give feedbacks. From the assessors’ feedbacks, both spinach quality and ambiance inside packaging bags were important factors that influenced consumers’ decisions in buying the product. Assessors defined damaged leaves as leaves that were teared, yellowish and brownish, wet, wilted, got white spots and possible insect bites. The amount of damaged leaves inside the bag needs to be considered. It was still acceptable to buy spinach bags that contained one or two damaged leaves while the rest of the leaves inside the bag were still fresh. As expected, Complete teared leaves with visible crack mark were found to be unacceptable. However, there were still assessors considered to buy the broken leaves as long as the leaves did not change colour and did not show sign of mushiness.

The assessors also suggested that discolouration of the spinach from perfect green colour to yellowish and brownish was the main criteria in rejecting the product. They stated that spinach leaves that were perfect dark green in colour are always better, fresher and more preferable to buy compared to leaves that got yellowing, browning and black spots. Allende et al. (2004) reported that discolouration, decay and development of strong unpleasant odour are the major problems associated with RTE spinach. During commercial, spinach with green colour received a best price. Obviously, perfectly intact leaves are such a bonus to buy. It is important to prevent and slow down the senescence process of the green leafy vegetables such as spinach to retain its quality and commercial value (Kaur et al. 2011). Attributes that clearly describe the physiological ageing phenomena of the leaf organ were associated with the visual appearance that includes green, moisture inside the package, leaf turgidity and leaf integrity (Lim & Nam 2007). Yellowing, loss of surface gloss, shrivelled leaves and stems were considered as decrease in freshness of the vegetables (Aked 2000). It was due to the chlorophyll loss Neal et al. (2010) as well as ethylene produced during senescence (Aked 2000). The rate and pathway of chlorophyll degradation is different between minimally and highly processed foods. In the active metabolic tissue, chlorophyll can degrade itself to non-favoured colourless or grey-brown compounds such as pheophytin and pheophorbide (Heaton & Marangoni 1996). Minimally processed vegetables such as packed salad maintained metabolic activity during storage thus chlorophyll degradation was influenced by genetic and environmental factors. On the other hand, highly processed vegetables such as blanched spinach lost their metabolic activity thus chlorophyll degradation was only affected by the environmental factors (Heaton & Marangoni 1996).

Majority of the assessors also agreed to more unlikely to buy spinach bags that contain high moisture drops which caused the leaves to turn wet, soggy and greenish-dark in colour immediately. The in-pack condensation caused tissue injury (leaves turned dark and yellow) and caused the leaves' decays in the spinach bag to be less noticeable (Lunadei et al. 2012). Clean packaging is important as it allows easier visual inspection for the users in order to avoid damaged product (Ragaert et al. 2004; Dinnella et al. 2014). Nevertheless, the relationship between freshness and acceptance level was quite weak, despite the expectations of freshness as the decisive attribute for consumers in making the decision (Péneau et al. 2006 and Péneau et al. 2007) reported. According to the assessors' feedbacks, sometimes, it is still acceptable to buy the product depending on how quick they are planning to consume them. At times, it is also acceptable to buy bags contain the damaged leaves when they were at reduced price. In some cases, when there were not many choices in the market, people tend to buy spinach bags that contain the damaged leaves. Usually, they will compare few spinach bags and pick the best bag that contained the least damage.

Apart from the above, the assessors also suggested that previous experience and shelf life date at purchase also influenced consumers in making overall judgement on the quality of the product. Similar responses were reported in studies of consumer's perception and choice towards minimally processed vegetables (Ragaert et al. 2004) and consumer's perception of freshness towards mixed salads (Dinnella et al. 2014). The consumers tend to look at the shelf life date on the packaging film as credence of freshness, healthiness, and nutritional value and the highest ratings were given towards products “very close to their original form” (Lund et al. 2006; Peneau et al. 2009).

6.4 Conclusion

Sensorial data including evaluations and perceptions from consumers helped in contributing to the knowledge of quality and shelf life of the RTE spinach as a whole. It is significant for the fresh produce retailer to have better understanding on consumers’ perceptions regarding the visibility of damage and the buying acceptance level of a specific product, thus precautions can be made and the quality of the product can be maintained and preserved throughout handling and distributing stages.

The degree of leaf injury and storage day significantly affect the visibility of decays and acceptance rate in buying the product. This shows that the higher the degree of leaf injury, the lower the shelf life of the fresh product thus the lower the acceptance rate in buying the product. The longer the storage day, the clearer the visibility of decays thus the lower the acceptance rate in buying the product. In some cases, damaged leaves were still acceptable if the product is on sale and it also depends on the time people plan to consume them.

The first limitation of this study would be a limited background of the assessors. All the 80 assessors participated in the study were students and staff from the School of Chemical Engineering, University of Birmingham. Other additional characteristic which may be beneficial in future research is to consider psychographic factor that include lifestyles, values, and personality (Zeithaml 1988; Grunert et al. 1993; Candel 2001; Vannoppen et al. 2002). Several other reasons that effect the perception of consumers towards spinach freshness were natural variation of spinach at different country of origin, demographic factor, socio-cultural background, cultural and environmental differences such as difference in cooking methods (e.g. Asian used to blanch or cook the spinach in soup whereas in the US or Europe, people consume the spinach more as a salad). As a result, the perception of quality and freshness differed as people who used the spinach as salad gave lower score to the freshness compared to those people who cooked the spinach (Jung et al. 2012). There has been study reported on the difference in freshness perception between citizen and soldier groups in the US (Cardello & Schutz 2003).

Secondly the study only included non-trained panellist. Having the expert or experienced consumers gave beneficial addition in the evaluations of the criteria during purchase well as after consumption (Gardial et al. 1994).

Lastly, during the making-decision process, the consumers depend more on the extrinsic properties in situation where relevant intrinsic properties cannot be evaluated in the market (Zeithaml 1988; Jung et al. 2012; Dinnella et al. 2014). As the visual attributes of the produce was also affected by the packaging system of the product Del Nobile et al. (2009), it is also necessary to investigate the effect of different packaging films towards respiration rate of the RTE spinach which is discussed in details in the next chapter.

CHAPTER 7

RESPIRATION RATE OF LEAFY VEGETABLES STORED AT DIFFERENT PACKAGING FILMS

7.1 Introduction

Spinach is a living material and it continues to respire and modified the atmosphere during postharvest storage. As spinach is highly perishable, it has high metabolism rate which leads to high respiration rate (Allende et al. 2004; Artes-Hernandez et al. 2009). High respiration rate is associated with short shelf life of the product (Watada et al. 1996). High O_2 level accelerates the respiration rate of the product which then lead to browning, however, too low of O_2 level lead to anaerobic respiration inside the package (Heimdal et al. 1995). The anaerobic respiration produced ketones, ethanol, and aldehydes (Ahvenainen 1996) where the production of these volatile compounds is associated with development of off-odour and microbial growth inside the package which may contribute to serious quality limiting factors of the products (Caleb et al. 2013; Deza-Durand & Petersen 2014). Thus, appropriate choice of film is important as to achieve optimum atmosphere within the system where extremely low O_2 or high CO_2 which could lead to anaerobic respiration, anaerobic microorganism growth and off-flavour could be prevented (Beaudry 2000; Lucera et al. 2011).

The O_2 and CO_2 concentrations inside the system were influenced by the O_2 intake and CO_2 production by the produce and by the gas diffusion through the packaging film (Zagory & Kader 1988). Thus, choosing a packaging film with a proper oxygen transmission rate (OTR) is crucial to develop an effective atmosphere for the minimally processed vegetables. Poor packaging always lead to the shelf life reduction of these highly respiring product at the retailer's levels which caused the retailer to sell them at huge discounts. Extensive works have been done to evaluate the respiration rates of fruits and vegetables at different packaging films (Berrang et al. 1990; McGill et al. 1996; Peppelenbos & van't Leven 1996; A. M. Piagentini & Güemes 2002; Piagentini et al. 2003; Kim et al. 2004b; Serrano et al. 2006; Lu 2007; Ayala et al. 2009; Del Nobile et al. 2009; Lucera et al. 2011; Pan & Sasanatayart 2016). However, there is still lack of information in the literature regarding the comparison of respiration rates between intact and damaged spinach leaves treated at different packaging films. It is important for the fresh produce retailers to have the basic data regarding the effects that the damaged leaves may have towards the respiration rates of the product, thus quality and shelf life of the product can be maintained throughout the processing, handling, and distributing stages. Therefore, in this chapter, the effects of mechanical damage towards respiration rate of RTE spinach stored under different packaging films are discussed.

7.2 Materials and method

In this work, 50g of spinach sample S_4 was compressed with 200N under uniaxial compression as described in Section 3.2. Besides the compressed leaves, Control leaves were also prepared. Respiration rates of spinach were measured using closed system as described in details in Section 3.9. Three different films were used in this study (Table 3.6). The respiration rates of the spinach were modelled using uncompetitive Michaelis-Menten model and exponential model. The two models were compared and the best model was determined by performing Akaike Information Criterion (AIC) test. Besides that, the effects of packaging films towards quality attributes of the spinach were also measured including fresh weight loss as described in Section 3.6, texture (Section 3.3), and colour changes (Section 3.10).

7.3 Results and discussions

7.3.1 Respiration rate of Control and compressed leaves at different packaging films

7.3.1.1 Oxygen consumption of spinach stored at different films

The reduction of O_2 concentration for Control spinach treated under three different packaging films is shown in Figure 7.1. As the storage time increased, the O_2 decreased due to the intake of the O_2 for the respiration process. The O_2 reduction was divided into three time-segments. From 0^{th} hour to 6^{th} hour, the O_2 reduction was pretty linear. This was due to the measurement that was taken in every 1 hour interval for the first 6 hours. There was not any difference detected between the different films treatments. The O_2 reduction between different films started to differ starting from 7^{th} hour and remained decreasing (film 1 and film 3) till

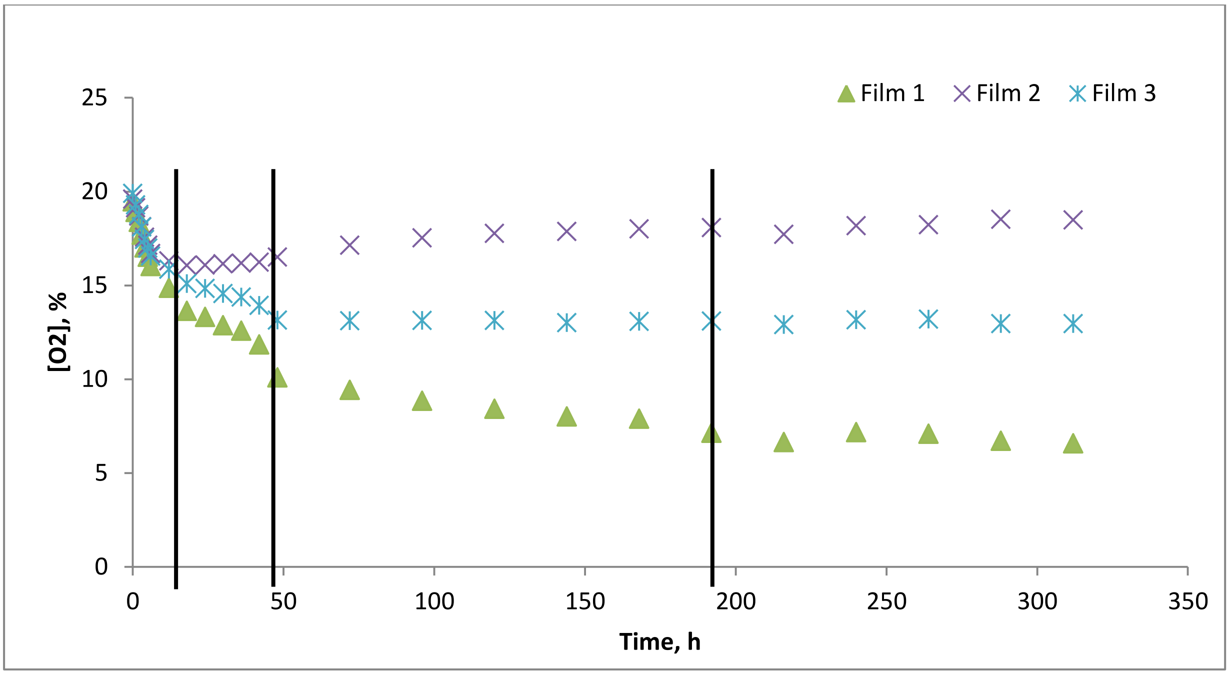


Figure 7.1: The percentage of O_2 concentration inside the closed jars for Control spinach treated under Film 1, Film 2, and Film 3.

48th hour. There was not any O_2 decrement for spinach treated with packaging film 2 within this time segment which indicates that the system has reached steady state. The spinach stored under film 3 reached the steady state starting from 49th hour whereas for spinach treated under film 1, the O_2 kept slightly decreasing until 192th hour and remained constant right after till the end of the set storage time. Kaur et al. (2011) reported that the headspace O_2 and CO_2 concentrations of spinach were in unsteady state until the first day (24 hours) of storage and reached steady state afterwards. It is necessary to pack the product as soon as possible as the respiration rate of the produce at the unsteady state is higher than at the steady state. Fonseca et al. (2002) also reported the same finding where the respiration rate of Galega kale at the unsteady state is threefold than that at steady state.

In terms of the magnitude of the O_2 concentration, spinach covered with film 2 maintained the highest O_2 concentration throughout the storage. This is because, film 2 has the highest OTR with 1200 cc/m^2/day compared to film 1 with 400 cc/m^2/day and film 3 with 1100 cc/m^2/day. This caused the diffusion rate of the O_2 from the outside into the system through film 2 to be the highest compared to film 1 and film 3. Besides the OTR factor, film 1 has the thickest film with 60 µm compared to film 2 and film 3 which have the film thickness of 35 µm. This caused the diffusion of O_2 from the outside into the system to be the lowest in film 1. This shows that packaging film type gave significant effect towards the headspace gas composition of the system (Kaur et al. 2011).

For spinach compressed under 200N, the first six hours showed similar trend as Control spinach (Figure 7.2). The O_2 reduction was linearly proportional to the storage time. As the storage time increased, the O_2 kept on decreasing until the 216th hour, where the O_2 started to reach 0% and remained constant at the 288th hour. This trend happened for spinach treated under all the films and there was not any difference between them. This shows that the respiration rates of the mechanically damaged spinach were not influenced by the type of packaging films used in this study. The ruptured plant cells increased the respiration rate, cut-surface browning, and ethylene production rate (Smyth et al. 1998). The open-cut surface leads to decay and yellowing of the leafy vegetables (Pan & Sasanatayart 2016). Fresh-cut vegetables have higher respiration rates compared to intact vegetables due to the damaged cells that lead to shelf life reduction (Watada et al. 1996; Kim et al. 2004a; Allende et al. 2004; Bolin & Huxsoll 1991). The higher respiration rate was due to the larger surface area on the cut surface (Bastrash et al. 1993) and physiological response towards the wound (Brecht 1995). The damaged cells and

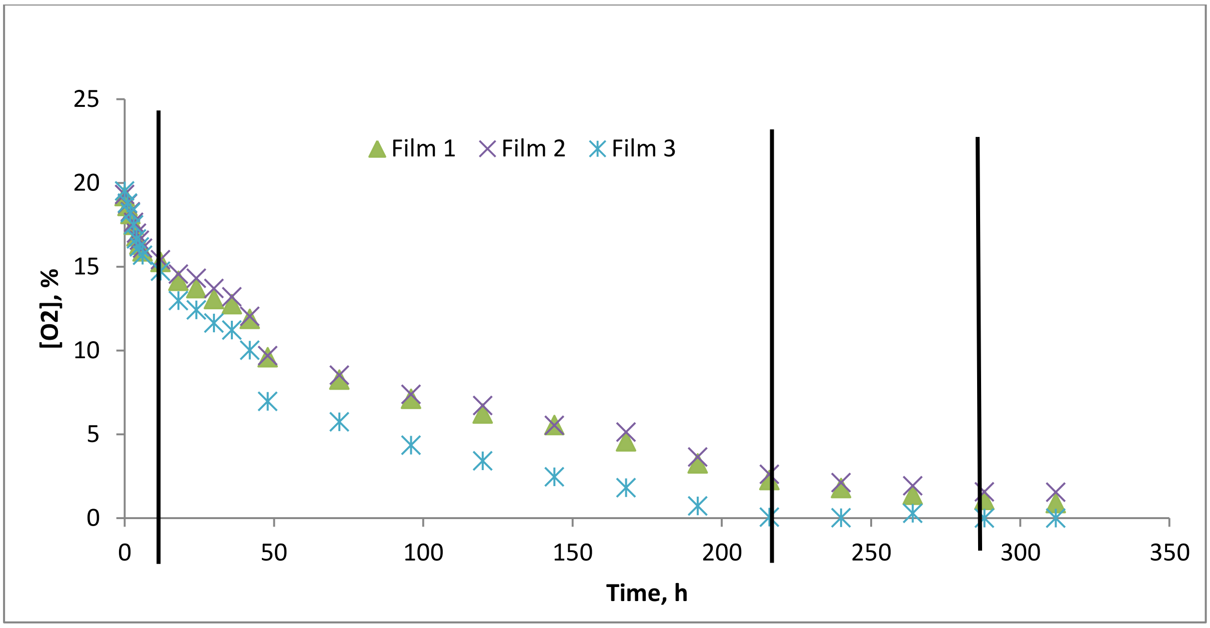


Figure 7.2: The percentage of O_2 concentration inside the closed jars for 200N-compressed spinach treated under Film 1, Film 2, and Film 3.

membranes altered the tissue metabolism such as increase the activity of browning enzymes, CO_2 and ethylene production, aroma and volatile profiles, off-flavour development, and water loss (Kaur et al. 2011). Studies reported that the respiration rate of shredded Galega kale was 2.8 times that of intact leaves (Fonseca et al. 2002) and 2 to 3 times for apple slices than that of intact fruit (Lakakul et al. 1999).

The O_2 concentration of the Control spinach did not reach 0% concentration within the storage time limit in this study. Longer storage time might be required for the O_2 concentration to reach its minimum limit. This shows that Control spinach has longer shelf life compared to mechanically broken spinach. Ko et al. (1996) recommended that spinach should be kept in less than 0.4% but above 0.2% O_2 in 5°C to maintain the quality of the spinach. 1-3% O_2 and 5-10% CO_2 have been suggested as an effective condition to maintain the quality of many green vegetables (Saltveit 2003). Hamza et al. (1995) reported that O_2 levels reduced to 1% helped to preserve the quality of minimally processed romaine lettuce due to the high CO_2 levels that helped in controlling the browning of the tissue. However, O_2 levels below 1% induced anaerobic fermentation of baby spinach (Neal et al. 2010). The anaerobic respiration caused the quality deterioration of baby spinach (Allende et al. 2004). It is important to have optimum atmosphere during storage to maintain the product quality as well as to prevent the development of off-odours (Tudela, Marín, Garrido, et al. 2013).

7.3.1.2 Carbon dioxide production of spinach stored at different films

In response to the intake of O_2, CO_2 was produced during the respiration. As the O_2 decreased, the CO_2 production increased (Figure 7.3). The high CO_2 slowed down the respiration rate of the spinach. Atmosphere with high CO_2 significantly inhibit the growth of aerobic microorganisms in fresh-cut spinach (Babic & Watada 1996) and broccoli (Berrang et al. 1990). High CO_2 atmosphere slowed the browning of lettuce (King et al. 1991) and the yellowing of spinach (Kader et al. 1989). However, the CO_2 concentration must remain below 18% to prevent anaerobic respiration. CO_2 concentration up to 13% developed off-odour in packed spinach (McGill et al. 1996). Decay, tissue softening, and development of off-odour are the main problems associated with baby spinach (Medina et al. 2012; Allende et al. 2004). For Control spinach, within the storage time limit in this study, the CO_2 concentrations remained below 18% for spinach treated under all the packaging films.

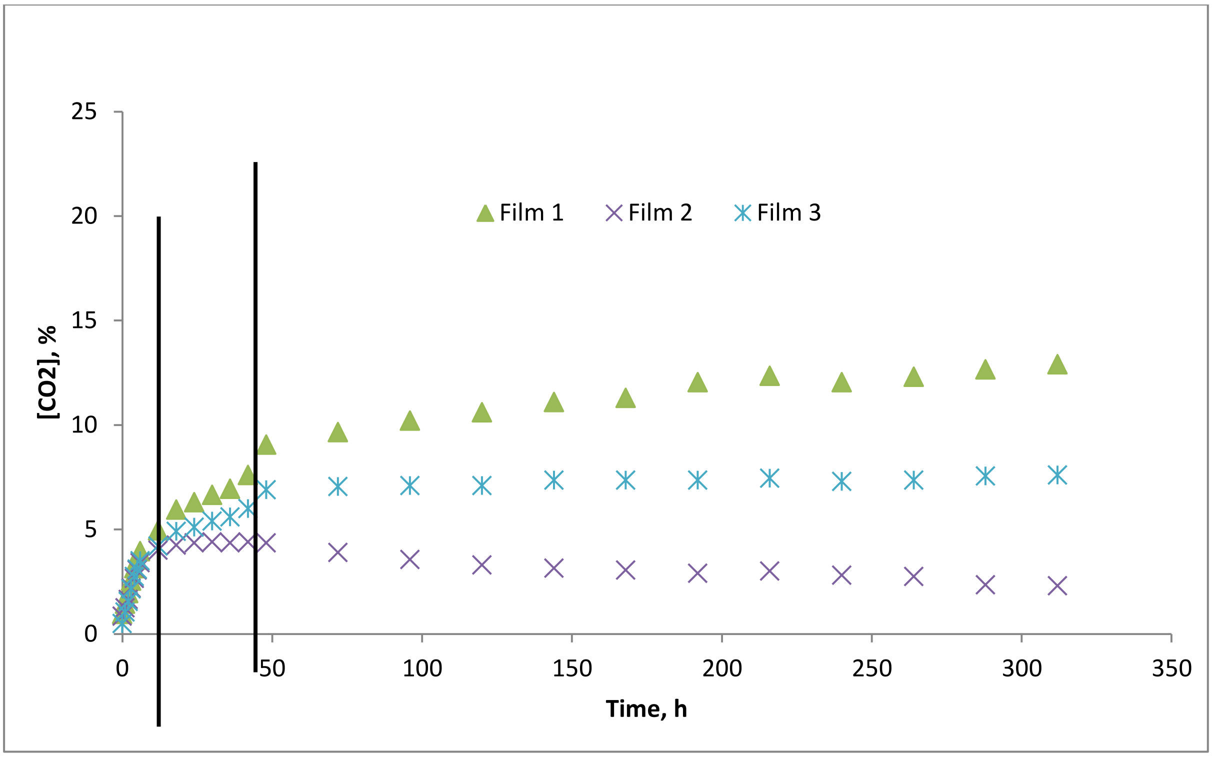


Figure 7.3: The percentage of CO_2 concentration inside the closed jars for Control spinach treated under Film 1, Film 2, and Film 3.

For 200N-compressed spinach, the CO_2 increased to 18% and then remained constant started at the 288th hour (Figure 7.4). Further storage could lead the spinach to undergo anaerobic respiration where the living cells take-in the excessive CO_2 for 'fermentation', which causes further deterioration of the leaves. Again, this confirmed that mechanically damaged spinach has shorter shelf life during storage compared to intact spinach. CO_2 production rate was lower than the O_2 consumption rate of the spinach which indicates that water vapour was also produced along with the CO_2. The water vapour transmission rate as well as the micro perforations may not be enough to diffuse the water outside the package thus lead to condensation inside the package and caused deterioration and reduction in sensory quality of the product (Kaur et al. 2011).

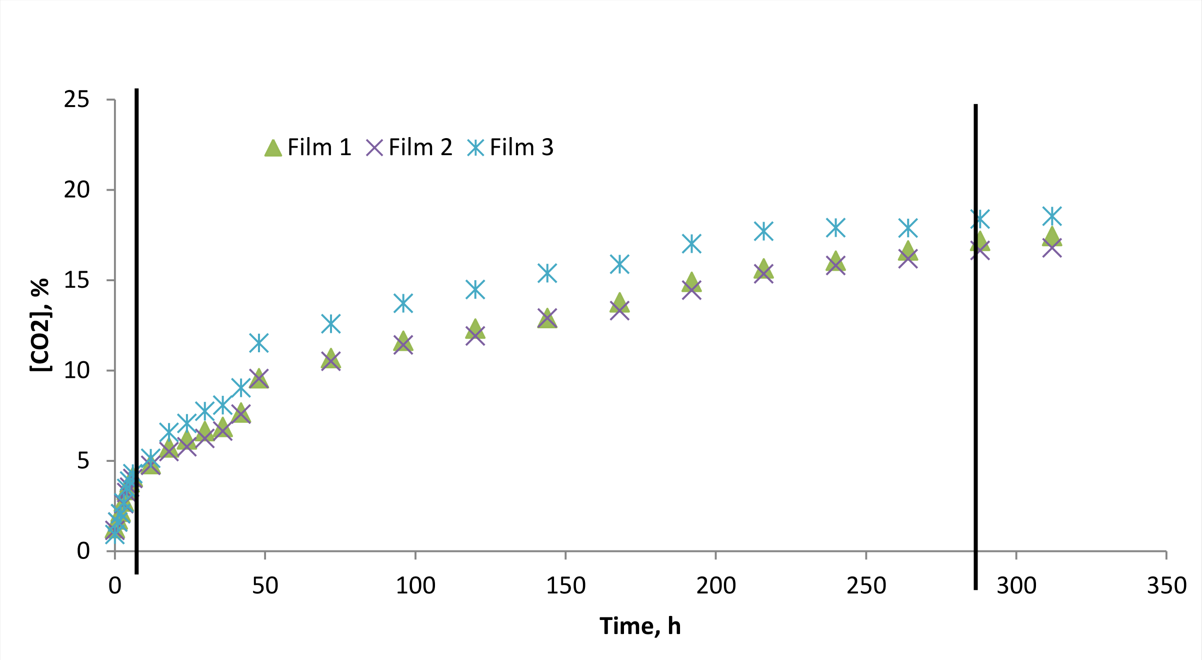


Figure 7.4: The percentage of CO_2 concentration inside the closed jars for 200N-compressed spinach treated under Film 1, Film 2, and Film 3.

7.3.2 Modelling the respiration rate of spinach

The respiration rate of the spinach was modelled using both uncompetitive Michaelis-Menten model and exponential model.

Both models showed good fit with the experimental data (Figure 7.5 and Figure 7.6) for uncompetitive Michaelis-Menten model and exponential model respectively). As the storage time increased, the respiration rate (RO_2) increased as the consumption of the O_2 for the respiration increased. In the beginning of the storage, there was still high O_2 concentration, thus there was steep increment of the RO_2. High O_2 concentration speed up the O_2 uptake as the respiration enzymes are exposed to higher levels of reaction substrate (Barrios et al. 2013). This relationship between O_2 concentrations and plant metabolism was also reported by (Li et al.

2009). After certain time, the RO_2 only increased slightly and remained stagnant as there was not much O_2 left for the respiration to happen. Oxygen availability is a requirement for the respiration to happen where the higher the respiration rate, the lower the shelf life of a commodity (Kader & Saltveit 2003). The negative values showed that the O_2 has been used by the spinach for the respiration.

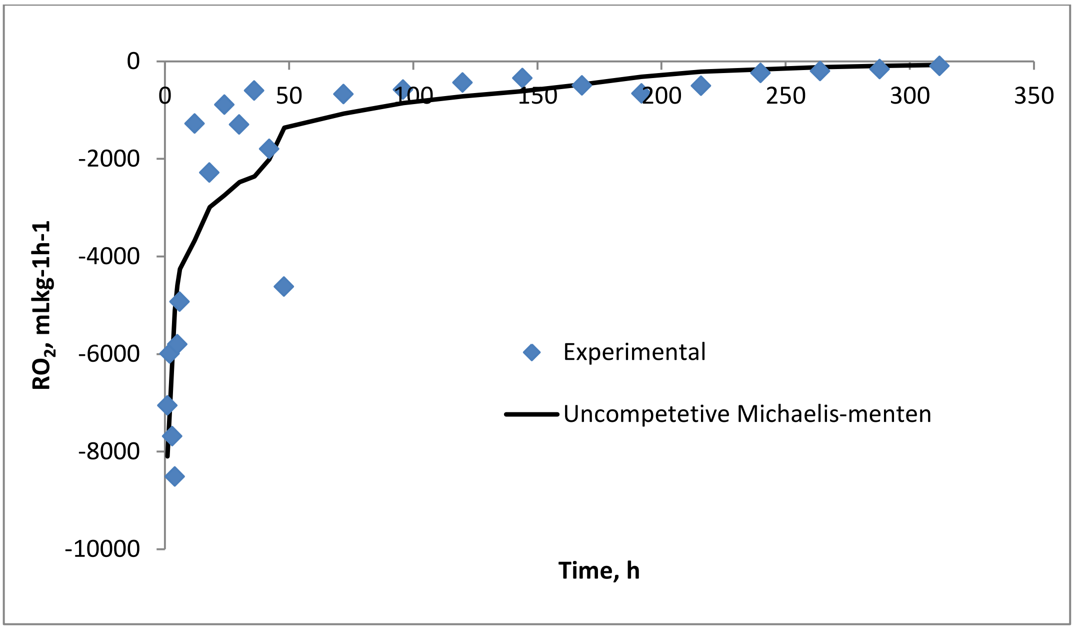


Figure 7.5: Uncompetitive Michaelis-Menten model for 200N-compressed spinach treated under Film 1.

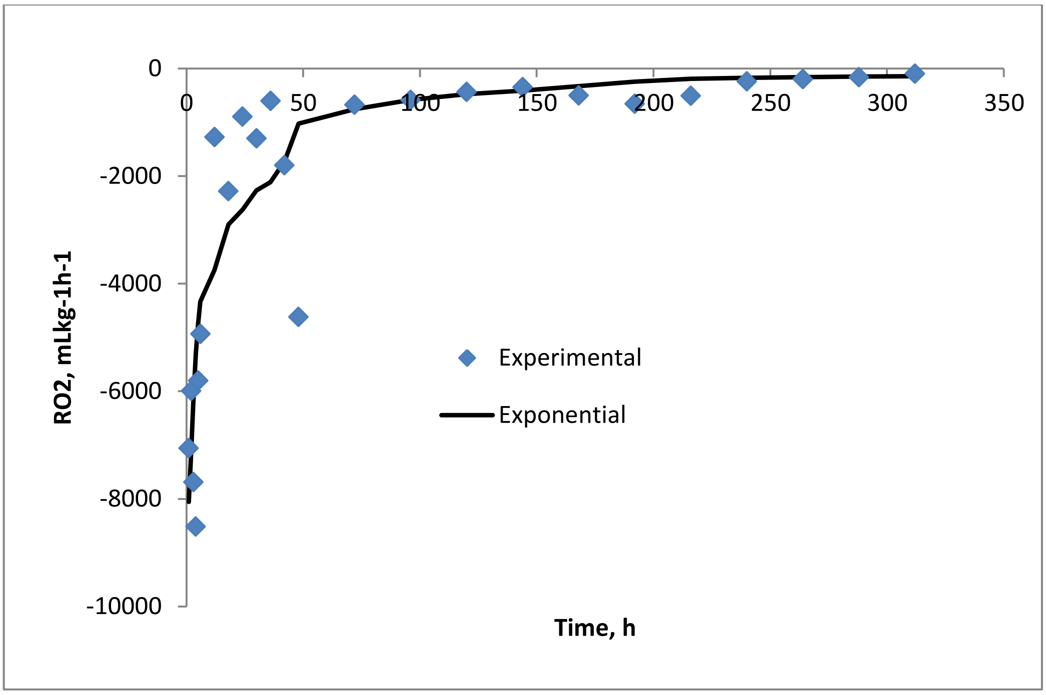


Figure 7.6: Exponential model for 200N-compressed spinach treated under Film 1.

The parameters for both models are tabulated in Table 7.1 and Table 7.2 for uncompetitive Michaelis-Menten model and exponential model respectively. The best model was determined by performing AIC test where model with the lowest AIC value shows the better fit. The AIC values showed almost similar values for both uncompetitive Michaelis-Menten model and exponential model. However, most of the AIC values from the exponential equation were lower than the AIC values from the uncompetitive Michaelis-Menten equation. This might suggest that exponential equation gave better fit to model the respiration rate of the spinach in this particular study.

Table 7.1: Predicted parameters for Uncompetitive Michaelis-Menten model equation of spinach sample S_4 for Control and 200N-compressed leaves.

Parameters	Control			200N		
	Film 1	Film 2	Film 3	Film 1	Film 2	Film 3
V_m	2.05E+09	9.19E+09	3.06E+09	2.06E+09	1.96E+09	3.39E+09
K_m	2.59E+06	1.88E+04	2.23E+06	2.57E+06	2.70E+06	4.93E+06
K_i	1.98	5.77E-04	0.54	2.05	2.14	4.19
AIC	351.23	377.21	360.15	352.14	352.70	359.96

Table 7.2: Predicted parameters for Exponential model equation of spinach sample S_4 for Control and 200N-compressed leaves.

Parameters	Control			200N		
	Film 1	Film 2	Film 3	Film 1	Film 2	Film 3
a	87.24	33.55	4.59	114.51	102.76	330.03
b	0.24	0.22	0.40	0.23	0.23	0.18
AIC	343.87	379.87	348.53	349.19	350.41	357.47

7.3.2.1 Model validation

For models validation, the oxygen transmission rate (OTR) for each film was inserted into the model validation equation.

$$Model\ validation = OTR\ term + Respiration\ rate\ (modelled\ )$$

The original unit for the OTR term was cm^3/m^2/day/atm. This unit was then converted to the similar unit as the respiration rate which was in mL/kg/h by multiplying with the surface area of the closed system (22.06 cm^2) and with the assumption of the atmospheric pressure of 1 atm. The OTR for Film 1 is 400 cm^3/m^2/day/atm, Film 2 is 1200 cm^3/m^2/day/atm, and Film 3 is 1100 cm^3/m^2/day/atm. By converting those OTR from cm^3/m^2/day/atm units to mL/kg/h, the OTR for Film 1 became 0.74 mL/kg/h, Film 2 was 2.21 mL/kg/h, and Film 3 was 2.02 mL/kg/h.

Inserting the OTR term into the model validation equation gave similar trend and fit as with the original modelled equations (Figure 7.7 and Figure 7.8). The changes of the fits were very small as the OTR terms values were small. The uncompetitive Michaelis-Menten model equation as well as exponential model equation appropriately described the effect of processing treatments and oxygen transfer rate of the film towards the respiration rate of spinach. The decision of these models was made based on the quality of the fit and simplicity of the model (Saenmuang, Al-Haq, Samarakoon, et al. 2012).

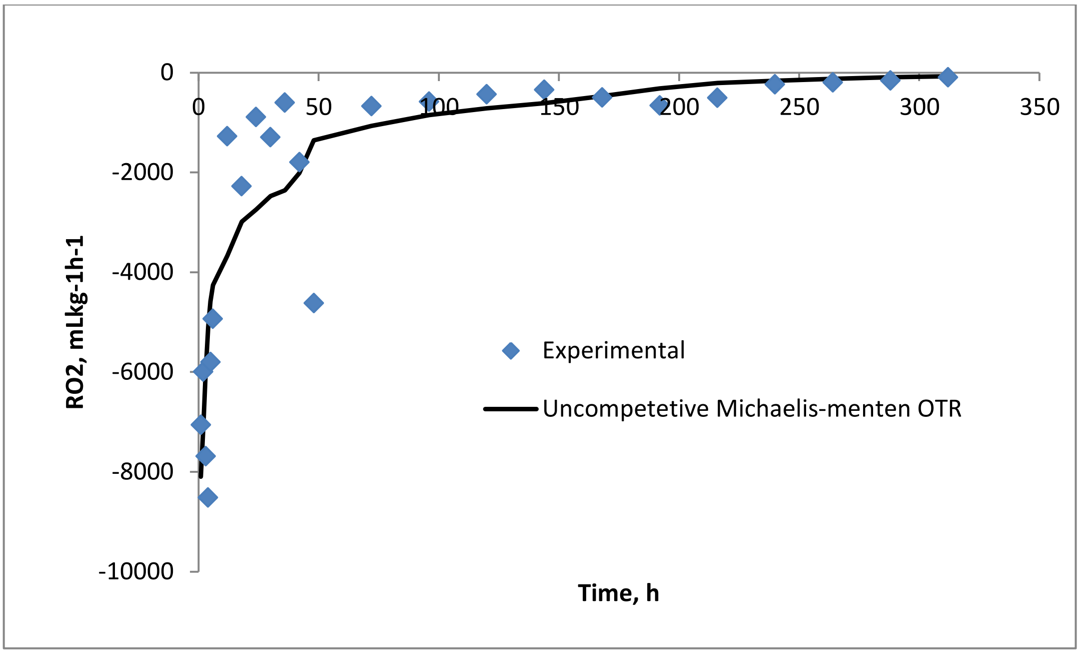


Figure 7.7: Uncompetitive Michaelis-Menten model with the OTR term for 200N-compressed spinach treated under Film 1.

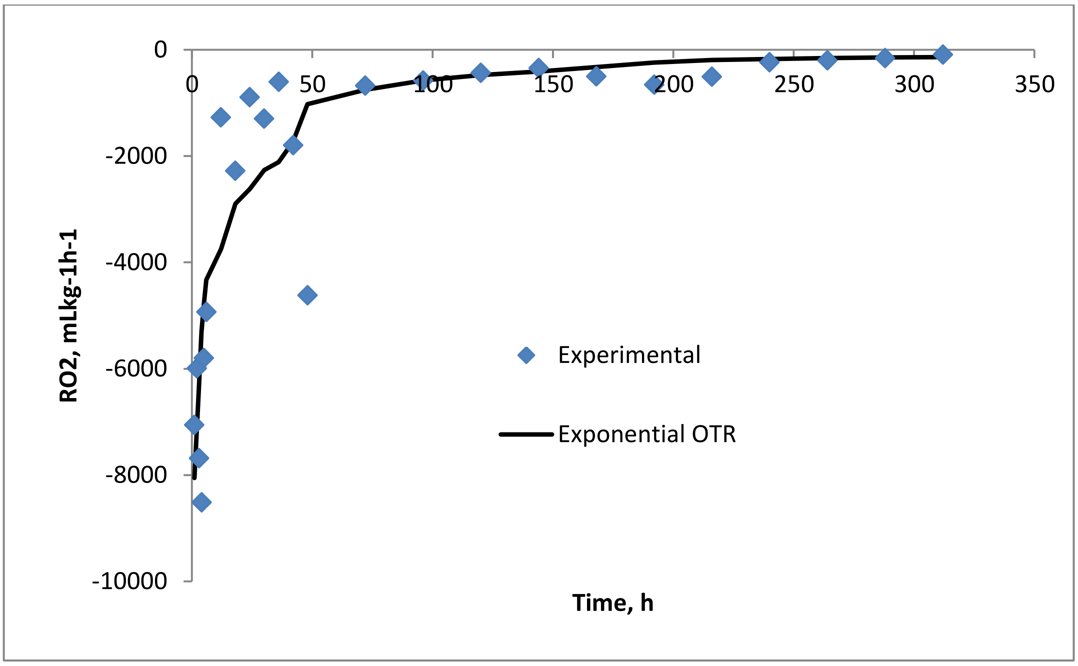


Figure 7.8: Exponential model with the OTR term for 200N-compressed spinach treated under Film 1.

7.3.3 The effect of packaging films towards quality attributes of spinach

7.3.3.1 Fresh weight loss

The percentage of fresh weight loss for Control and 200N-compressed spinach stored at Film 1, Film 2, and Film 3 is shown in Table 7.3 and Figure 7.9.

Table 7.3: The percentage of fresh weight loss for Control and 200N-compressed spinach stored at different packaging films. The data represent the mean of three replicates ± standard deviation.

Films	Leaf category	Fresh weight loss, %			
		Day 4	Day 8	Day 11	Day 14
Film 1	**Control**	0.33 ± 0.26 [a]	0.56 ± 0.33 [ab]	0.86 ± 0.24 [b]	1.2 ± 0.30 [c]
	200N	0.17 ± 0.15 [a]	0.51 ± 0.18 [ab]	0.74 ± 0.21 [bc]	1.17 ± 0.28 [c]
Film 2	**Control**	0.4 ± 0.07 [a]	0.93 ± 0.29 [bc]	1.05 ± 0.11 [cd]	1.38 ± 0.07 [d]
	200N	0.51 ± 0.02 [a]	1.11 ± 0.22 [bc]	1.41 ± 0.06 [c]	1.84 ± 0.13 [d]
Film 3	**Control**	0.38 ± 0.04 [a]	0.84 ± 0.15 [ab]	1.3 ± 0.23 [bc]	1.70 ± 0.25 [c]
	200N	0.48 ± 0.03 [a]	1.14 ± 0.12 [b]	1.69 ± 0.13 [c]	2.17 ± 0.20 [d]

**going across the row, similar letter shows similarity.

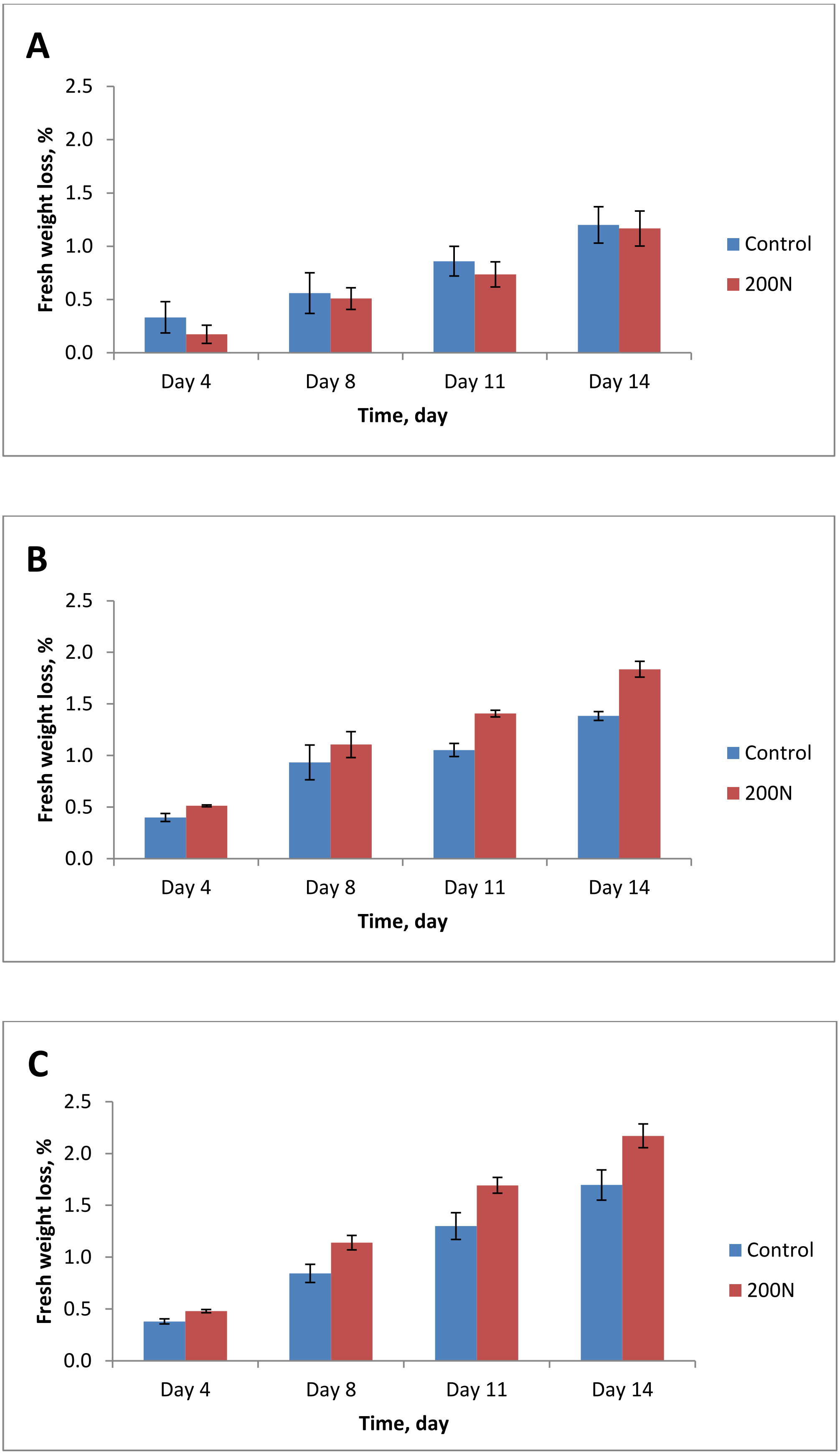


Figure 7.9: Fresh weight loss for Control and 200N-compressed spinach stored at A) Film 1; B) Film 2; and C) Film 3.

The percentage of weight loss increased as the storage time increased. The weight loss measured after 14 days of storage was higher than the weight loss measured after 7 days of storage regardless of the treatment and film used (Piagentini et al. 2002). One-way ANOVA with post hoc Tukey test ($P < 0.05$) was used to find the significant difference for each Control and 200N-compressed leaves throughout storage. Overall, spinach stored in Film 1 showed the lowest percentage of fresh weight loss for both Control and 200N-compressed leaves compared to spinach stored in Film 2 and Film 3. All the packaging films used in this study were micro-perforated BOPP films with Film 1 with the lowest OTR value. It has been reported that the micro-perforated film that has the lowest OTR gave the best performance in prolonging the shelf life of fresh-cut broccoli (Lucera et al. 2011).

For spinach stored in Film 1, the percentage of fresh weight loss for Control spinach was higher than the damaged spinach throughout the storage. This is in agreement with the results of fresh weight loss discussed in Chapter 5, Section 5.3.2.4. On the other hand, for spinach stored in Film 2 and Film 3, the percentage of fresh weight loss for the damaged spinach was higher than Control spinach throughout the storage. This may be related to the high OTR as well as thinner film for Film 2 and Film 3 compared to Film 1. Film 1 has thickness of 60 µm, whereas Film 2 and Film 3 both have film thickness of 35 µm. The high OTR and thin film allowed high O_2 diffusion into the packages thus increased the respiration rates of the product. OTR and film thickness were found to be significant in affecting the quality attributes of the packed fresh products (Ayala et al. 2009; Del Nobile et al. 2009; Oliveira et al. 2010; Lucera et al. 2011; Pan & Sasanatayart 2016). As discussed earlier, mechanically broken leaves have higher respiration rates than intact leaves. Increased in the respiration rates caused the CO_2 production and water vapour to increase which then caused the fresh weight loss to increase. Weight loss can be limited by using appropriate packaging as well as controlling the storage humidity and temperature (Kaur et al. 2011).

7.3.3.2 Texture

The maximum forces (MaxF) required to break the leaves under Penetration test are shown in Figure 7.10 and Figure 7.11 for Control and 200N-compressed spinach respectively, whereas the maximum distance (MaxD) required to break the leaves are shown in Figure 7.12 and Figure 7.13 for Control and 200N-compressed spinach respectively. The MaxF is also known as the burst strength of the leaves whereas the MaxD is associated with the toughness of the leaves. From the plots, both storage day and type of packaging film did not have effect towards both burst strength and toughness of the spinach leaves. Piagentini et al. (2002) reported that research has been done to study the effect of packaging film towards sensory qualities of spinach, asparagus and broccoli. The study showed that different packaging film did not have effect towards visual appearance, browning, colour, and leaf wilting of the respective products. Film types did affect the development of off-odour which is related to the headspace gas composition, however, visual sensory attributes were not affected (Piagentini & Güemes 2002). MaxF was found to be not vary significantly for spinach stored at different MAP conditions during storage (Tudela et al. 2013) and fresh-cut Romaine lettuce showed similar crispiness before and after storage (Martinez-Sanchez et al. 2011). The variations in the increment and decrement of the MaxF values were due to the variations of the spinach leaves. The raw data of both MaxF and MaxD were tabulated in Table 7.4 and Table 7.5.

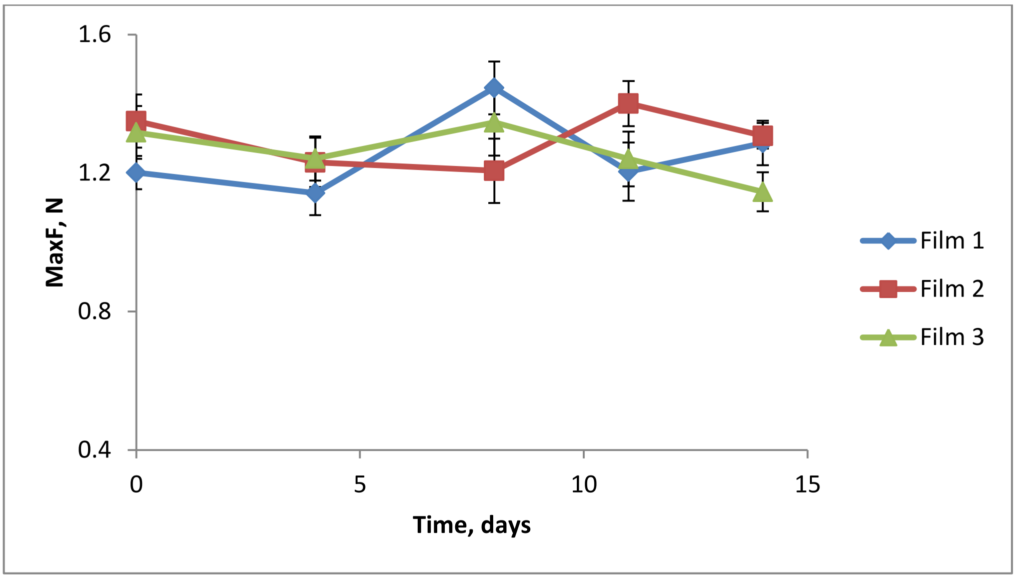


Figure 7.10: MaxF for Control spinach sample S_4 stored under Film 1, Film 2, and Film 3.

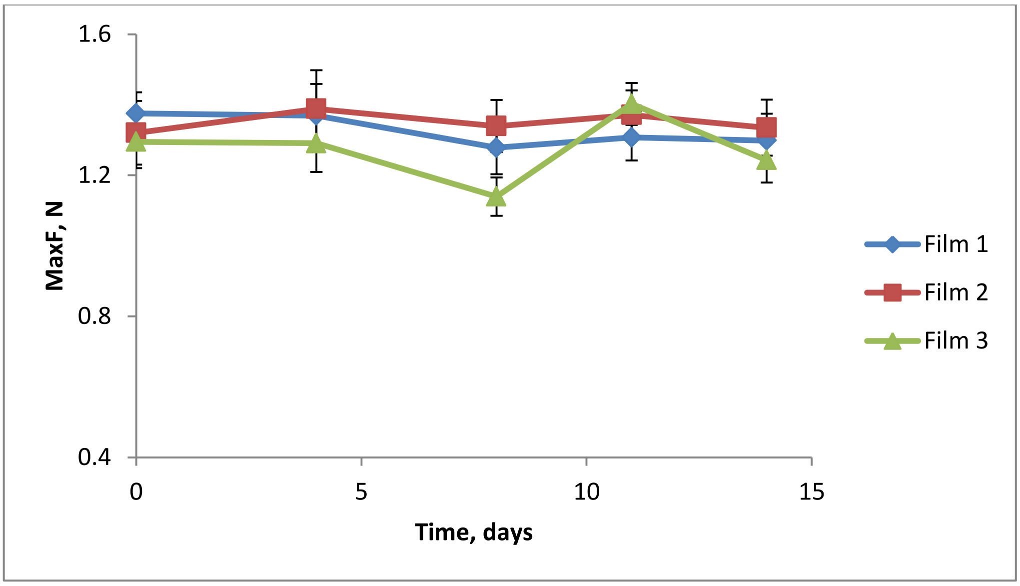


Figure 7.11: MaxF for 200N-compressed spinach sample S_4 stored under Film 1, Film 2, and Film 3.

Table 7.4: The average values of MaxF, N. The data represent the mean of 20 replicates ± standard deviation.

Films	Leaf category	Day 0	Day 4	Day 8	Day 11	Day 14
Film 1	Control	1.20 ± 0.21	1.14 ± 0.28	1.45 ± 0.34	1.2 ± 0.38	1.29 ± 0.29
	200N	1.37 ± 0.27	1.37 ± 0.40	1.28 ± 0.34	1.31 ± 0.29	1.30 ± 0.34
Film 2	Control	1.35 ± 0.34	1.23 ± 0.32	1.21 ± 0.41	1.40 ± 0.29	1.31 ± 0.16
	200N	1.32 ± 0.40	1.39 ± 0.49	1.34 ± 0.33	1.37 ± 0.31	1.33 ± 0.36
Film 3	Control	1.32 ± 0.34	1.24 ± 0.29	1.35 ± 0.43	1.24 ± 0.35	1.15 ± 0.25
	200N	1.29 ± 0.34	1.29 ± 0.36	1.14 ± 0.24	1.40 ± 0.27	1.24 ± 0.28

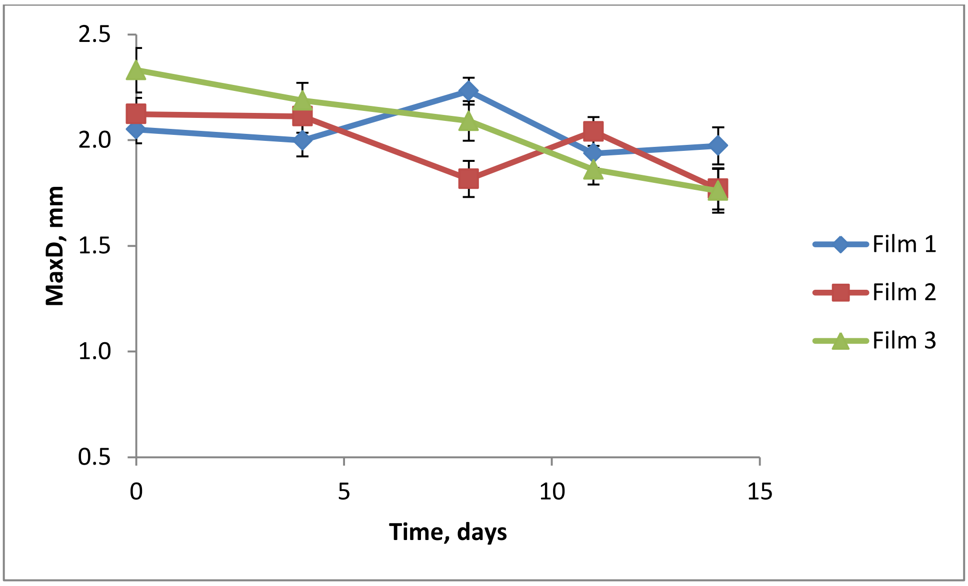


Figure 7.12: MaxD for Control spinach leaves stored under Film 1, Film 2, and Film 3.

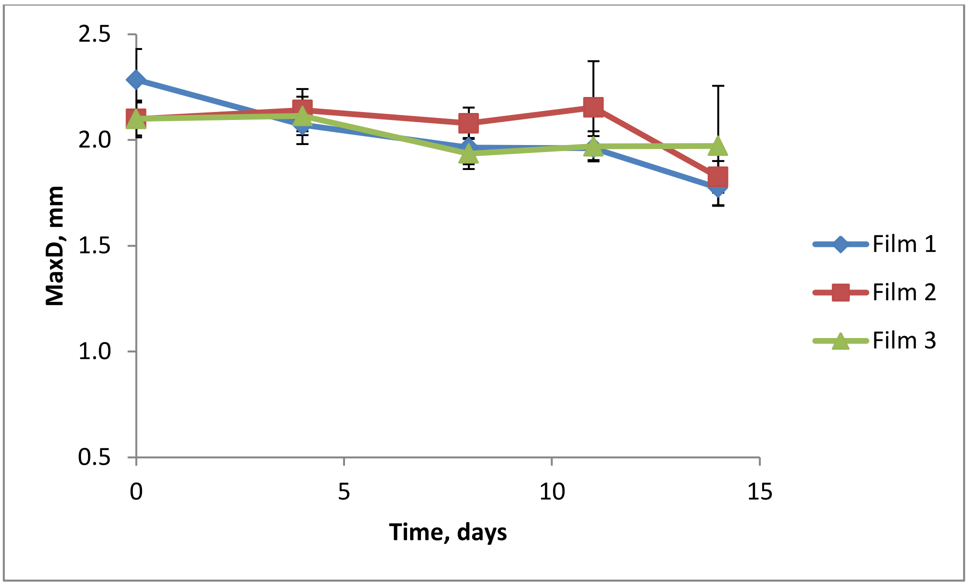


Figure 7.13: MaxD for 200N-compressed spinach leaves stored under Film 1, Film 2, and Film 3.

Table 7.5: The average values of MaxD, mm. The data represent the mean of 20 replicates ± standard deviation.

Films	Leaf category	Day 0	Day 4	Day 8	Day 11	Day 14
Film 1	**Control**	2.05 ± 0.30	2.00 ± 0.34	2.23 ± 0.29	1.94 ± 0.31	1.97 ± 0.39
	200N	2.28 ± 0.65	2.07 ± 0.41	1.96 ± 0.35	1.96 ± 0.26	1.77 ± 0.37
Film 2	**Control**	2.12 ± 0.34	2.11 ± 0.35	1.82 ± 0.38	2.04 ± 0.31	1.77 ± 0.44
	200N	2.1 ± 0.39	2.14 ± 0.45	2.08 ± 0.33	2.15 ± 0.98	1.82 ± 0.34
Film 3	**Control**	2.33 ± 0.47	2.19 ± 0.37	2.09 ± 0.42	1.86 ± 0.32	1.76 ± 0.47
	200N	2.1 ± 0.35	2.11 ± 0.41	1.94 ± 0.33	1.97 ± 0.32	1.97 ± 1.27

7.3.4 Developing colour analysis technique to measure colour changes of spinach

The L*, a*, b* values measured using colorimeter are shown in Table 7.6 and Table 7.7 for both Control leaves and 200N-compressed leaves respectively. Overall, as the storage day increased, the L* value which is reflecting lightness and b* value which is reflecting yellowness increased, and 200N-compressed leaves always showed higher L* and b* values compared to Control leaves. This showed that the damaged leaves undergone the yellowing process faster than the intact leaves. As for the a* value which represents the greenness, the values decreased as the storage day increased. The decreased in a* values was associated with the yellowing process of the leafy vegetables (Toivonen & Brummell 2008). There was non-uniform colour changes which could be associated to the increment of the enzymatic activity caused from tissue disruption at the cut surfaces, depending on the local substrate composition and the amount of enzyme that initiate the discolouration process (Lunadei et al. 2012). There were also inconsistent increase/decrease of L* and b* values due to either the leaves turned yellow or turned dark due to the deterioration. The modified atmosphere accumulate significant levels of volatiles within the headspace area (Kader et al. 1989) such as released of ammonia due to protein catabolism (Cantwell et al. 2010). The accumulation of ammonia induced dark deterioration of the leaves (Tudela et al. 2013). The changes of the L*, a*, b* values measured from the colorimeter were also compared to the L*, a*, b* values converted from the RGB images. The RGB values were converted to L*, a*, b* values using a coding in MATLAB and the values are tabulated in Table 7.8 and Table 7.9 for Control and 200N-compressed leaves respectively.

Table 7.6: L*, a*, b* values measured using colorimeter for Control spinach stored under Film 1, Film 2, and Film 3. The data represent the mean of 24 replicates ± standard deviation.

Control	FILM 1			FILM 2			FILM 3		
Days	**L**	**a**	**b**	**L**	**a**	**b**	**L**	**a**	**b**
0	41.18 ± 1.72	-15.58 ± 0.96	23.90 ± 1.85	40.50 ± 1.89	-15.37 ± 0.96	23.16 ± 2.10	41.30 ± 1.79	-16.06 ± 1.22	24.56 ± 2.24
1	40.68 ± 2.03	-15.54 ± 1.03	23.60 ± 2.04	39.74 ± 2.69	-15.35 ± 1.12	23.03 ± 2.62	39.91 ± 1.86	-15.79 ± 1.20	24.00 ± 2.24
4	40.77 ± 2.20	-16.03 ± 0.99	24.68 ± 2.04	39.86 ± 3.35	-15.85 ± 1.24	24.35 ± 3.30	39.91 ± 1.94	-16.29 ± 1.16	25.35 ± 2.41
8	42.34 ± 2.88	-17.08 ± 0.90	27.82 ± 2.90	41.42 ± 4.21	-16.85 ± 1.33	27.57 ± 4.30	40.80 ± 2.92	-17.12 ± 1.27	28.28 ± 3.32
11	44.97 ± 4.15	-17.95 ± 0.76	32.10 ± 4.55	43.50 ± 5.03	-17.48 ± 1.41	31.04 ± 5.39	42.46 ± 3.73	-17.70 ± 1.33	31.40 ± 4.15
14	47.14 ± 5.19	-18.20 ± 0.86	35.93 ± 5.68	45.07 ± 5.47	-17.89 ± 1.34	34.25 ± 5.89	43.44 ± 4.27	-17.64 ± 1.26	33.16 ± 4.55

Table 7.7: L*, a*, b* values measured using colorimeter for 200N-compressed spinach stored under Film 1, Film 2, and Film 3. The data represent the mean of 24 replicates ± standard deviation.

200N	FILM 1			FILM 2			FILM 3		
Days	**L**	**a**	**b**	**L**	**a**	**b**	**L**	**a**	**b**
0	41.42 ± 2.45	-15.38 ± 1.44	23.49 ± 2.97	41.52 ± 1.95	-15.43 ± 1.24	23.62 ± 2.42	41.85 ± 2.27	-15.65 ± 1.25	24.30 ± 2.71
1	41.29 ± 2.71	-15.46 ± 1.55	23.89 ± 3.47	41.10 ± 2.77	-15.37 ± 1.37	23.94 ± 3.26	40.65 ± 2.09	-15.38 ± 1.30	23.85 ± 2.87
4	41.95 ± 3.21	-16.09 ± 1.53	25.88 ± 4.20	42.46 ± 3.59	-16.21 ± 1.31	26.65 ± 4.20	41.01 ± 2.56	-15.90 ± 1.34	25.79 ± 3.70
8	43.99 ± 4.23	-17.13 ± 1.35	29.55 ± 4.90	44.56 ± 4.61	-17.03 ± 1.10	30.10 ± 4.65	42.01 ± 3.42	-16.54 ± 1.32	28.89 ± 4.36
11	47.29 ± 4.80	-17.73 ± 0.83	34.11 ± 5.11	46.86 ± 4.50	-17.47 ± 1.02	33.81 ± 4.34	43.90 ± 3.67	-16.78 ± 1.21	32.10 ± 4.53
14	49.32 ± 4.97	-17.62 ± 0.70	37.19 ± 4.75	48.31 ± 4.90	-16.91 ± 1.74	35.51 ± 4.43	45.24 ± 3.40	-16.17 ± 1.45	34.06 ± 4.21

Table 7.8: L*, a*, b* values converted from RGB values for Control spinach stored under Film 1, Film 2, and Film 3. The data represent the mean of 24 replicates ± standard deviation.

Control	FILM 1			FILM 2			FILM 3		
Days	**L**	**a**	**b**	**L**	**a**	**b**	**L**	**a**	**b**
1	40.39 ± 5.66	-20.67 ± 2.27	21.00 ± 2.62	40.49 ± 3.60	-21.13 ± 2.05	21.21 ± 2.60	40.37 ± 4.28	-21.03 ± 2.00	21.23 ± 2.56
4	38.90 ± 4.48	-20.34 ± 1.65	21.09 ± 2.27	41.04 ± 6.07	-21.19 ± 1.67	21.61 ± 2.62	39.52 ± 5.68	-20.44 ± 1.98	20.93 ± 2.50
8	41.82 ± 6.02	-20.94 ± 1.96	22.40 ± 2.78	42.02 ± 6.58	-21.14 ± 1.96	22.42 ± 3.18	40.05 ± 7.97	-19.50 ± 1.87	20.77 ± 2.67
11	43.74 ± 3.88	-23.05 ± 1.67	25.61 ± 3.12	43.07 ± 3.78	-22.62 ± 1.85	24.47 ± 3.39	38.03 ± 4.02	-19.84 ± 2.09	21.09 ± 3.00
14	44.77 ± 4.84	-22.92 ± 1.50	26.76 ± 3.34	43.49 ± 3.79	-22.20 ± 2.07	25.00 ± 4.01	42.42 ± 7.55	-20.87 ± 1.89	22.81 ± 3.16

Table 7.9: L*, a*, b* values converted from RGB values for 200N-compressed spinach stored under Film 1, Film 2, and Film 3. The data represent the mean of 24 replicates ± standard deviation.

200N	FILM 1			FILM 2			FILM 3		
Days	**L**	**a**	**b**	**L**	**a**	**b**	**L**	**a**	**b**
1	42.45 ± 3.60	-21.89 ± 1.76	22.69 ± 2.97	42.17 ± 4.62	-21.60 ± 1.65	22.37 ± 2.44	39.75 ± 4.74	-20.08 ± 2.00	20.57 ± 2.92
4	41.90 ± 5.71	-21.40 ± 2.18	22.77 ± 3.28	42.73 ± 5.50	-21.94 ± 2.02	23.54 ± 3.46	39.24 ± 5.06	-19.91 ± 1.92	20.83 ± 3.14
8	43.76 ± 6.25	-21.94 ± 1.66	24.49 ± 3.46	44.05 ± 6.40	-21.84 ± 1.79	24.51 ± 3.87	40.18 ± 8.86	-18.81 ± 2.19	20.53 ± 3.82
11	45.23 ± 4.12	-23.41 ± 1.80	27.40 ± 3.86	45.45 ± 4.77	-22.92 ± 1.75	26.50 ± 4.06	38.86 ± 4.33	-19.44 ± 1.60	21.41 ± 3.18
14	44.84 ± 7.12	-21.34 ± 1.29	26.22 ± 2.97	45.44 ± 6.68	-22.28 ± 1.58	27.20 ± 4.37	40.81 ± 6.27	-19.55 ± 1.45	22.43 ± 3.13

The L*, a*, b* values from the RGB were also converted to the L*, a*, b* values respective to the original values from colorimeter by using linear models obtained from the calibrated colour curves (Figure 7.14).

Using linear model,

$$f(x) = p1\,x + p2 \tag{19}$$

with 95% confidence bounds, the values for the parameters are tabulated in Table 7.10.

Table 7.10: Parameters values for the calibrated colour curves of L*, a*, and b*.

Colour value		p1	p2	R^2
L*	X < 93	0.78	-6.51	0.86
	X > 93	2.70	-181.20	0.40
a*		0.91	14.13	0.84
b*		0.92	-6.31	0.85

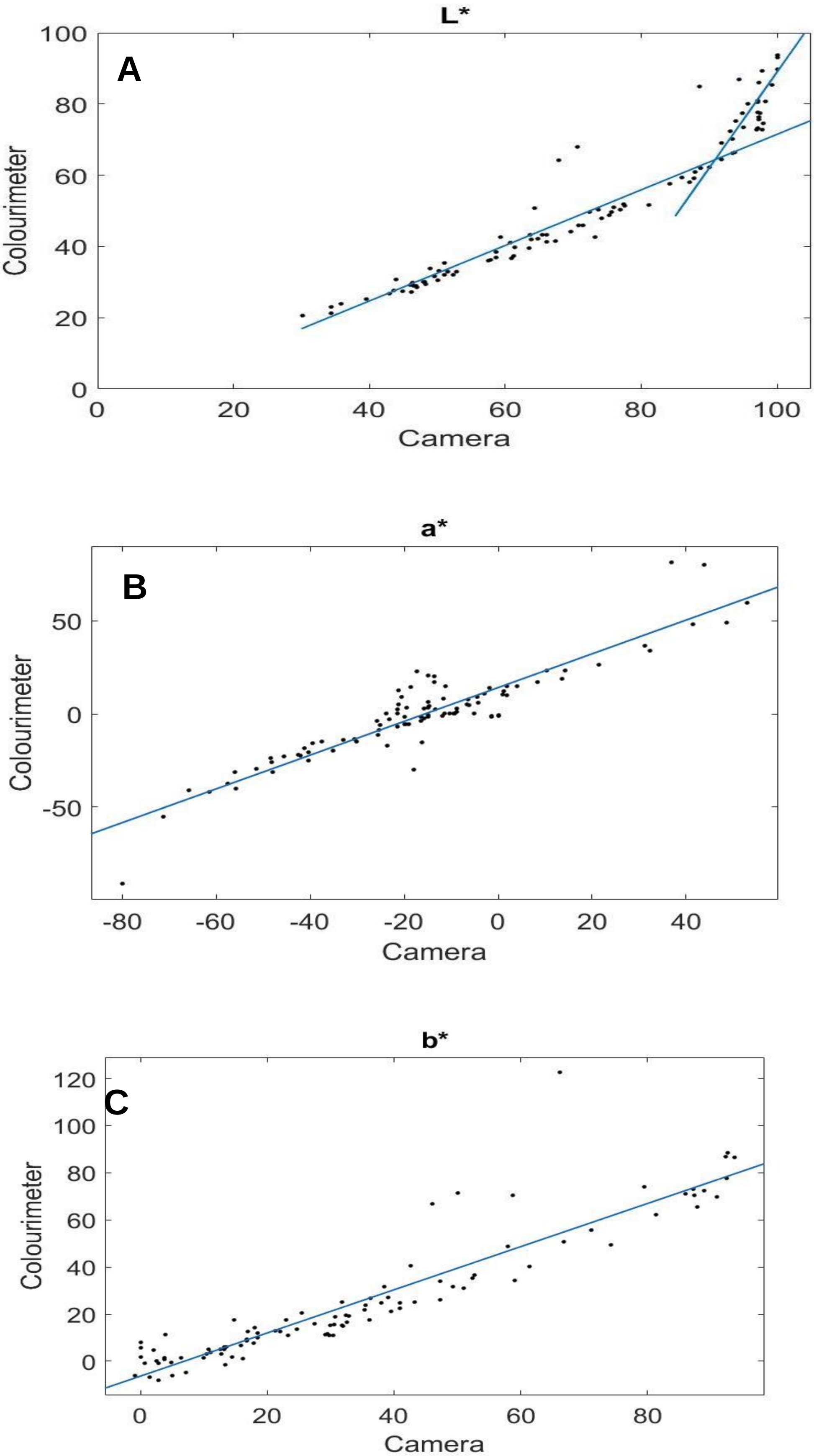


Figure 7.14: Calibration colour curves for A) L*, B) a*, C) b* for values from colorimeter against camera.

The converted Lab values to the respective colorimeter values presented in Table 7.11 and Table 7.12 have around 1.5 times lower than the original colorimeter values. Overall, majority of the values showed similar trend as values measured using colorimeter. However, there were quite lots of inconsistencies as well as the RGB images hardly differentiate the Control and damaged leaves (Table 7.13 and Figure 7.15). This possibly due to the error that came from the colour charts where the Lab values for the calibrated colour curves were derived from. The colour charts used was made of semi-matte material which may affect the reflection of lights when images were captured using the digital camera.

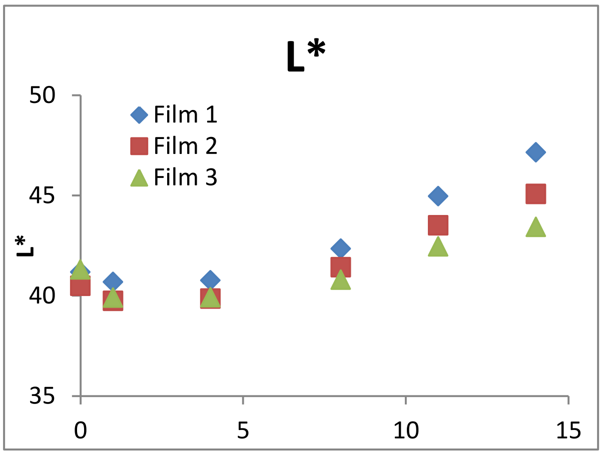
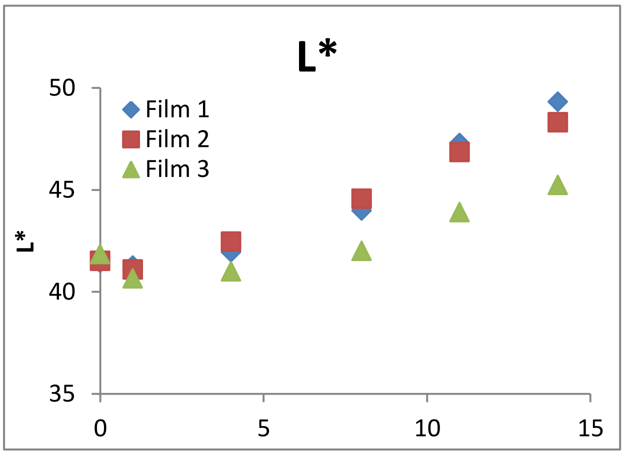
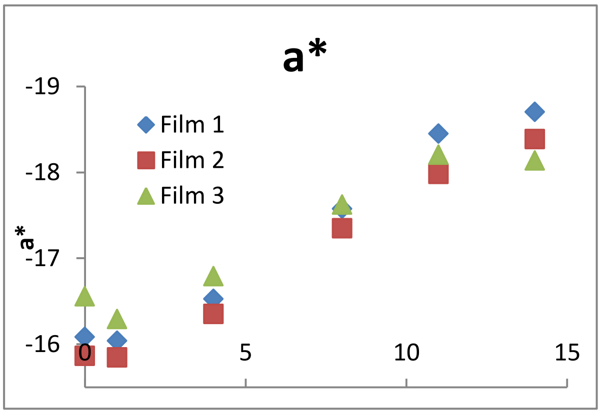
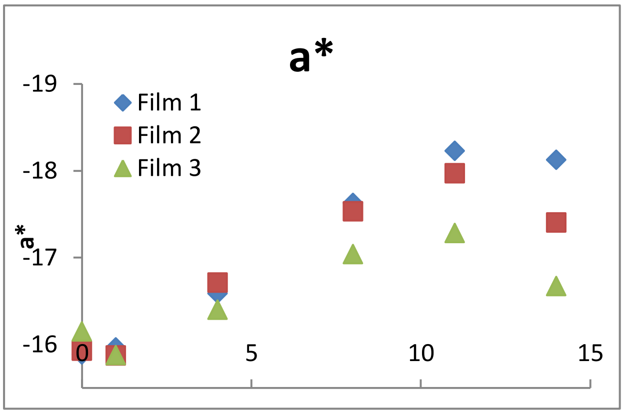
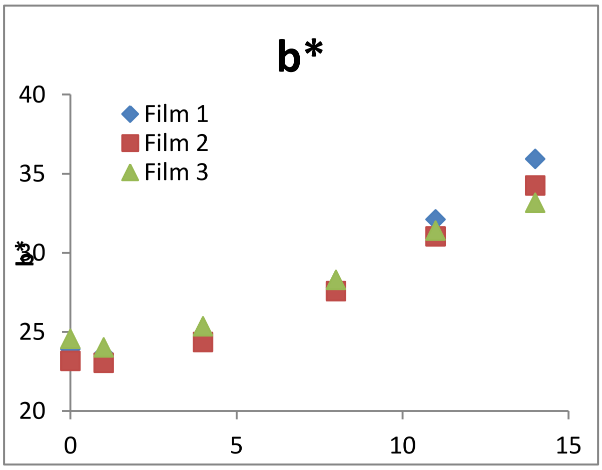
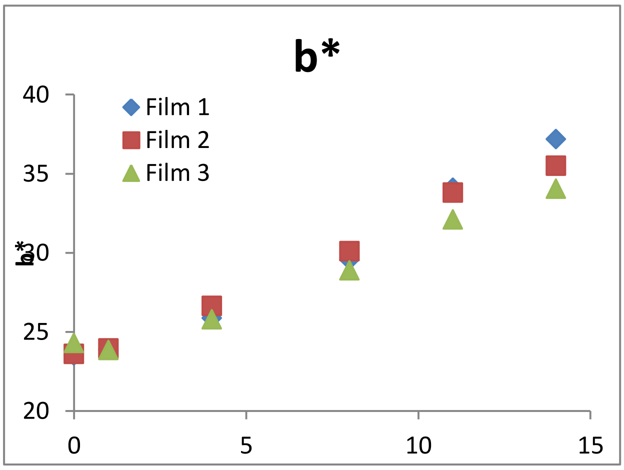
Table 7.11: L*, a*, b* values from RGB values and converted to the respective colorimeter values using calibration curves for Control spinach stored under Film 1, Film 2, and Film 3. The data represent the mean of 24 replicates ± standard deviation.

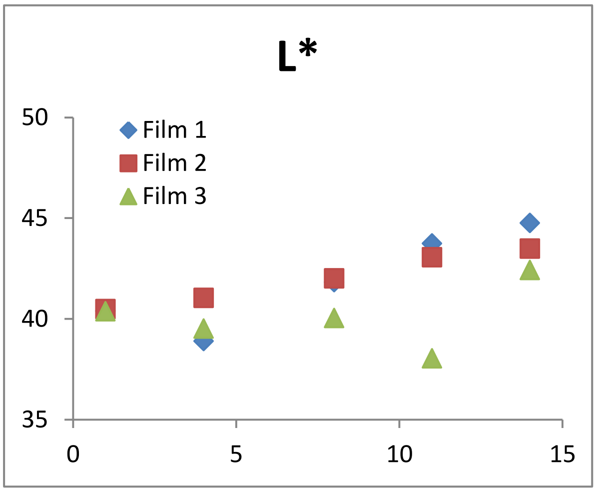
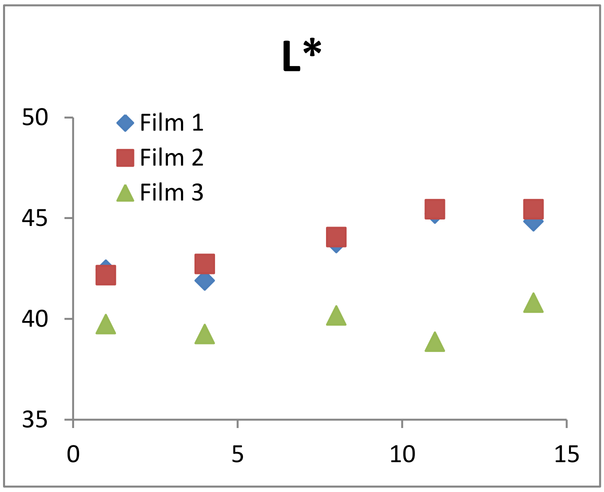
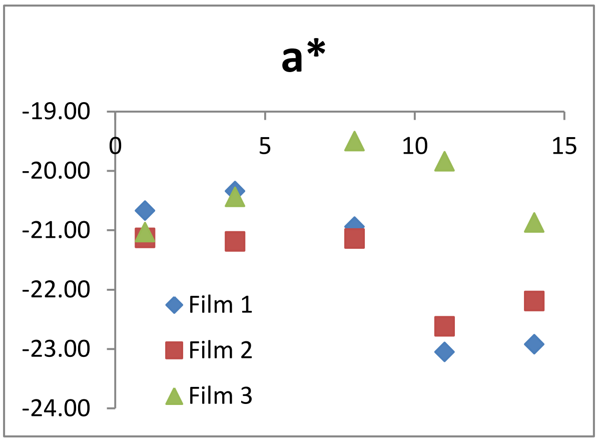
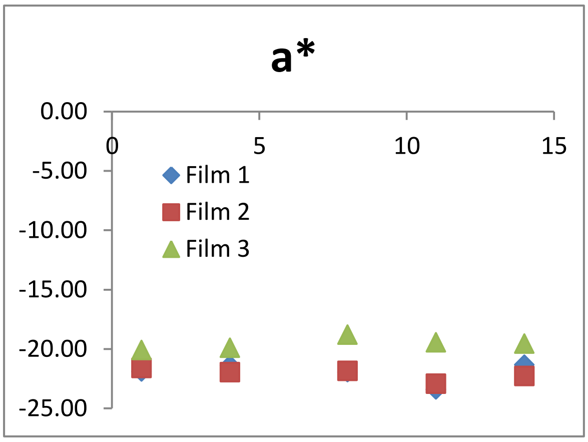
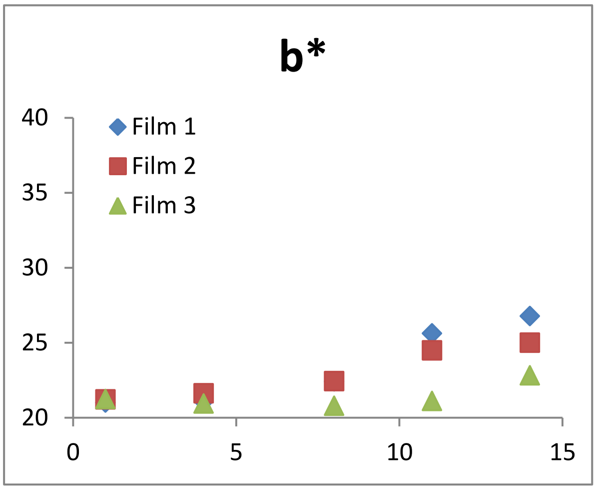
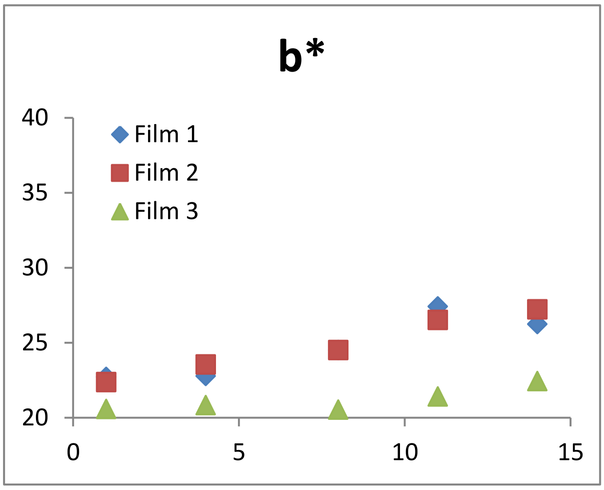
Control	FILM 1			FILM 2			FILM 3		
Days	L	a	b	L	a	b	L	a	b
1	24.84 ± 4.40	-4.59 ± 2.06	12.92 ± 2.40	24.91 ± 2.79	-5.00 ± 1.86	13.11 ± 2.38	24.82 ± 3.32	-4.92 ± 1.81	13.14 ± 2.35
4	23.68 ± 3.48	-4.29 ± 1.49	13.00 ± 2.08	25.34 ± 4.71	-5.06 ± 1.51	13.48 ± 2.39	24.15 ± 4.41	-4.38 ± 1.80	12.86 ± 2.29
8	25.95 ± 4.67	-4.83 ± 1.77	14.20 ± 2.54	26.10 ± 5.11	-5.02 ± 1.77	14.23 ± 2.91	24.57 ± 6.18	-3.53 ± 1.69	12.71 ± 2.45
11	27.43 ± 3.01	-6.74 ± 1.51	17.15 ± 2.86	26.91 ± 2.93	-6.35 ± 1.68	16.10 ± 3.10	23.01 ± 3.12	-3.84 ± 1.89	13.01 ± 2.75
14	28.23 ± 3.76	-6.63 ± 1.36	18.19 ± 3.06	27.24 ± 2.94	-5.97 ± 1.87	16.58 ± 3.67	26.41 ± 5.86	-4.77 ± 1.71	14.58 ± 2.89

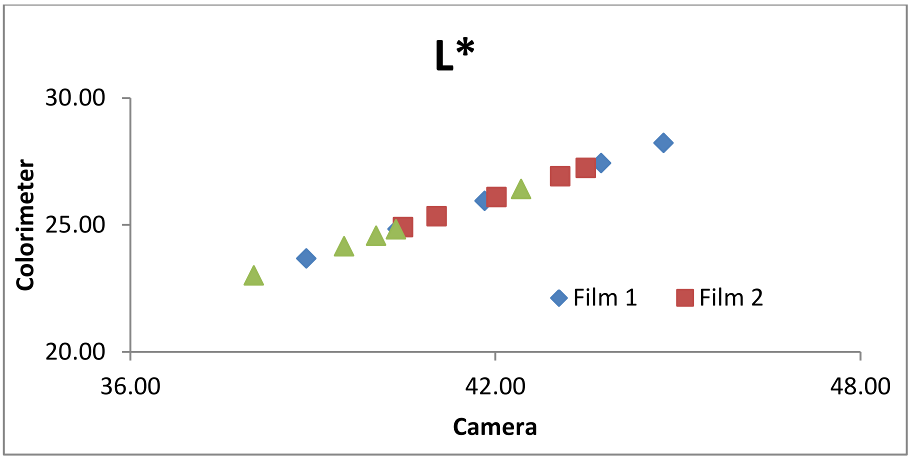
Table 7.12: L*, a*, b* values from RGB values and converted to the respective colorimeter values using calibration curves for 200N-compressed spinach stored under Film 1, Film 2, and Film 3. The data represent the mean of 24 replicates ± standard deviation.

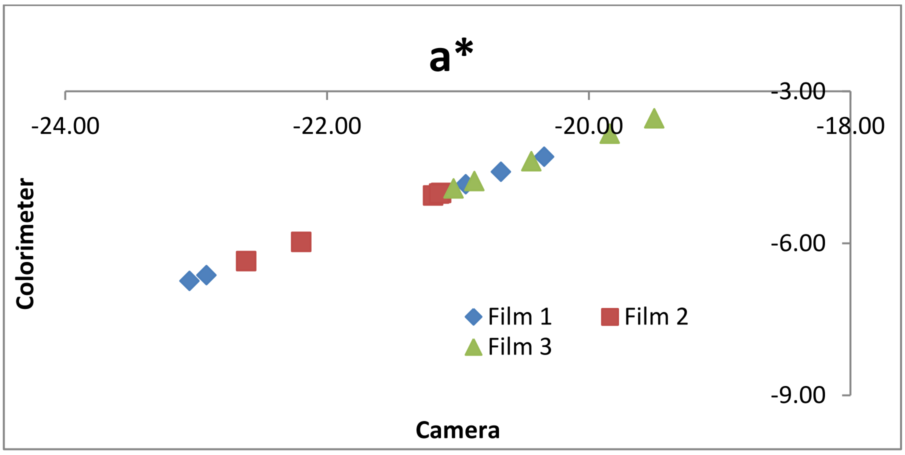
200N	FILM 1			FILM 2			FILM 3		
Days	L	a	b	L	a	b	L	a	b
1	26.43 ± 2.79	-5.70 ± 1.59	14.47 ± 2.72	26.22 ± 3.59	-5.43 ± 1.49	14.18 ± 2.23	24.34 ± 3.67	-4.06 ± 1.81	12.53 ± 2.68
4	26.00 ± 4.43	-5.25 ± 1.98	14.55 ± 3.01	26.65 ± 4.27	-5.74 ± 1.83	15.24 ± 3.17	23.94 ± 3.93	-3.90 ± 1.74	12.76 ± 2.88
8	27.45 ± 4.85	-5.74 ± 1.51	16.12 ± 3.17	27.68 ± 4.96	-5.65 ± 1.62	16.13 ± 3.55	24.67 ± 6.88	-2.90 ± 1.98	12.49 ± 3.50
11	28.59 ± 3.20	-7.07 ± 1.63	18.78 ± 3.54	28.76 ± 3.70	-6.63 ± 1.58	17.96 ± 3.72	23.65 ± 3.36	-3.47 ± 1.45	13.30 ± 2.91
14	28.29 ± 5.53	-5.19 ± 1.17	17.70 ± 2.72	28.75 ± 5.18	-6.05 ± 1.43	18.60 ± 4.00	25.16 ± 4.86	-3.57 ± 1.31	14.23 ± 2.87

Table 7.13: Plots of L*, a*, and b* values measured using A) colorimeter, and B) RGB converted to L*, a*, b* for both Control and 200N-compressed spinach.

A Colorimeter Control	Colorimeter 200N
	
	
	

B	Digital Control	Digital 200N
		
		
		





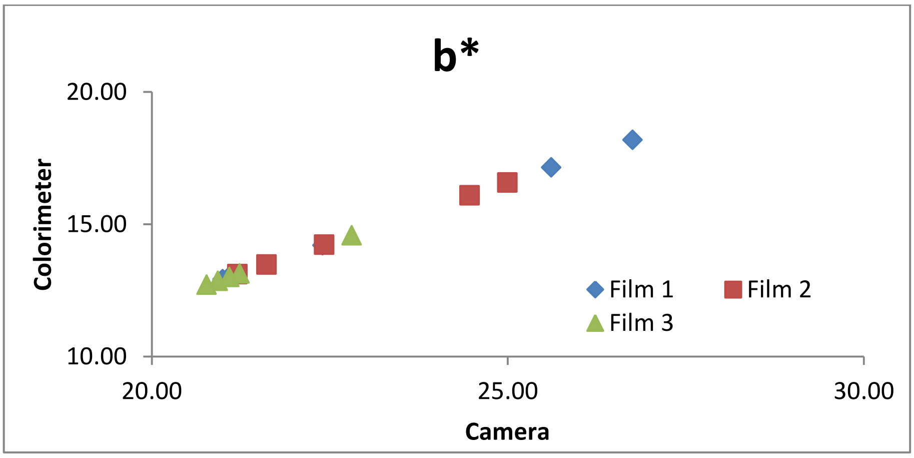


Figure 7.15: Plots of Lab values measured using both colorimeter and conversion of RGB images.

7.4 Conclusion

Mechanically damaged spinach leaves have higher respiration rates compared to intact leaves; which accelerate quality degradations and shelf life reduction of the product. The OTR gives effect towards the quality attributes of the spinach. The OTR significantly affected the package atmosphere where the similar film material with lower yet appropriate OTR maintained longer shelf-life and higher product quality during storage (Pan & Sasanatayart 2016; Kim et al. 2004a). The OTR significantly affected the sensory quality of baby spinach (Allende et al. 2004). However, this effect may be not applicable to the mechanically damaged leaves, which showed similar responses when treated under different packaging films. Surface dehydration, loss of firmness, and loss of green colour to yellowing are major quality losses which lead to shelf life reduction of the fresh produce. It is beneficial for the fresh produce industry to have the information regarding the effect of the mechanical damage towards the respiration rates of the fresh products thus any accidental damage towards the products could be minimised during processing, handling and distributing. This help to improve the quality and shelf life of the fresh product throughout storage.

For the colour analysis technique, there seems to be ‘inaccuracy’ of the calibration curves as the L*, a*, b* values reported after using the conversion model equations showed 1.5 times lower than the original L*, a*, b* values from the colorimeter. This may be due to the effect of light reflection while taking the RGB digital image. Although the process took place in a control box with controlled light, as the colour charts used in this study were semi-matte, the surfaces of the colour charts were partly glossy thus caused the light reflection. This reduced the quality of the RGB images and affected the L*, a*, b* values of the images. Improvement could be made in the future to use specifically designed matte tiles with several colours as the colour charts for the calibration of the digital colour system. Those specific tiles take some time to be made and the price is quite costly which these two reasons made me took the alternative to use the semi-matte colour charts. This image analysis technique would be really beneficial in the food industries as they could apply the technique to any product especially those dealing with irregular shape and size of food samples while at the same time do not have to worry about unfit aperture or lens size for the food product as what usually experienced by colorimeter.

CHAPTER 8

CONCLUSIONS AND FUTURE WORK

The compression method was successfully implemented to study the effect of processing towards quality and shelf life of the RTE leafy vegetables. Findings from the works have sufficiently met the objectives of the project.

The types of spinach gave effects toward the mechanical properties and qualities of the spinach. Organic spinach was found to be the best in resisting stress and damage compared to Teen, Salad and Baby spinach due to its morphology. Physical appearance was found to be effective in representing damage that occurred on the leaves. However, microstructure hardly tells the damage occurred within the leaves. This was due to the compression of bulk spinach where for each compression, not all the leaves got breakage. The compression caused cell bursting and released its contents which caused the blurry images found under the microscope. Appropriate weight of spinach inside the packaging bag reduced the possibility of the leaves inside the bag to be compressed due to crowdedness inside the bag thus lowered the risk for accidental mechanical leaf damage. There was not significant different between texture of spinach before and after storage, however, the texture was somewhat found to be different between different varieties.

The population test method developed in this study has successfully able to distinguish different degrees of leaf injuries and their effects towards quality and shelf life of the RTE spinach during postharvest storage. Force 200N was suggested to be the minimum required to pick up differences of the leaves deteriorations before and after storage. Uncompressed spinach leaves were found to have a shelf life of 14 days. Higher degrees of leaf injuries which are Halfway teared and Complete teared showed obvious decay since before storage, whereas Minor teared leaves started to show decay after eight days of storage. This method is practical and could be applicable to study the injuries experienced by other fruits and leafy vegetables.

Sensorial data including evaluations and perceptions from assessors helped in contributing to the knowledge of quality and shelf life of the RTE spinach as a whole. The degree of leaf injury and storage day significantly affect the visibility of decays and acceptance rate in buying the product. This shows that the higher the degree of leaf injury, the lower the shelf life of the fresh product thus the lower the acceptance rate in buying the product. The longer the storage day, the clearer the visibility of decays thus the lower the acceptance rate in buying the product. Main factors affecting assessor’s decision in buying the product include quality of the spinach inside the bag and ambiance inside the bag. Assessors defined damaged leaves as leaves that were teared, yellowish and brownish, wet, wilted, got white spots and possible insect bites. As expected, any Complete teared leaves or visible crack marks were totally unacceptable. In some cases, damaged leaves were still acceptable if the product is at reduced price and it also depends on the time people plan to consume them. Sometimes, the broken leaves were tolerable as long as the leaves do not change colour and do not show sign of mushiness. Spinach bags that contained high moisture caused most consumers unlikely to buy the product.

Mechanically damaged spinach leaves was found to have higher respiration rates compared to intact leaves; which accelerate quality degradations and shelf life reduction of the product. The OTR gives effect towards the quality attributes of the spinach. However, this effect may be not applicable to the mechanically damaged leaves, which showed similar responses when treated under different packaging films. The respiration rate data in this study sufficiently fit the Michaelis-Menten and exponential models.

Image analysis technique would be really beneficial in the food industries as they could apply the technique to any product especially those dealing with irregular shape and size of food samples while at the same time do not have to worry about unfit aperture or lens size for the food product as what usually experienced by colorimeter. However, results found in this study showed some ‘inaccuracy’ of the calibration curves as the L*, a*, b* values reported after using the conversion model equations showed 1.5 times lower than the original L*, a*, b* values from the colorimeter. Although the process took place in a controlled-light environment, because of the semi-glossy surface due to semi-mate property, there was light reflection which affected the quality of the RGB images.

Data from studies conducted in this thesis could provide basis to both fresh produce industry and retailer to have understanding on the effect of processing, which in this case focused on compression, towards the quality and shelf life of the RTE leafy vegetables. Precaution and optimisation could be made during processing and handling to prevent and minimise any possible compression thus improve the quality and shelf life of the product.

RESEARCH IMPROVEMENTS

If the research were to start again, few changes could be made for improvement of overall responses from the experiment. First improvement would be to plant the spinach in a controlled environment rather than bought the spinach from the supermarket. This will minimise the variation of the leaves by having standard and fixed treatment during plantation, harvest, processing, and storage.

Another improvement that could be made is to use specifically designed matte tiles with several colours as the colour charts for the calibration of the digital colour system. The full matte tiles could prevent any reflection of light during image capture thus minimise any technical error that could happen during the image acquisition process.

Studying the response of the spinach leaves harvested at different season would also be a beneficial addition to the quality and shelf life of fresh leafy vegetables area. During winter for example, the ambiance is more wet and cold, whereas during summer, the ambiance is dry and hot. These two differences in humidity and temperature could affect the quality and shelf life of the spinach leaves and their responses are something worth to look at.

Apart from that, a model equation of deterioration due to mechanical damage experienced by packed spinach could also be built. By having a proper ratio of work in the lab to the real work in the industry, the industry can straight away use the model equation to predict the total decay of the leaves in a bag thus precautions could be made during handling and overall loss could be minimised.

FUTURE WORKS

Based on the conclusions developed from this thesis, it is recommended to further the study from the effect of processing and handling to the study of the effect of postharvest storage treatment. The suggested postharvest treatment is light treatment. This is because, during postharvest storage, fruits and vegetables are exposed to changes of light intensities. The light exposure stimulated the opening of stomata (Figure 8.1). Previously, we have work on the effect of light treatment towards qualities of spinach leaves. However, during this study, it was quite challenging to control the light treatment while at the same time maintaining the storage temperature. Since the control box was quite big, it was a hassle to get the sharing space in the cold room shared by other researchers. People walking in and out of the cold room also affected the temperature inside the cold room. Further improvement regarding the set-up of the system should be made in the future.

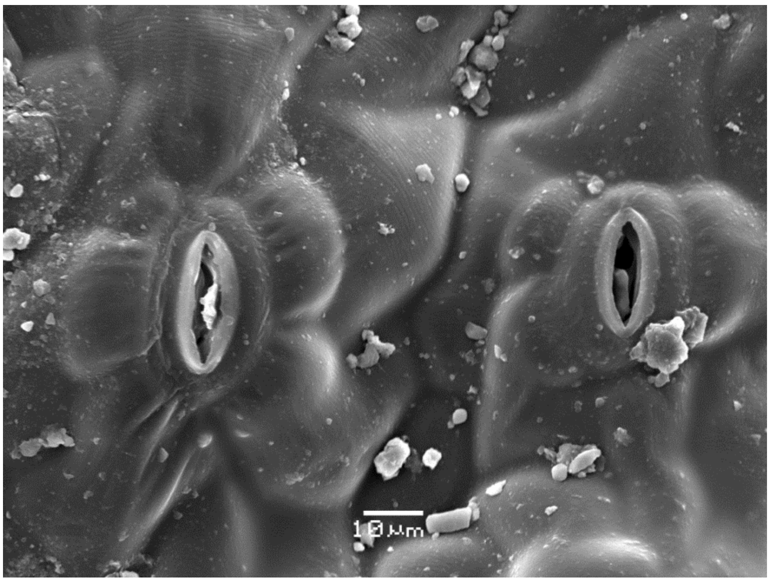


Figure 8.1: Stomata opening of spinach leaves under light treatment. The image was observed using SEM.

Light intensities may bring both advantage and disadvantage to the fresh product. Glowacz et al. (2015) reported that high light exposure resulted in oxidative stress causing tissue damage; however, low light gave more promising results as it improved the nutritional value of spinach while maintaining its texture. Lester et al. (2010) also reported that spinach leaves exposed to continuous light of simulated retailer were overall more nutritionally dense than leaves stored in continuous darkness. Further studies should be conducted to identify the threshold of light intensity that could improve the nutritional value while at the same time does not compromise the quality of the spinach thus recommendations can be made to the supply chain and retailers.

Part of this work has been published in:

Ariffin, S.H., Gkatzionis, K. & Bakalis, S., 2017. Leaf injury and its effect towards shelf-life and quality of ready-to-eat (RTE) spinach. Energy Procedia, 123, pp.105–112. Available at: http://dx.doi.org/10.1016/j.egypro.2017.07.265.

APPENDIX

A.1 SPSS results from ANOVA for textural differences of spinach throughout storage

Baby spinach

ONE WAY ANOVA

Burst Strength

- No significant difference throughout storage days for every groups
- No significant difference between group Control and group 100N, 150N, and 200N

2 WAY ANOVA

Multiple comparisons between storage days (P = 0.007 < 0.05)

- Overall results show that there is AT LEAST ONE significant difference throughout the storage days
  - There is AT LEAST ONE significant difference between Day 0 and Day 8 & Day 12

Multiple comparisons between treatment (P = 0.404 > 0.05)

- No significant difference for all comparisons at all

Table A.1.1: 2 Way ANOVA- Overall comparisons for Average MaxForce (Burst Strength) between groups and storage days

Tests of Between-Subjects Effects

Dependent Variable: MaxForce

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.808[a]	11	.073	1.755	.071
Intercept	128.278	1	128.278	3064.000	.000
StorageDays	.439	2	.220	5.247	.007
Treatment	.123	3	.041	.983	.404
StorageDays * Treatment	.245	6	.041	.977	.445
Error	4.522	108	.042		
Total	133.608	120			
Corrected Total	5.330	119			

a. R Squared = .152 (Adjusted R Squared = .065)

ONE WAY ANOVA BETWEEN BABY AND YOUNG SPINACH

Burst Strength

- There is significant difference between Baby 100N with Young 100N & Young 200N at Day 0
- There is significant difference between Baby 150N with Young 100N & Young 200N at Day 0
- There is significant difference between Baby 150N with Young Control Day 12
- There is significant difference between Baby 200N with Young 100N and Young 200N at Day 0

Young spinach

ONE WAY ANOVA

Burst Strength

- No significant difference throughout storage days for every groups
- No significant difference between group Control and group 100N, 150N, and 200N

2 WAYS ANOVA

Multiple comparisons between groups (P = 0.577 > 0.05)

- No significant difference for all comparisons at all

Multiple comparisons between days (P = 0.829 > 0.05)

- No significant difference for all comparisons at all

Table A.1.2: 2 Way ANOVA- Overall comparisons for Average MaxForce (Burst Strength) between groups and storage days

Tests of Between-Subjects Effects

Dependent Variable: MaxForce

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.355[a]	11	.032	.679	.756
Intercept	189.908	1	189.908	3994.412	.000
Treatment	.094	3	.031	.662	.577
StorageDays	.018	2	.009	.188	.829
Treatment * StorageDays	.243	6	.040	.850	.534
Error	5.135	108	.048		
Total	195.397	120			
Corrected Total	5.490	119			

a. R Squared = .065 (Adjusted R Squared = -.031)

A.2 Raw data of textural changes of spinach leaves throughout storage

Baby spinach

Table A.2.1: Values of MaxF for 10 Replicates of Baby spinach for Control, 100N, 150N, and 200N Compressed leaves at Day 0.

Day	Control		Day	100N		Day	150N		Day	200N	
	Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)
0	1	0.94	0	1	0.87	0	1	0.63	0	1	0.86
	2	1.18		2	0.65		2	0.89		2	1.01
	3	1.26		3	1.02		3	1.21		3	0.94
	4	0.87		4	1.06		4	0.89		4	0.86
	5	1.44		5	1.02		5	0.88		5	0.70
	6	0.82		6	0.85		6	0.83		6	0.82
	7	0.83		7	0.91		7	0.91		7	1.08
	8	1.02		8	0.78		8	0.91		8	0.75
	9	1.31		9	0.74		9	0.99		9	1.09
	10	0.96		10	1.11		10	1.19		10	0.88
TrimMean(10)		**1.05**	**TrimMean(10)**		**0.90**	**TrimMean(10)**		**0.94**	**TrimMean(10)**		**0.90**
AverageMean		**1.06**	**AverageMean**		**0.90**	**AverageMean**		**0.93**	**AverageMean**		**0.90**
Std Dev		**0.22**	**Std Dev**		**0.15**	**Std Dev**		**0.17**	**Std Dev**		**0.13**
Std error		**0.07**	**Std error**		**0.05**	**Std error**		**0.05**	**Std error**		**0.04**

Table A.2.2: Values of MaxF for 10 Replicates of Baby spinach for Control, 100N, 150N, and 200N Compressed leaves at Day 8.

Day	Control		Day	100N		Day	150N		Day	200N	
	Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)
8	1	0.97	8	1	1.20	8	1	1.06	8	1	1.24
	2	1.10		2	0.78		2	1.01		2	1.32
	3	0.92		3	1.28		3	0.71		3	0.92
	4	1.11		4	1.24		4	0.86		4	1.10
	5	0.87		5	0.75		5	0.81		5	1.25
	6	1.17		6	0.91		6	1.53		6	1.14
	7	1.01		7	1.13		7	1.07		7	1.06
	8	1.13		8	1.25		8	1.39		8	1.12
	9	0.91		9	0.88		9	1.08		9	0.90
	10	1.01		10	1.14		10	1.07		10	1.29
TrimMean(10)		1.02	**TrimMean(10)**		1.07	**TrimMean(10)**		1.04	**TrimMean(10)**		1.14
AverageMean		1.02	**AverageMean**		1.05	**AverageMean**		1.06	**AverageMean**		1.13
Std Dev		**0.10**	**Std Dev**		**0.21**	**Std Dev**		**0.25**	**Std Dev**		**0.15**
Std error		**0.03**	**Std error**		**0.06**	**Std error**		**0.08**	**Std error**		**0.05**

Table A.2.3: Values of MaxF for 10 Replicates of Baby spinach for Control, 100N, 150N, and 200N Compressed leaves at Day 12.

Day	Control		Day	100N		Day	150N		Day	200N	
	Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)
12	1	1.03	12	1	0.74	12	1	1.02	12	1	1.20
	2	1.46		2	0.84		2	1.07		2	1.17
	3	1.30		3	0.99		3	1.15		3	1.21
	4	0.79		4	1.45		4	1.03		4	0.79
	5	1.94		5	0.89		5	1.00		5	1.23
	6	1.49		6	1.62		6	1.14		6	1.10
	7	1.10		7	1.06		7	0.90		7	0.81
	8	0.97		8	0.94		8	1.08		8	1.02
	9	0.86		9	1.25		9	1.11		9	0.94
	10	0.85		10	0.86		10	0.91		10	1.11
TrimMean(10)		1.13	**TrimMean(10)**		1.04	**TrimMean(10)**		1.05	**TrimMean(10)**		1.07
AverageMean		1.18	**AverageMean**		1.06	**AverageMean**		1.04	**AverageMean**		1.06
Std Dev		**0.37**	**Std Dev**		**0.29**	**Std Dev**		**0.09**	**Std Dev**		**0.16**
Std error		**0.12**	**Std error**		**0.09**	**Std error**		**0.03**	**Std error**		**0.05**

Young's spinach

Table A.2.4: Values of MaxF for 10 Replicates of Young spinach for Control, 100N, 150N, and 200N Compressed leaves at Day 0.

Day	Control		Day	100N		Day	150N		Day	200N	
	Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)
0	1	1.00	0	1	1.31	0	1	1.01	0	1	1.53
	2	1.03		2	1.23		2	1.19		2	1.25
	3	1.18		3	1.52		3	1.24		3	1.15
	4	1.47		4	1.29		4	1.11		4	0.96
	5	1.68		5	1.21		5	1.34		5	1.33
	6	1.21		6	1.25		6	1.06		6	1.17
	7	1.06		7	0.85		7	1.22		7	1.01
	8	1.22		8	1.29		8	1.25		8	1.84
	9	1.32		9	1.51		9	1.26		9	1.61
	10	1.13		10	1.44		10	1.04		10	1.07
TrimMean(10)		**1.20**	**TrimMean(10)**		**1.32**	**TrimMean(10)**		**1.19**	**TrimMean(10)**		**1.26**
AverageMean		**1.23**	**AverageMean**		**1.29**	**AverageMean**		**1.19**	**AverageMean**		**1.29**
Std Dev		**0.21**	**Std Dev**		**0.19**	**Std Dev**		**0.11**	**Std Dev**		**0.29**
Std error		**0.07**	**Std error**		**0.06**	**Std error**		**0.03**	**Std error**		**0.09**

Table A.2.5: Values of MaxF for 10 Replicates of Young spinach for Control, 100N, 150N, and 200N Compressed leaves at Day 8.

Day	Control		Day	100N		Day	150N		Day	200N	
	Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)
8	1	1.30	8	1	1.05	8	1	0.91	8	1	0.97
	2	1.28		2	1.59		2	1.43		2	1.21
	3	1.02		3	1.02		3	1.07		3	1.57
	4	1.32		4	1.23		4	1.72		4	1.39
	5	0.93		5	1.04		5	1.11		5	1.57
	6	1.39		6	1.45		6	1.37		6	0.99
	7	1.35		7	1.01		7	1.48		7	1.16
	8	1.02		8	1.13		8	1.30		8	1.13
	9	1.33		9	1.56		9	1.28		9	1.62
	10	1.50		10	1.38		10	0.88		10	1.07
TrimMean(10)		1.25	**TrimMean(10)**		1.23	**TrimMean(10)**		1.24	**TrimMean(10)**		1.26
AverageMean		1.24	**AverageMean**		1.25	**AverageMean**		1.25	**AverageMean**		1.27
Std Dev		**0.19**	**Std Dev**		**0.23**	**Std Dev**		**0.26**	**Std Dev**		**0.25**
Std error		**0.06**	**Std error**		**0.07**	**Std error**		**0.08**	**Std error**		**0.08**

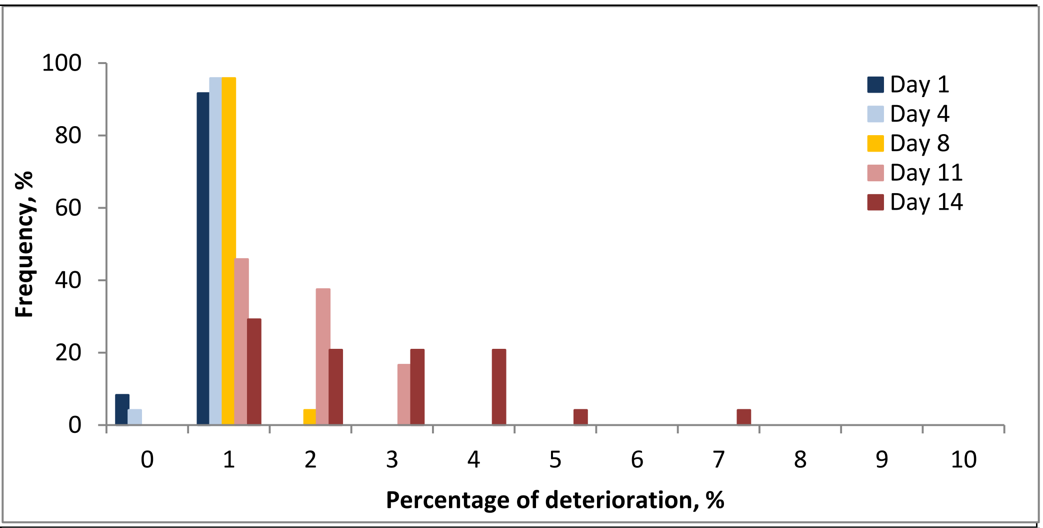
Table A.2.6: Values of MaxF for 10 Replicates of Young spinach for Control, 100N, 150N, and 200N Compressed leaves at Day 12.

Day	Control		Day	100N		Day	150N		Day	200N	
	Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)		Replicate	MaxF (N)
12	1	1.31	12	1	1.21	12	1	1.32	12	1	1.17
	2	1.70		2	1.72		2	1.51		2	1.08
	3	1.51		3	1.11		3	1.09		3	1.13
	4	1.14		4	1.45		4	0.89		4	1.27
	5	1.63		5	1.29		5	1.30		5	1.38
	6	1.49		6	1.26		6	1.09		6	0.99
	7	1.44		7	0.97		7	1.29		7	1.13
	8	1.07		8	1.32		8	1.63		8	1.27
	9	1.07		9	1.21		9	1.16		9	1.47
	10	1.55		10	1.41		10	0.91		10	1.05
TrimMean(10)		**1.39**	**TrimMean(10)**		**1.28**	**TrimMean(10)**		**1.21**	**TrimMean(10)**		**1.19**
AverageMean		**1.39**	**AverageMean**		**1.29**	**AverageMean**		**1.22**	**AverageMean**		**1.20**
Std Dev		**0.23**	**Std Dev**		**0.20**	**Std Dev**		**0.24**	**Std Dev**		**0.15**
Std error		**0.07**	**Std error**		**0.06**	**Std error**		**0.08**	**Std error**		**0.05**

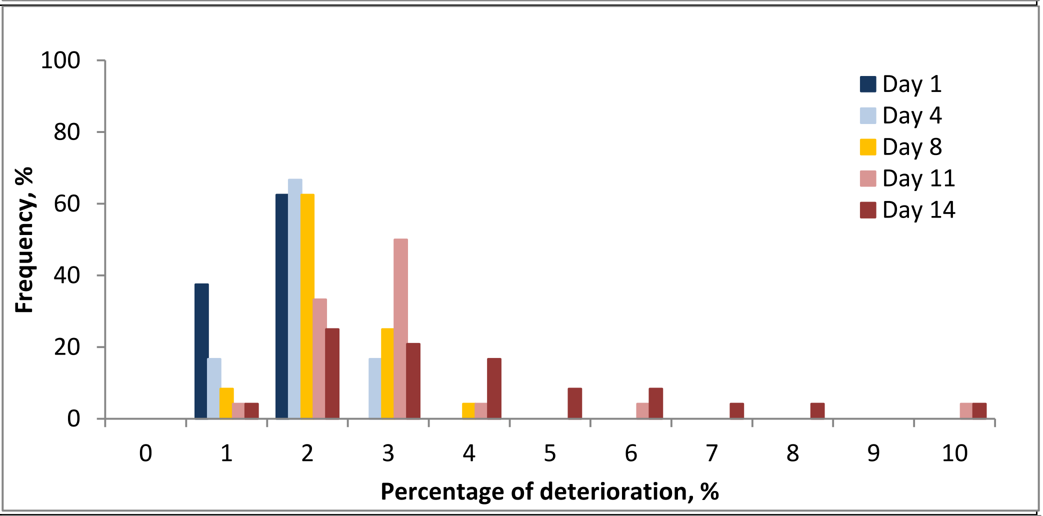
A.3 Distribution of deteriorations of spinach leaves under compression

Spinach leaves (S_4) compressed under 50N

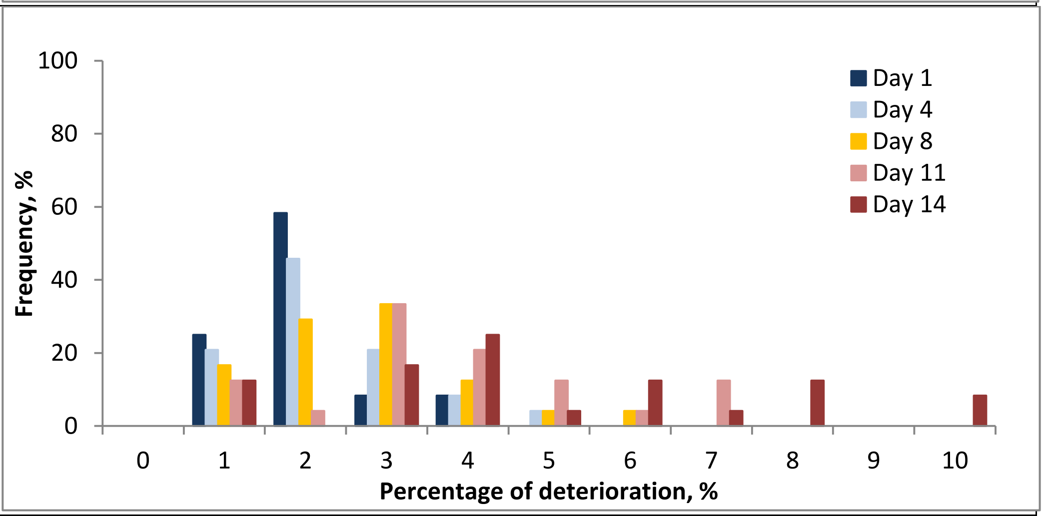
Undamaged



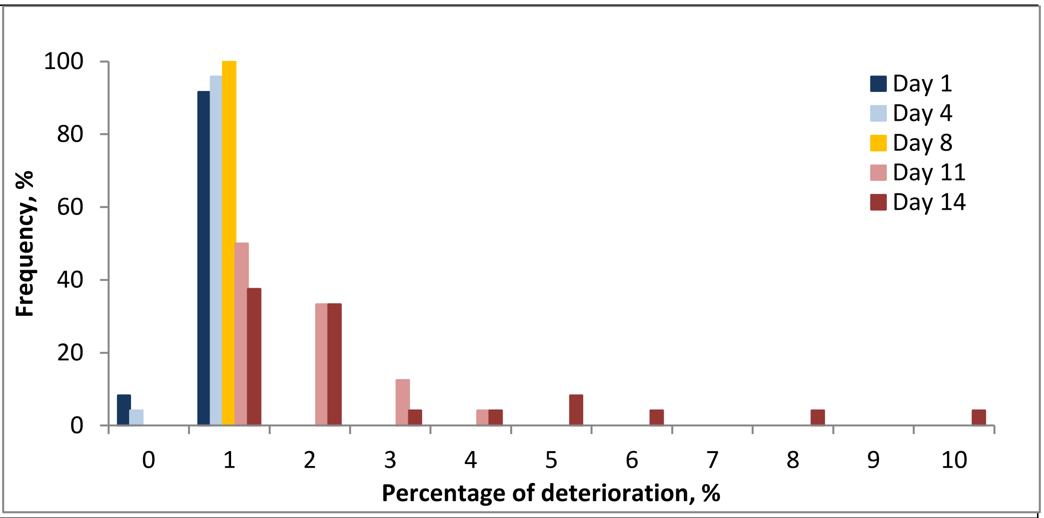
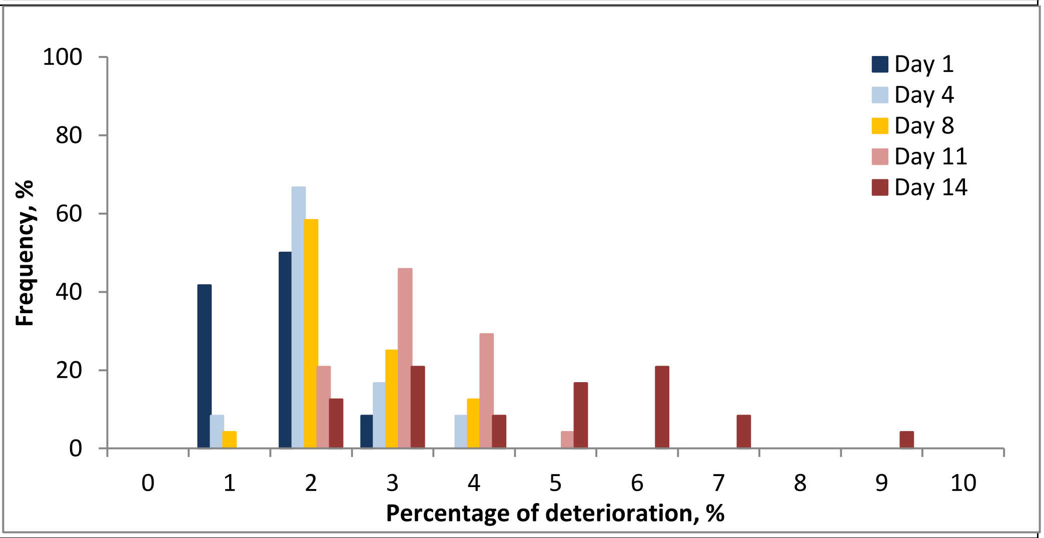
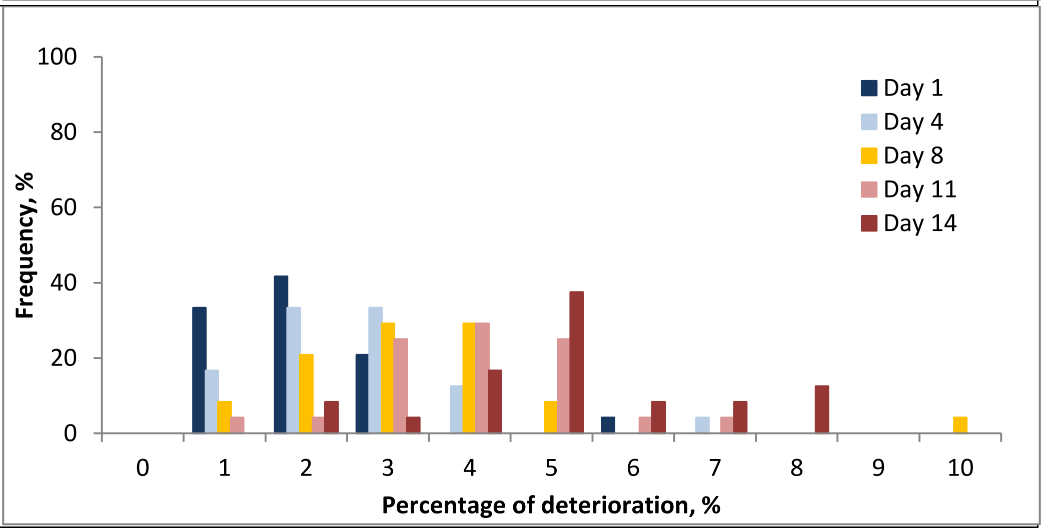
Halfway teared



Complete teared



Spinach leaves (S_4) compressed under 150N

Undamaged	
Halfway teared	
Complete teared	

A.4 Questionnaire for sensory test.

Name ________________________________

Please **OBSERVE** the **VISUAL APPEARANCE** of each set of the spinach bags

Set A

504 594 700 140 662

Please rank the **VISIBILITY OF DETERIORATION/DAMAGE** of the spinach leaves. One code only per line – no ties allowed

Most visible _____ _____ _____ _____ _____ Least visible

Comments:

Please indicate how much you would like to **BUY** the sample by ticking the most appropriate phrase below for each of the samples codes.

Samples codes	**504**	**594**	**700**	**140**	**662**
I certainly would buy					
I might buy					
I might buy/I might not buy					
I might not buy					
I certainly would not buy					

Comments:

Set B

900 464 156 966 938

Please rank the **VISIBILITY OF DETERIORATION/DAMAGE** of the spinach leaves. One code only per line – no ties allowed

Most visible _____ _____ _____ _____ _____ Least visible

Comments:

Please indicate how much you would like to **BUY** the sample by ticking the most appropriate phrase below for each of the samples codes.

Samples codes	**900**	**464**	**156**	**966**	**938**
I certainly would buy					
I might buy					
I might buy/I might not buy					
I might not buy					
I certainly would not buy					

Comments:

Set C

044 878 160 785 866

Please rank the **VISIBILITY OF DETERIORATION/DAMAGE** of the spinach leaves. One code only per line – no ties allowed

Most visible _____ _____ _____ _____ _____ Least visible

Comments:

Please indicate how much you would like to **BUY** the sample by ticking the most appropriate phrase below for each of the samples codes.

Samples codes	**044**	**878**	**160**	**785**	**866**
I certainly would buy					
I might buy					
I might buy/I might not buy					
I might not buy					
I certainly would not buy					

Comments:

Set D

639 234 381 663 729

Please rank the **VISIBILITY OF DETERIORATION/DAMAGE** of the spinach leaves. One code only per line – no ties allowed

Most visible _____ _____ _____ _____ _____ Least visible

Comments:

Please indicate how much you would like to **BUY** the sample by ticking the most appropriate phrase below for each of the samples codes.

Samples codes	**639**	**234**	**381**	**663**	**729**
I certainly would buy					
I might buy					
I might buy/I might not buy					
I might not buy					
I certainly would not buy					

Comments:

Set E

711 463 641 084 879

Please rank the **VISIBILITY OF DETERIORATION/DAMAGE** of the spinach leaves. One code only per line – no ties allowed

Most visible _____ _____ _____ _____ _____ Least visible

Comments:

Please indicate how much you would like to **BUY** the sample by ticking the most appropriate phrase below for each of the samples codes.

Samples codes	**711**	**463**	**641**	**084**	**879**
I certainly would buy					
I might buy					
I might buy/I might not buy					
I might not buy					
I certainly would not buy					

Comments:

GENERAL QUESTIONS

1. Nationality/country of origin: ___________________

2. Gender: Male _______ Female ________

3. Age: 18-25 26-35 36-45 46-55 56-65 65+

4. How often do you purchase ready-to-eat (RTE) spinach? (Please circle **ONLY ONE** number under **ONLY ONE** category)

Per day Per week Per month

1X 2X 3X 1X 2X 3X-4X 5X-6X 1X-3X 4X-6X

5. Do you observe the visual appearance of the spinach before buying?

YES NO SOMETIMES

6. If your answer in 5 is YES, do you mind to buy bag of spinach that contains deteriorated/damaged leaves?

YES NO SOMETIMES

Additional comments:

____

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