Linking the Quality of Sweet Basil Leaves to the Quality of Pesto

A thesis presented in partial fulfilment of the
requirements for the degree of

Master
in
AgriScience (Horticulture)

at Massey University, Palmerston North,
New Zealand

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2017

ABSTRACT

The method for linking basil quality to pesto quality has not been invented but the urgency is certain. If the quality loss of pesto is due to the loss of basil leaves quality, then financial loss suffered by basil-based producer could be reduced by only maintaining basil leaves quality. Through several batches of experiments, the methods of measuring senescence and chilling injury (CI) of basil leaves and the blackening rate of pesto were developed to investigate the impact of pre-processing factors (leaf washing, blanching, and storing) on basil and pesto quality. The final aim was to test whether there was a correlation between senescence or CI in basil leaves and blackening rate of the pesto. The methods included the selection of spectrophotometer proxy, number of replication, processing duration, pesto setting, fitting curve, sampling, cooling techniques, factors that influence all variables, and also solving some issues regarding data and pesto appearance.

The result produced several findings. First, measuring senescence or CI of basil leaves could be simply done by cooling basil leaves to a certain level of cold temperature and duration, left them in room temperature for a day and used percentage of rotten leaves and weight loss as the indicators of the symptoms severity. Second, measuring blackening rate of pesto could be done by using L value as the proxy of spectrophotometer and transformed the data into negative exponential to find the K value. Third, there was no correlation between senescence or CI in basil leaves and blackening rate of their pesto. Fourth, pre-processing factors impacted basil quality but not the pesto quality. Washing basil leaves before pesto processing could raising up the initial L value of pesto, blanching could created darker pesto, and storing basil leaves could lead to CI or senescence development, but those factors never affect the blackening rate of pesto.

Keywords: *Ocimum basilicum,* basil, chilling injury, senescence, blackening rate, blanching, temperature, cold storage

ACKNOWLEDGEMENTS

Al-ḥamdu li-llāhi rabbi l-ʿālamīn, all the praises and thanks be to Allah, the Lord of the worlds, who has been bless me with numerous gifts, protect me in all causes and to whom I always surrender my fate after I give all my best effort.

I would like to express my sincere gratitude to my family who support me all the way, especially my parents whose prays follow me in every step I make. The some goes to my little sister and her family who always remind me the reality beyond books and journals, and no, I am not forgetting to create my own.

Also, to my friends and neighbours, both in New Zealand and Indonesia, who directly or indirectly affect my work. I can say that good people are much alike to each other regardless of the colour of their skins and cultures. Thank you for making my life a lot easier. I enjoy staying in New Zealand as much as I enjoy living in Indonesia.

I also would like to express my best gratitude to my supervisor at Massey University, Prof. Julian Heyes and my co-supervisor Dr. Erin O'Donoghue who have been so patient in guiding me and magically turn my 'puzzling' words into a clear thesis and fulfil the requirement. It has not been easy, but I am so glad to have both of you in this vessel.

Last but not least, I would like to thank to my government, especially The Indonesian Agency for Agricultural Research and Development (IAARD)-The Ministry of Agriculture, who have given me this opportunity. Through the SMARTD scholarship that they provided, I could see the other side of the world no longer from behind a glass but actually touch it and feel it. It is more than a study experience for me but also a life experience. I will share these to the people of Indonesia as I promise, *Insya Allāh*.

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CHAPTER 1. INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is an ornamental and culinary herb of the Lamiaceae family (Miele, Dondero, Ciarallo, & Mazzei, 2001). It originated from Indo-Malayan regions and is important as a culinary herb, medicine (Demirhan & Özbek, 2009; Khair-ul-Bariyah, Ahmed, & Ikram, 2012), phytotherapeutant (Miele et al., 2001) and cosmetic material (Filho et al., 2006). Due to the importance of this plant, it is necessary to conduct study on maintaining its quality and prolonging its shelf-life during storage period. A transit of basil from plant field to factory sometimes takes days and appropriate post-harvest handling is required because quality loss could lead to financial loss for basil-based producers (A. Parkin, personal communication, 9 February, 2016).

Figure 1 Basil plant (*Ocimum basilicum L.*)
(http://loghouseplants.com/plants/shop/basil-genovese-ocimum-basilicum/)

The quality of herbs, in general, can be determined from their visual appearance or freshness, the uniformity of size, shape, colour, and the lack of damage caused by decay or improper handling after harvesting (Cantwell & Reid, 1993; Tijsken, Schijvens, & Biekman, 2001). Sometimes specific attributes are also assessed e.g. flavour, aroma (Arthey, 1975) and essential oils (EO) content (Cantwell & Reid, 1993). However, colour measurement is probably the most common method of assessment to evaluate the change in quality related to temperature (Shin & Bhowmik, 1995) since it is easy to do. Moreover, colour of the food is an important aspect of food quality since it can stimulate appetite of consumers. In human perspective, colours indicate the flavour and taste (Downham & Collins, 2000).

Shelf-life of a horticultural product is closely related to the decay process/ senescence (Aubry, Mani, & Hortensteiner, 2008) and for tropical or subtropical products, such as basil, chilling injury/ CI (the lesion that is caused by low temperature) can become another important term. Senescence symptoms on green detached leaves usually involve discoloration such as yellowing (Able, Wong, Prasad, & O'Hare, 2003; Lomaniec, Aharon, Aharoni, & Lers, 2003), browning, blackening, reddening or even greening (Adams & Brown, 2007). On the basil leaves, CI symptoms usually involve blackening (Wongsheree, Ketsa, & van Doorn, 2009).

Leaf discoloration (in this case blackening) decreases basil quality. It is really important for the food industry with basil-based products, for example, pesto sauce producers (Carlo, Silvia, Stefano, & Paolo, 2013). Standard preservative methods used in pesto processing, such as pasteurization, sterilization, and modified atmosphere packaging (MAP) (Luo and Barbosa-Canovas, 1996; Vicini & Previdi, 1992 cited in Fabiano, Perego, Pastorino, & Del Borghi, 2000), can cause unexpected colour changes, loss of taste and aroma (Fabiano et al. 2000; Pavlović et al., 2010). Food additives may be used as an alternative to preservation in accordance with the regulations or 'Generally Recognized as Safe' (GRAS) status. However, consumers are wary of chemical additives which limits the choices for suitable additives (Downham & Collins, 2000; Reyes-Moreno, Parr-Inzunza, Milan-Carillo, & Zazueta-Niebla, 2001). Therefore, another method should be developed to reduce subsequent colour change of basil. Choosing the right temperature regime to store basil during the transport from the field to processing factory may be enough for this purpose since there might be a connection between the quality of basil and the quality of pesto.

Generating the best storage method for basil is difficult because it is chilling sensitive and the optimal storage temperature may vary between varieties (Cantwell & Reid, 1993; Costa, Millan Montano, Carrión, Rolny, & Guiamét, 2013; Goto, Murakami, & Yamada, 1993; Lange & Cameron, 1994; Lers et al., 2010; Rolny, Costa, Carrion, & Guiamét, 2011). Also, in the 'real world', optimal storage regimes are never achieved; products may be exposed to long or short periods of time outside their ideal storage temperature range.

Changes in herb quality are also difficult to measure (Arthey, 1975). The degradation symptoms, such as senescence-related blackening (decay) or blackening

caused by CI are not evenly spread on the leaves and can look very similar. Visual assessment is the only practical way to summarize basil quality.

Assessing blackening rate on simplified pesto (a blended mixture of basil leaves and olive oil), on the other hand, is more straightforward. Pesto blackening begins at the surface where pesto is in contact with air. It can be assessed objectively with a tool such as a spectrophotometer. What needs to be determined is whether there is a relationship between basil leaf quality and pesto blackening; there is no guarantee that there is a close relationship between these processes.

Many studies regarding basil quality or its processed products have been reported (Aharoni, Dvir, Chalupowicz, & Aharon, 1993; Cantwell & Reid, 1993; Costa et al., 2013; da Silva et al., 2005; Fabiano et al., 2000; Lers et al., 2010; Pavlović et al., 2010; Wongsheree et al., 2009). However, there have been no reports concerning the relationship between leaf quality and pesto quality. In the meantime, a New Zealand processing company has found that there are some cases when several batches of pesto behaved differently in terms of blackening rate and has been wondering if the prior condition of the basil affects the quality of the pesto (A. Parkin, Personal communication, 9 February, 2016). Therefore, the objectives of this research are:

1. To develop methods of measuring senescence and CI of basil leaves and the blackening rate of pesto;
2. To investigate whether there is a correlation between senescence or CI in basil leaves and blackening rate of pesto made from these leaves;
3. To assess the impact of pre-processing factors (leaf washing, blanching, and storing) on pesto quality.

CHAPTER 2. LITERATURE REVIEW

2.1 Sweet basil (*Ocimum basilicum* L.)

Ocimum basilicum is one of the most popular species of genus *Ocimum* in scientific records. Others are *O. tenuiflorum* (holy basil) and *O. citriodorum* (lemon basil). All of them are known for their various flavours and essential oils. *Ocimum* genus is known by several names such as basil and 'tulsi' (Mukherjee & Data, 2007). However, *Ocimum basilicum* is usually called sweet basil or French basil (Fleisher, 1981).

Sweet basil has a broad habitat. It is found in many tropical and subtropical regions in the world, such as Southeast Asia (Filho et al., 2006; Mukherjee & Data, 2007; Khair-ul-Bariyah et al., 2012), Central Africa (Das, 2002; Data et al., 2010; Filho et al., 2006), Brazil (Filho et al., 2006), Israel and Southern Europe (Dudai et al., 2002).

2.1.1 The importance of sweet basil

Apart from being used for direct consumption (Lee, Umano, Shibamoto, & Lee, 2005) and a key ingredient for processed products such as pesto sauce (Fabiano et al., 2000), sweet basil is used for many other purposes because of its chemical constituents. Basil's essential oils (EOs) are useful for preserving food as they have antifungal properties (Abdolahi, Hassani, Ghosta, Bernousi, & Meshkatalsadat, 2010), and are also reported to be useful as treatments for headaches, coughs, diarrhoea, constipation, warts, worms, and kidney malfunction (Khalid, 2006), as food additives and cosmetics (Marotti, Piccaglia, & Giovanelli, 1996).

2.1.2 Essential oils (EOs) of sweet basil

EOs are the main constituent of sweet basil. EOs can be separated into 2 groups: terpenes (monoterpenes and sesquiterpenes) and phenylpropenes (Croteau & Karp, 1991; Croteau, Kutchan, & Lewis, 2000; Gang et al., 2001). Monoterpenes includes R-linalool, 1,8-cineole, limonene, thymol, citral, geraniol and camphor. Sesquiterpenes include germacrene D and α-bergamotene (Grayer et al., 1996; Lachowicz et al., 1996). Phenylpropenes consists of eugenol and methyl-chavicol/estragole (Koeduka et al., 2006). Some other EOs in basil are methyl-eugenol and methyl-cinnamate (Gupta, 1994; Lewinsohn et al., 2000; Miele et al., 2001; Nacar & Tansi, 2000; Simon, Quinn, & Murray, 1990), and many more.

EOs can be derived from each other, e.g. methyl-eugenol is derived from eugenol and methyl-chavicol is derived from chavicol. Both are products of enzyme conversion by methyltransferase, one of the key enzymes that influence the composition of EOs (Lewinsohn et al., 2000).

The EO constituents of sweet basil vary within and between plants and depend on the condition and the developmental stage of the plant (Data et al., 2010). Some authors stated that the most dominant oils, linalool and eugenol, make up around 15-87% and 10-24% of total oil concentration respectively (Chang, Alderson, & Wright, 2005; Díaz-Maroto, Palomo, Castro, Viñas, & Pérez-Coello, 2004; Riaz, Shadab, & Chaudhury, 1999; Slougui, Mohammed, & Rolando, 2003). However, sweet basil from Europe is dominated by linalool and estragole respectively 40.5-48.2% and 28.9-31.6% (Charles & Simon, 1990; Fleisher, 1981).

2.1.3 The composition and distribution of sweet basil's chemical material

Sweet basil has a huge genetic variation that makes it difficult to classify (Data et al., 2010). This may be because the species hybridises readily with over 149 other species in the *Ocimum* genus. Basil reproduction requires cross pollination by bees (*Apis florae*, *A. cerana indica*, *Amegilla sp*., and *Pseudapis oxybeloies*) or butterflies (*Surandra queretroum*) (Raju, 1989) since the biological characteristics of the flowers do not allow them to be self-pollinated (Nation, Janick, & Simon, 1992). In nature, a new species could easily arise from cross-pollination between two species. Lemon basil (*Ocimum citriodorum* Vis) is a hybrid from *Ocimum basilicum* and *Ocimum americanum* (Paton & Putievsky, 1996; Viña & Murillo, 2003). All species of basil could possibly generate viable seeds after cross pollination with sweet basil (Paton, 1992). This could be an issue since commercial hybrid varieties of basil may be contaminated by alternative pollens. In addition, parts of basil on the same plant have different aroma characteristics and different chemical compounds (Rubiyanto, Sastrohamidjojo, & Anwar, 2015; Simon et al., 1990). When the process of cultivation, harvesting and delivering of the samples were not in control of the researcher, there are many more factors regarding chemical compound that possibly affect the parity of the samples, some will be briefly explained in the next paragraphs.

Chemical amount and composition of basil could also vary with plant age and stages of development. There is controversy over developmental changes in EOs; one study found that EOs are maximal at full bloom stage (Fleisher, 1981), another suggested that EO concentration in most basil species reaches its peak when 50% of its seeds are mature (Gupta, 1994). Others believe EOs in the leaves decrease during basil maturation. This probably relates to the fact that trichomes, which are the site of EO biosynthesis (Gang et al., 2001; Grayer et al., 1996; Lachowicz et al., 1996; Lewinsohn et al., 2000), decline in density through the age of the leaf (Gershenzon, McConkey, & Croteau, 2000; Paton, Harley, & Harley, 1999). Furthermore, EO can evaporate by the time leaves gain weight (Fischer, Nitzan, Chaimovitsh, Rubin, & Dudai, 2011).

The differences in EO composition of young and old leaves have been recorded in several studies (Dudai, Larkov, Ravid, Putievsky, & Lewinsohn, 2001; McConkey, Gershenzon, & Croteau, 2000; Szabo & Bernarth, 2002). The composition of EO in young leaves of sweet basil is usually dominated by eugenol (~53%) and the old leaves by methyl-eugenol (~68%) (Fischer et al., 2011). This condition might occur because during the aging, the methyltransferase enzyme is active to convert eugenol to methyl-eugenol (Lewinsohn et al., 2000). Other types of EO might also change through the biosynthesis of monoterpenes (McConkey et al., 2000). In the case of lemon basil, estragole is the EO that is affected by basil development. Its production would be higher during pre-flowering and the flavour would be maximized during post-flowering (Al-Kateb & Mottram, 2014).

EO content also changes along with the number of harvests as well as the level of antioxidant activity (AOA) (Carlo et al., 2013). The highest AOA is found in the first and second harvests of sweet basil and then decreases sharply in the third cut (Carlo et al., 2013) but other EOs increase with each harvest (Zheljazkov, Cantrell, Tekwani, & Khan, 2008).

Harvest time also influences the composition and the amount of EO within sweet basil. The maximum EO content for basil grown in Brazil, was in January (2.26%) and the lowest concentration was found in August (1.06 %) (da Silva et al., 2005). Furthermore, the best time during the day for harvesting for sweet basil that is grown in Brazil is between 8 and 12 noon, when the EO especially linalool reaches the highest point (Filho et al., 2006).

Similarly, growing temperature will probably influence the concentration of EO in basil leaves. Two-weeks-old basil has been reported to have a change in content and composition of EOs by exposure to different growing temperatures. At temperatures around 25°C, EOs are dominated by eugenol and cis-ocimene, while at 15°C, camphor and trans-beta farnesene are present in greatest amounts (Chang et al., 2005). Basil might also lose some of its EO in storage since other leafy products (2 cultivars of watercress) were found to have a decreasing EO concentration after storage in low temperature (Spence & Tucknot, 1983).

In lemon basil, flowers, branches and leaves have different concentrations of chemical compounds. Flowers have more abundant linalool and cis-3-hexenylacetate than do other plant parts. Flowers contain 13% linalool in the total volatile compounds, while branches and leaves have 11% and 3% of it respectively. Branches have higher level of monoterpenes, hydrocarbons, oxygenated compounds, sesquiterpenes, cis-chrysanthemol, and b-caryophyllene than other parts of the plant (Al-Kateb & Mottram, 2014; Laakso, Laakso, Wolf, Kuhnel, & Knobloch, 1990).

The EO concentration and composition in sweet basil are related to aroma (Fischer et al., 2011). It is suggested that linalool, cineole, and eugenol are the EOs responsible for the aroma of sweet basil (Fleisher, 1981). Linalool and cineole are known as volatile compounds that attract pollinators at the flowering stage (Croteau et al., 2000). Linalool is found abundant in basil leaves when they are extracted for the purpose of retarding decay on grapes (Abdolahi et al., 2010) and potatoes (Vaughn & Spencer, 1991). It is also known to have the finest odour of all other EOs and is probably the main constituent of basil (Paton et al., 1999). Cineole is a repellent to herbivores and found toxic for other plant competitors (Croteau et al., 2000). Eugenol, on the other hand, has the highest antioxidant capacity, compared to the other EOs (Politeo, Jukic, & Milos, 2007).

2.1.4 Storing sweet basil

Sweet basil has some different attributes compared to other herbs. It is categorized as a chilling sensitive herb and it needs to be stored at temperature between 9-20°C (Cantwell & Reid, 1993; Costa et al., 2013; Goto et al., 1993; Lange & Cameron, 1994; Lers et al., 2010; Rolny et al., 2011). Meanwhile, less chilling-sensitive herbs, such

as parsley, tarragon, etc, can be refrigerated and would have a shorter shelf life if kept above 10°C (Cantwell & Reid, 1993). At room temperature (about 20°C), sweet basil shelf life can last up to 5 days and 12 days at 15°C (Cantwell & Reid, 1993). Storage of basil in temperatures below 9°C is likely to have blackening caused by CI (Cantwell & Reid, 1993; Costa et al., 2013), and the severity will depend on the period of exposure, pre-harvest treatment, age of the leaf, the time of harvest, the species and cultivar (Cantwell & Reid, 1993). This variability is the reason why studies regarding cold exposure could vary in their results.

During the period of storage, sweet basil not only changes its chemical compounds as is the case in lemon basil (Rubiyanto, Anwar, & Sastrohamidjojo, 2009), but most of the EOs, except eugenol and linalool, will also decrease. It also can lose some of its chlorophyll, and it becomes more likely to have mold and yeast colonies (da Silva et al., 2005) due to natural aging and/or environmental factors (Lange & Cameron, 1994; Lange & Cameron, 1997). Therefore, the following sections will describe some of the change processes during basil storage and pesto manufacture. All are important to illustrate the events that might occur during the experiments.

2.2 Senescence

The senescence process of a plant can be defined as the process of cell degeneration or cell death which is manifested by the loss of the cell membrane's integrity and its ability to maintain itself (Noodén, Guiamét, & John, 1997). It is a genetic program for specific organs (e.g. leaf, flower, and fruit) that is related to the developmental stages and environmental conditions (Kim, Chang, & Tucker, 2015). This process causes quality to decline (Cantwell &Reid, 1993; Meir, Ronen, Lurie, & Philosoph-Hadas, 1997; Philosoph-Hadas, Meir, Akiri, & Kanner, 1994) and can lead to economic losses after harvest (Hassan & Mahfouz, 2010; Lers, Jiang, Lomaniec, & Aharoni, 1998).

2.2.1 Mechanism of senescence

The mechanism of plant senescence has been generating controversy due to its complexity (Noodén et al., 1997; Yamada et al., 2014). It seems that there is no similar answer for every case of senescence (Cantwell & Reid, 1993; Degl'Innocenti, Guidi, Pardossi, & Tognoni, 2005; Sklensky & Davies, 1993). Some have said that senescence

starts with chromatin condensation and changes in nuclear material (Biradar & Lane Rayburn, 1994; Kuran, 1993), while others think that it depends on the conditions, as will be briefly discussed in the next paragraphs.

Senescence during reproduction and plant growth is probably induced by sugar/starch flux (da Silva et al., 2005; Wingler, Purdy, MacLean, & Pourtau, 2006). It occurs to allow for transport of assimilated nutrients from old tissues to storage or young tissues (Sklensky & Davies, 1993; Gan & Amasino, 1997) and is regulated by a series of Senescence Associated Genes (SAGs) (Gan & Amasino, 1997). This condition may overlap with senescence caused by environmental stress since the genes that regulate both kind of senescence are probably the same (Weaver, Gan, Quirino, & Amasino, 1998).

It is also possible that senescence is triggered by the accumulation of ammonium and/or the oxidation of chlorophyll, lipids and protein/amino acids (Cantwell & Reid, 1993; Chen, Hung, & Kao, 1997; Clarke, 1994; Rolny et al., 2011). However, senescence of detached leaves might only be regulated by ammonium accumulation (Chen et al., 1997; Lauriere & Daussant, 1983).

Ammonium is closely related to the senescence process of the leaves of wheat and rice stored in the dark conditions (Chen et al., 1997; Lauriere & Daussant, 1983). It seems that after leaves are cut from the plants, abscisic acid (ABA) increases ammonium levels by decreasing glutamine synthetase activity and increasing the reduction of nitrate, and this eventually promotes the sensitivity of the leaf to ethylene (Chen et al., 1997). Ammonium can be toxic for plants (Givan, 1979) and ethylene is involved in chlorophyll degradation (Clarke, 1994). However, a study on broccoli found that ammonium started to accumulate 4 days after detachment or just about after chlorophyll rapidly decreased (Clarke, 1994). Starting point of senescence might still be arguable, but it seems that all agree that the process is promoted by hormones (Leopold & Noodén, 1984) and regulated by genes (Sarwat, Naqvi, Ahmad, Ashraf, & Akram, 2013).

2.2.2 Hormones of senescence

Hormones that are involved in senescence are cytokinin, gibberellin, ABA (Sklensky & Davies, 1993; Weaver et al., 1998), salicylic acid, strigolactones (Yamada et

al., 2014), jasmonic acid (Kim et al, 2015), and ethylene (Burg, 1968; Gan & Amasino, 1997; Kim et al., 2015; Leopold & Noodén, 1984).

Cytokinin is known as an inhibitor of senescence (Gan & Amasino, 1996), and is able to hamper the process by reducing the sensitivity of the leaf to ethylene (Kao & Yang, 1983). Plants that over produce this hormone (regulated by the IPT gene, a gene encoding isopentenyl transferase) show delayed senescence (Gan & Amasino, 1995). However cytokinin may only delay senescence before it has started, i.e. it can only prevent plants from early aging, instead of stopping or slowing it down (Weaver et al., 1998). The application of exogenous cytokinin is effective in delaying leaf senescence of broccoli (*Brassica oleracea*) in the field (Chen, Hwang, Chang, Sun, & Yang, 2001), lettuce (McCabe et al., 2001), tobacco and wheat (*Triticum aestivum*) (Sýkorová et al., 2008).

Gibberellin is another hormone that retards leaves senescence while either attached or detached (Sklensky & Davies, 1993). It works as the antagonist of ethylene (Dubois et al., 2013). Gibberellin is also known to contribute to the response to other environmental stresses such as salt, cold, or osmotic stress (Colebrook, Thomas, Phillips, & Hedden, 2014) and the effect of the light (van Doorn & van Lieburg, 1993). An example of gibberellin use for delaying senescence is a study on peas (Proebsting, Davies, & Marx, 1978) and cut flowers (Ferrante, Mensuali-Sodi, & Serra, 2009).

ABA increases during leaf senescence and exogenous treatment can accelerate senescence (Yang, Zhang, Wang, Zhu, & Liu, 2003). It is also known that ABA works synergistically with ethylene (Kim, Chung, & Woo, 2011). It promotes senescence in rice, soybean (Lindoo & Noodén, 1978) and detached leaves of *Arabidopsis thaliana* (Weaver et al., 1998). It is a hormone that also regulates the change in response to dehydration in plants by mediating communication between roots and shoots (Colebrook et al., 2014).

Strigolactone is a hormone that is activated by nitrogen and phosphorous deficiency. It is a hormone that is responsible for transferring nutrient from old leaves to young tissues and starting the senescence process in the field. There is a possibility that this hormone is only produced in old leaves, but not in the young ones (Yamada et al., 2014). A study on rice and arabidopsis leaf segments shows that applying this hormone to strigolactone-deficient mutants will accelerate senescence, but the

insensitive mutants do not show this response. Strigolactone works as a promotor of senescence by increasing the effect of ethylene (Ueda & Kusaba, 2015).

Leaves produce only small amounts of ethylene within their tissues. However, they are very sensitive to this gas in the atmosphere (Kader, 1985; Philosoph-Hadas, Pesis, Meir, Reuveni, & Aharoni, 1989). External ethylene can accelerate the senescence process in many species of plants (Lers et al., 1998). The threshold concentration of ethylene in the environment is around 1 µl/l for rocket salad (*Eruca sativa*) (Koukounaras, Siomos, & Sfakiotakis, 2006) and 0.1-1.0 µl/l for sensitive herbs, such as sweet basil (Cantwell & Reid, 1993) but the impact (accelerated yellowing and leaf abscission) will be maximized if leaves are exposed to around 1-10 µl/l (Reid, 1987; Ryall & Lipton, 1979). This can be a major problem for leafy products since a normal atmosphere may contain ethylene as a contaminant and cold stores often contain fruits which can generate high amounts of ethylene (Cantwell & Reid, 1993). During the transfer of leaf samples from Superherb company to the laboratory, contamination like this is also possible.

Ethylene is a stress-related hormone, but unlike cytokinin, ethylene is a hormone that promotes senescence (Gan & Amasino, 1997). The actions of ethylene depend on the age of the plant (Lomaniec et al., 2003), the tissue sensitivity (Iqbal et al., 2017) and environmental stresses including temperature (Apeland, 1971; Cantwell & Reid, 1993), darkness, detachment, water and nutrient deficiencies (Lomaniec et al., 2003).

The biosynthesis of ethylene varies between age of the leaves. It is higher during leaf formation, but declines when leaves mature and increases again during senescence (Iqbal et al., 2017). The varied responses to ethylene are also found in leafy vegetables (Reid, 1987; Ryall & Lipton, 1979). The most common responses are yellowing, abscission, wilting, necrosis (Cantwell & Reid, 1993; Iqbal et al., 2017), and blackening on basil (Adams & Brown, 2007).

2.3 Discoloration

Discoloration in agricultural products causes not only visual quality loss, but also changes in flavours and nutrient compositions (Luo & Barbosa-Canovas, 1997). The type of discoloration depends on phenolic compounds and the enzymes that catalyse the process. The non-nitrogenous compounds will generate browning, while nitrogenous

compounds create blackening (Adams & Brown, 2007). As for yellowing, the phenolic compounds are likely contributors since it can be related to nitrogen deficiency (Hanaoka, 2002). However, blackening is not very commonly described as an outcome of discoloration apart from basil. The closest process to blackening is probably browning. Therefore, the next section about discoloration is dominated by browning.

Substances that influence discoloration

1. Polyphenol oxidase

The main material that influences discoloration is polyphenol oxidase (PPO). PPO is an enzyme that contains ionic copper (Cu^{2+}) and can be found in the chloroplast of the leaves (Mayer & Eitan, 1979; van Gelder, Flurkey, & Wichers, 1997). This enzyme is present in the latent state and becomes activated with the presence of many environmental stresses (Fan, Wang, & Zou, 2005). Environmental stresses disrupt cell membrane integrity and induce a phenylpropanoid pathway. This pathway produces phenolic compounds which are the main substrates that are converted by PPO into o-diphenols and then into o-quinones with the presence of oxygen (Matheis & Whitakerz, 1984; Mayer & Eitan, 1979). *O*-quinones are closely related to the discoloration process and reduction of the quality of the leaf (Matheis & Whitakerz, 1984; Mayer & Eitan, 1979)

PPO shares many functions with peroxidase (POD) and both have a synergistic activity in the wounding mechanism (Richard-Forget & Gauillard, 1997). POD is known to turn some of the compounds of vegetables into dark pigments. In broad bean leaves, POD regulates browning by changing dihydroxyphenylalanine into melanin (Degl'Innocenti et al., 2005; Takahama, 2004). In tobacco leaves and lettuce, POD induces browning by oxidizing chlorogenic acid. Meanwhile, in onion, it starts browning by oxidizing quercetin (Degl'Innocenti et al., 2005; Takahama, 2004).

2. Gibberellin

Gibberellin not only affects senescence, but it is also inversely correlated with discoloration, including browning and blackening. This hormone regulates the decreasing of calcium uptake which is important to the stability of membranes and mechanism of defence against abiotic stresses (White & Broadley, 2003). The loss of membrane stability leads to the accumulation of Reactive Oxygen Species (ROS),

followed by the oxidation of phenolic compounds and ending up with discoloration (Adams & Brown, 2007). ROS are known to induce lipid peroxidation, increasing membrane permeability that disrupts metabolic activity (Ranwala & Miller, 2000) and signalling genes that regulate cell death (Foyer & Noctor, 2005).

Exogenous gibberellin is reported to retard leaf yellowing or preserve chlorophyll in some species of cut flowers such as *Matthiola incana* L. (Ferrante et al., 2009), *Alstroemeria* sp. and *Lilium longiflorum* (Jordi, Stoopen, Kelepouris, & van der Krieken, 1995). In *Lilium longiflorum*, the effect of gibberellin is noticeable after the samples are removed from cold storage to room temperature (Ranwala & Miller, 2000).

3. Vitamin C/ascorbic acid (ASA)

One of the most useful antioxidants to reduce discoloration is vitamin C/ascorbic acid (ASA). It might also delay blackening in basil since it hampers browning process in citrus juice (Li, Sawamura, & Yano, 2014) and preserving celeriac flakes (Elzbieta, Janusz, & Katarzyna, 2007; Weaver et al., 1998). It is also suggested that ASA is involved in lettuce resistance to browning since the amount increases during storage in a browning resistant cultivar and decreases in a susceptible one (Degl'Innocenti et al., 2005).

The mechanism of ASA delaying effect is by turning *o*-quinones back to diphenols (Alscher, Donahue, & Cramer, 1997). *O*-quinones are known to be related to discoloration (Matheis & Whitakerz, 1984; Mayer & Eitan, 1979). They produce dark substances by reacting with other *o*-quinones or protein (Eskin, 1990).

The use of ASA to preserve food is not a new method in fruit and vegetable processing. However, ASA application can be a problem since there is a possibility that it may be oxidized back into dehydroascorbate acid (DHA). DHA is known as a substrate that can easily be degraded into dark pigment (Barry-Ryan & O'Beirne, 1999; Li et al., 2014; Petersen & Berends, 1993). It wass the reason that ASA was not used in this study.

2.4 The factors that influence senescence and discoloration

Senescence and discoloration can be influenced by both the biological factors of the leaf and environmental stresses (Weaver et al., 1998). The biological factors include

the age of leaves (Cantwell & Reid, 1993; Viacava, Gonzalez-Aguilar, & Roura, 2014), harvest time (Hasperué, Chaves, & Martínez, 2011; Hasperué, Gómez-Lobato, Chaves, Civello, & Martínez, 2013), the position of the leaf (Fischer et al., 2011; Viacava et al., 2014), drought, darkness, detachment (Weaver et al., 1998), temperature change (Ceni et al., 2008), wounding (Boss, Gardner, Janssen, & Ross, 1995; Constabel & Ryan, 1996; Mayer & Eitan, 1979), pH level, competitor elements (Broothaerts et al., 2000), light, and humidity (Aguero, Barg, Yommi, Camelo, & Roura, 2008). Most of these factors appear to change the PPO activity within the leaves.

1. **The age of the leaf**

   The age of the leaf influences the amount of chlorophyll, and rates of respiration and transpiration (Cantwell & Reid, 1993). Chlorophyll concentration and respiration rate decline with leaf ages, but after harvest, they tend to decline less in older leaves compared to younger leaves (Cantwell & Reid, 1993). As for transpiration, younger leaves and enclosed tissues (e.g. within a bud) have a lower transpiration rate than do old and open tissues (Adams & Brown, 2007).

   Assimilates can move from older leaves to younger leaves . Consequently, old leaves senesce faster and are more sensitive to cold temperature than are younger leaves (Ranwala & Miller, 2000).

2. **Harvest time**

   Research on broccoli shows that the different times of harvest affect its shelf-life (Hasperué et al., 2011; Hasperué et al., 2013). Broccoli that is harvested at sunset shows the lowest rate of discoloration compared to broccoli harvested at dawn and midday. This might be due to the fact that sunset-harvested broccoli has more starch to degrade into simple sugars which are then involved in delaying senescence after harvest (Hasperué et al., 2011).

3. **Position of the leaves**

   Leaf position in lettuce determines the quantity of carotenoids, chlorophyll, phenolics (the substrate for PPO), and antioxidants. ASA is found to be at maximum amount in the middle leaves and the other compounds are dominant in the outer leaves (Viacava et al., 2014). Thus, the outer leaves are more vulnerable to discoloration since they have more phenolics and less ASA.

4. Temperature

A study on mate tea leaves shows that the inhibition of PPO increases along with the rise of temperature and the time of exposure if they are placed at 40-80°C, the activity of PPO fully stops at 80°C in 6 minutes (Ceni et al., 2008). This means that PPO is likely to remain active in basil processing unless it can be stopped by e.g. blanching or pasteurisation.

5. Wounding

Wounding is also another factor that affects PPO. It increased PPO activity in the leaf of some plant species (Mayer & Eitan, 1979), including apples (Boss et al., 1995), tomatoes (Constabel & Ryan, 1996) and lettuce (Degl'Innocenti et al., 2005). Browning caused by wounding is also known as a defence mechanism system for plants against pests and disease (Broothaerts et al., 2000).

6. pH level

Acidity (pH) level is important for PPO activity. A study in apple leaves shows that the optimum pH for PPO activity is 6. Below this, PPO activity will reduce sharply (Broothaerts et al., 2000). pH also will influence the behaviour of some inhibitors that might be used to retard PPO activity since they can only work optimally if the pH level is around 3.5-5.0 (McEvily, Iyengar, & Otwell, 1992).

7. Competitors

There are some inhibitors of browning that are commonly used in cut fruits and vegetables that act as competitors because of their structural similarity to PPO (McEvily et al., 1992). These are 4-hexylresorcinol (Buta & Abbott, 2000; Reyes-Moreno et al., 2001) and tropolone (Khan & Andrawis, 1985).

1) 4-Hexylresorcinol

   4-Hexylresorcinol is found to preserve the freshness of cut-pears (Buta & Abbott, 2000), potatoes (Reyes-Moreno et al., 2001) and apples (Lou&barbosa, 1997). The extent of its inhibition would depend on the PPO sensitivity of the products itself (Buta & Abbott, 2000). Unfortunately, the use of 4-hexylresorcinol for fresh fruits and vegetables has not been legally approved (Reyes-Moreno et al., 2001).

2) Tropolone

Tropolone is known as a competitor of Cu^{2+} (Khan & Andrawis, 1985), the ion that is essential for the PPO enzyme. The addition of 0.5 mM tropolone inhibits 85% of PPO activity in tomatoes leaves, 2 mM of tropolone inhibits 95% PPO activity in apple leaves; whereas 10 mM of tropolone inhibited 80% of PPO activity in tobacco (Broothaerts et al., 2000) and it is reported that tropolone is the most effective inhibitor for PPO of basil (Dogan, Turan, Dogan, Alkan, & Arslan, 2007). However, up to now there has been no record regarding the safety or regulation of tropolone application on basil leaves.

8. Light

Light is involved in senescence because of its relationship to phytochromes that regulate developmental processes within the leaf (Paul & Khurana, 2008; Rousseaux, Hall, & Sanchez, 1996), which includes plant growth (Quail, 2002) and the increase of carbohydrate. In some species, these two developmental processes are related to the retarding of senescence process (Ranwala & Miller, 2000). Light involvement in senescence might also correlate with pH balance since it could decrease pH level of chloroplast and vacuole (Smith & Raven, 1979). pH level is known as an important factor affecting PPO activity and inhibitor concentration (Broothaerts et al., 2000; McEvily et al., 1992). Others also suggest that light contributes to reduction of nitrate (Chang, Yang, & Riskowski, 2013), which related to the sensitivity of the leaf to ethylene (Chen et al., 1997).

Light can be both beneficial and harmful for leafy products since they have their own optimum amount. For spinach, it can be around 26.9 µmol $m^{-2}s^{-1}$ (Zavaleta-Mancera, Thomas, Thomas, & Scott, 1999), for basil approximately 30–37 µmol $m^{-2}s^{-1}$ (Costa, et al., 2013), and broccoli 24 µmol $m^{-2}s^{-1}$ (Zhan, Hu, Li, & Pang, 2012).

It is known that leaves lose their chlorophyll and protein if the flux of radiant energy per unit area is below the light compensation point for CO_2 fixation, which is approximately 10 µmol $m^{-2}s^{-1}$ (Cantwell & Reid, 1993). As for basil, light intensity seems to contribute to the nitrate concentration since basil that is harvested after 3 hours of daylight has the higher nitrate concentration than that harvested at other times during the same 24-hour period (Chang et al., 2013).

Light is reported to reverse senescence, as is the case in nicotiana leaves (Zavaleta-Mancera et al., 1999). In the optimum amount, the ability of light to maintain leaf function correlates with the level of antioxidant enzymes and metabolites which are responsible for plant's defence mechanism system against abiotic stress (Alscher et al., 1997). Beyond that range, light can have an unwanted side effect since it may increase respiration as was found in leeks (Ayala, Echávarri, Olarte, & Sanz, 2009) dill and tarragon (Cantwell & Reid, 1993). It can also expedite chlorophyll degradation and increase transpiration as in cauliflower (Olarte, Sanz, Federico Echávarri, & Ayala, 2009).

Respiration takes part in senescence since the level of oxygen (O_2) and carbon dioxide (CO_2) is also crucial. CO_2 is known as an ethylene competitor in some products after harvest (Philosoph-Hadas, Aharoni, & Yang, 1986). In general, with a lower level of oxygen and higher level of carbon dioxide, the deterioration process can be retarded (Isenberg, 1979; Philosoph-Hadas et al., 1986; Saltveit, 1985; Weichman, 1977). However, the concentration of oxygen below 0.5% and carbon dioxide higher than 10% at 5°C can result in unpleasant odours on broccoli (Weichman, 1977). For basil, the shelf-life can increase 2.5 times that in untreated air if the level of oxygen is maintained at 1.5% with 0% carbon dioxide at 20°C (Cantwell & Reid, 1993).

Transpiration is related to water loss which is important for maintaining weight of leafy products and may affect the defensive ability against pathogens. Water loss has been used as an indicator of fresh herb quality since wilting is caused by water loss. Most leafy products will wilt after more than about 8% water loss. However, during storage, some herbs can absorb water under certain conditions (Cantwell & Reid, 1993). As a result, there are some cases when sweet basil gains weight after cold storage, especially if water-stressed leaves are placed into a high humidity package with free moisture.

9. Humidity

The optimum humidity for leafy vegetable storage is usually around 95-98 % RH. In this range, the water content within the products will be in a balanced condition. This range shall prevent early decay and dehydration while avoiding surface moisture that might promote pathogen growth (Aguero et al., 2008).

2.5 Chilling injury

As mentioned earlier, sweet basil is a chilling-sensitive product. This characteristic is common among tropical and subtropical plants (Aharoni et al., 1993). Cold storage will cause CI in basil which might appear in the form of blackening (Sevillano, Sanchez-Ballesta, Romojaro, & Flores, 2009), oxidative stress (Sevillano et al., 2009), loss of aroma (Cantwell & Reid, 1993) and essential oils (Spence & Tucknot, 1983).

2.5.1 CI mechanism

CI probably starts with damage to cell membranes (Lyons, 1973). CI initiates when the permeability of cell membranes is disrupted. In the process of CI, liquid-crystalline cell membranes solidify into a gel structure (Lyons, 1973; Rui et al., 2010). The change is due to both the direct effect of lowering the temperature and an increase in the degree of saturation membrane of fatty acids through peroxidation; and changes in the ratio of sterol to phospolipid, coupled with the degradation of galactolipid. CI becomes irreversible whenever membranes rupture as indicated by electrolyte leakage or the rise of malonyl-dialdehyde production (Lijuan, Jianguo, Yongkang, Guohua, & Mujumdar). Malonyl-dialdehyde is the final product of fatty acid peroxidation (Hodges, DeLong, Forney, & Prange, 1999; Wise & Naylor, 1987).

The mediators of CI might be the reaction of LOX and antioxidant (Wongsheree et al., 2009) or the activity of PPO enzyme (Degl'Innocenti et al., 2005) and or phenylalanine ammonia lyase (PAL) and POD (Degl'Innocenti et al., 2005; Mayer & Eitan, 1979). LOX is the enzyme that is responsible for lipid peroxidation process (Pinhero, Paliyath, Yada, & Murr, 1998). Antioxidants serve as a plant's defence mechanism system, especially to protect chloroplasts and mitochondria against ROS that are caused by environment stress. The factor that sets chilling defence apart from other defence mechanisms is the action site. In chilling, the site of ROS production and detoxification is the cell membrane but it will take place in the chloroplast if chilling stress is combined with other abiotic stresses, such as intensive light and toxic substances (Alscher et al., 1997).

Two antioxidants that help protect against CI are ASA and glutathione. They can protect the chloroplast by regulating the transcription of stress tolerant genes (Alscher et al., 1997). Ascorbatic acid relates to the chilling tolerance of tomato fruit (Stevens et

al., 2008), while a study on pea leaves shows that the activity of glutathione reductase increases when they are exposed to cold temperature (Edwards, Enard, Creissen, & Mullineaux, 1994). Both ASA and glutathione are more abundant in the leaves of chilling-tolerant tomato, *Lycopersicon hirsutum,* rather than chilling-sensitive tomato, *L. esculentum* after they are exposed to chilled temperature of 2℃ (Walker & Mckersie, 1993).

CI symptoms are not always immediately visible once the products are removed from cold storage. A period of temperature at 20°C may be required to generate visible blackening since the rate of blackening is slow at lower temperatures. It is known that during transfer from cold to room temperature, catalase activity decreases sharply, as does superoxide dismutase (Ferrante et al., 2009) that is responsible for ROS scavenging and this condition exacerbates the oxidative stress caused by cold storage (Ranwala & Miller, 2000).

A period of time with temperature of 20°C for visible blackening can vary among products. For example, it takes around 3 days for peaches (Wang, Chen, Kong, Li, & Archbold, 2006), and one day for tomato (Artes & Escriche, 1994). However, there is no record regarding the period of 20°C required for visible blackening on basil.

2.5.2 Blackening

Blackening is a process in which the colour of the agricultural products becomes darker than their original tint (Wongsheree et al., 2009). Blackening usually relates to chilling injury on *Ocimum* species such as lemon, holy, and sweet basil (Wongsheree et al., 2009). In most cases with sweet basil leaves, blackening tissues/leaves could turn slimy and some might develop mould. At this stage, the symptom can hardly be differentiated from natural senescence since it may involve changes in the same genes that also cause senescence (Gan & Amasino, 1997; McConchie, Lang, Lax, & Lang, 1994).

Figure 2 Blackening on basil (Preliminary research, unpublished)

2.5.3 Oxidative stress

Oxidative stress is a condition where an imbalance in cells between reductive or oxidative processes occurs (Alscher et al., 1997). CI can generate a secondary stress, such as oxidative stress since low temperature can contribute to the loss of membrane function by increasing ROS that promotes lipid peroxidation (Sevillano et al., 2009). A study conducted on CI-resistant and susceptible loquat fruit, Qingzhong and Fuyang, shows that the ratio of unsaturated fatty acid and saturated fatty acid of Qingzhong is higher than that of Fuyang. The level of SOD and catalase (CAT) which are responsible for ROS scavenging is also higher, while LOX remains lower (Cao, Yang, Cai, & Zheng, 2011).

Resistance levels of leaves to oxidative stress can be influenced by the ages of the leaf. Young leaves are found to be more resistant to oxidative stress due to the fact that they have a higher and more stable antioxidant activity than do old leaves (Alscher et al., 1997).

2.6 The techniques that could be used to preserve sweet basil

2.6.1 Low temperature storage

Low temperature storage is one of the post-harvest strategies to preserve agricultural products. It can delay senescence by reducing respiration rate and slowing down the development of fungal disease (Aghdam & Bodbodak, 2013).

To a certain point, low-temperature would be beneficial since phenolic compound production is limited during low-temperature storage (Wongsheree et al.,

2009). Thus, blackening processes occur more slowly after chilling treatment. However, basil and other chilling-sensitive products are more likely to show different results.

Herb leaves like basil are very likely to decay easily since their metabolism rate is fast. Low temperature can reduce respiration and increase water loss, which is the major cause of quality degradation during storage (Aharoni et al., 1993). Furthermore, the amount of phenolic compounds in one basil species (lemon basil) has nothing to do with the rate of blackening in low temperature storage (Wongsheree et al., 2009). There are probably other factors that also influence blackening caused by CI on basil.

Basil will develop CI if the storage temperature falls some degrees below the threshold, but CI is also markedly affected by the duration of storage. The visible symptoms of CI will appear when basil is stored at 4-8°C for 4 days (Rolny et al., 2011) or at 6-12°C if it is stored for more than 6 days (Lers et al., 2010) and more than 9 days at 10-12°C (Chen et al., 1997; da Silva et al., 2005).

A study on the effect of cold water immersion to the leaf of african violets or *Saintpaulia* spp. (hydrocooling) shows that CI occurs only when the storage temperature drops too fast (fast cooling). The CI level decreases if the leaf has temperature drop of less than 3°C per second even if the first temperature is significantly different from the last one (Yang, Hayashi, Hosokawa, & Yazawa, 2003). Likewise, step-down cooling or intermittent warming (1 day at 20°C after cold storage) is reported to reduce CI symptoms on mature-green tomato fruits (Artes & Escriche, 1994).

It is important to note that cooling techniques using water may yield different results compared to other techniques since with the same volume of air, water is capable of removing more heat (Sargent, Talbot, & Brecht, 1988). This leads to the possibility that CI is not solely dependent on the level of temperature, duration, and cooling rate. It might also be influenced by the method of cooling. Precooling can improve the quality and the shelf life of the products of fruits and vegetables (Chonhenchob & Singh, 2003)

Because cooling rates are proportional to the difference in temperature between the product and its environment, they slow dramatically as the product nears its equilibrium temperature. A convenient way to compare cooling rates is to note the 7/8 cooling time. It is a period taken to achieve 7/8 of the maximum possible cooling; e.g. moving a leaf from 20°C to 4°C, the 7/8 cooling time is time to reach 6°C.

2.6.2 Blanching

Blanching is the process by which plant tissues are exposed to heat (in the form of hot steam or hot water) at a certain temperature and duration (Barrett & Theerakulkait, 1995). It can be used to reduce the activity of the enzyme that is mainly responsible for discoloration in vegetables products. When broccoli is blanched using steam for 90 seconds, LOX, POD, and cysteine lyase are deactivated (Barrett, Garcia, Russell, Ramirez, & Shirazi, 2000). Meanwhile, for green beans, blanching at 93°C for 0.5 and 2 minutes, respectively is needed to deactivate LOX and POD (Barrett & Theerakulkait, 1995). The temperature and duration should be limited since the level of tolerance to temperature will vary among different species and even cultivars. Abusive temperature may change the colour, flavour, texture, and chemical quality (Barrett et al., 2000). Therefore, a small test in Batch 4 was conducted, to see whether blanching changes the appearance of basil leaves and their pesto.

2.7 Pesto

Pesto whose main ingredient is basil is the name of pasta sauce that originated in Italy (Pavlović et al., 2010). It is also known as Ligurian spaghetti sauce and marketed mostly in North America.

Main problems in the manufacturing of this sort of product are the growth of microbiological organisms and chlorophyll degradation which could lead to shortening its shelf-life. Microbiological growth could be inhibited by stabilizing pH level to around 4.5, use a proper treatment of pasteurization (Food and Drugs Administration, 2002) and the addition of humectants such as sugars, salts, polyols and protein derivatives to control water activity (a_w) (Taoukis, Breene, & Labuza, 1988). To avoid chlorophyll degradation, the producers usually use acidification which is hardly accepted by consumers since it could change the taste (Severini, Corbo, Derossi, Bevilacqua, & Giuliani, 2008). Other pesto methods to prolong the shelf life are modified atmosphere, coating, and refrigeration. Pesto sauce shelf life could be up to 30 to 120 days by these preservation methods (Fabiano et al., 2000; Severini et al., 2008).

The original pesto was a mixture of basil and other materials such as cheese, walnut, olive oil, and sunflower oil (Pavlović et al., 2010), others also add garlic, potato starch, cashews and pine kernels (Severini, et al., 2008). However, none of them are to

be added for this research, for two reasons: first, they would complicate data interpretation; and secondly, olive oil alone is enough to delay the browning process of basil (Pavlović et al., 2010). This is partly because the oil provides a barrier layer that serves to limit oxygen access to cut cells in the tissue.

Olive oil is a vegetable oil that is extracted from olives (*Olea europaea*). It is known as an antioxidant material since it contains a high polyphenol concentration, the substance that is considered to influence enzymatic browning (Georgalaki, Sotiroudis, & Xenakis, 1998). By adding olive oil in the pesto processing, basil will avoid early blackening while the leaves are chopped. Blackening will only occur as pesto is exposed to oxygen by spreading it on an absorptive material like a paper towel; this can be used to develop a blackening assay (Pavlović et al., 2010).

The quality of pesto depends on the cultivar, age and developmental age of basil. Pesto quality will be better if the basil comes from the younger and earlier cuts since (a) the level of basil fiber becomes higher during development and (b) the basil composition will vary, with changes in essential oils, phenolics and antioxidant constituents. Higher fiber may increase the time required for processing (Carlo et al., 2013) and time is crucial in blackening process. These are important since one of the purpose of this study is to develop method of measuring blackening rate of pesto. It would be beneficial to know some factors that might influence pesto quality during processing, to ensure that pesto is produced consistently and the measured results are comparable.

CHAPTER 3. MATERIALS AND METHODS

3.1 Preliminary research

This thesis built on a special topic research as the basis of its method. Special topic research was a small project conducted during academic year of the Master Degree Program in Agriscience (horticulture). The aim of this project was to generate a replicable assay for blackening rate of pesto made from basil leaves and then use it to test the varying blackening rate of pesto made from old and young leaves.

3.1.1 Plant Materials

Sweet basil plants for special topic were taken from two different sources, basil plants from Superherb Company and basil plants that were reared from seeds in Plant Growth Unit (PGU) of Massey University. The seeds came from Lefroy Valley Company, planted on 3 July 2015 during winter at a temperature of approximately 16–23.5°C. They were transplanted from seed bed to small pots (1-2 plants per pot) after 35 to 49 days. The plants from Superherb arrived at the PGU on 2 September 2015 and were also transplanted from their original media to larger pots a month later.

3.1.2 Sampling and pesto processing

Samples for preliminary research were used for two purposes: to develop a suitable method for assessing pesto blackening rate and to test the blackening rate of pesto made from different parts of the plant (old and young leaves). For the first purpose, samples were taken from plants that had never been transplanted or were still growing in the seed bed. All parts of the plants, except the roots, were blended into pesto. For the second purpose, samples were taken from transplanted plants, older and younger leaves were separated into different pesto samples.

Both groups of samples were harvested in the morning since harvest time during the day might affect substrate composition in the basil leaves (Fischer et al., 2011; Makri & Kintzios, 2008). Roots were cut seconds before they were made into pesto to ensure the freshness of the leaves.

A simplified ‘pesto’ was made from 20-50 g of basil blended with olive oil in the ratio of 1:1 by weight (Pavlović et al., 2010) for approximately 20 seconds. This was important for pesto since the duration of the blending probably affects the starting

colour of the pesto and the rate of blackening. The mixture was then placed in a 75 mL plastic jar and closed.

3.1.3 The Blackening Assay

Pesto measurement was conducted in the postharvest laboratory, Riddet complex of Massey University. The temperature of the room was maintained at approximately 20°C. For the first purpose, pesto were placed in the two different conditions in between the measurements. The first treatment was placed in the same room where the measurement was conducted and the second was put into a lamp box, a white box with the lamp inside that kept the temperature in the box higher than 20°C and thus made pesto potentially deteriorate faster. This was done to test whether temperature conditions affected the rate of pesto blackening.

Each ca. 5 g of pesto sample was taken from the jar and immediately spread on a two-ply paper towel in a pre-marked circle approximately 18 mm in diameter. A rubber O-ring, 20 mm in diameter and 3.5 mm in thickness, was used to separate samples from a reflectance spectrophotometer (Figure 4). This was done to prevent physical contact between the oily pesto and the spectrophotometer optical chamber; any stains on its surface or interior chamber may cause differences in the readings.

As the olive oil in the mixture was absorbed slowly by the paper towels (Phifer & Costello, 1992) the surface was exposed to oxygen and started blackening. A series of colour measurements were made immediately after pesto had been smeared and the process continued every 5 minutes for the next 30-60 minutes. The readings (Lightness/ L value, chroma/ C value and hue angle/ h value) were plotted against time and used in calculations to assess the blackening rate.

(A) (B)

Figure 3 Instruments of the experiment (A) O-ring placed on spread pesto and (B) reflectance spectrophotometer

A Konica Minolta CM-2600d spectrophotometer was used in this study since it is capable of determining the colour change of pesto. There are many possible units produced by spectrophotometer. They are L value for measuring the brightness of pesto, ranging from 0-100 that respectively represents black to white, h value for the type of colour (the hue) and browning index (or rate of colour change, C value for saturation or the vividness of colour and many other attributes that could be used to describe the colour resulting from enzymatic or non-enzymatic browning (Barreiro, Milano, & Sandoval, 1997; Lijuan et al., 2005; Lozano & Ibarz, 1997). All units can be measured in two ways: SCE (Specular Component Excluded) and SCI (Specular Component Included). For the prelimanary test, both SCE and SCI were used to compare the result, but the seven batches of experiment were only using SCE.

3.2 Seven batches of experiment

Basil samples for the main experimental series were taken from the plants cultivated and harvested by Southernfresh company in Hamilton, New Zealand. The age of the plants was at least a month and some stems turned woody. Basil side shoots were harvested and sent in the form of stalks by an overnight courier to the laboratory of postharvest within less than one day. Especially for Batch 4, plants were purchased in pots cultivated by Superherb company and sold by Pak ‘n’ Save. The age of these plants was unknown but seedlings were young with soft stems.

Seven batches of basil were used for these pesto blackening studies. Basil stems were made into pesto using the method of preliminary study with some modifications.

Most importantly, only freshly-plucked leaves were used for pesto making, to reduce variability resulting from stem ageing. Black (possibly rotten) leaves that might occur in some experiments were included in the pesto processing. Also, a minimum weight of 40 g of basil leaves was used per jar to eliminate variability from occasionally small samples in the earlier work.

During assessment of the effects of storage temperature, basil stalks were put into 20 x 30 cm polyethylene plastic bags with 4 punched holes each ca. 8 mm in diameter. Bags were placed in cardboard boxes with or without polyliner plastic wraps. Bags and contents were weighed separately to allow for an accurate measurement of weight loss during storage (in mg). Some condensation water might be included in the weight of the leaves after storage as it remained inside the plastic bag when they were weighed. In addition, basil quality was assessed after storage. Quality was primarily defined as the proportion of rotten leaves. These blackened leaf parts were found to be the major form of deterioration for NZ-grown basil. It was not possible to distinguish the cause of this leaf blackening and tissue collapse; it could be a result of age-related senescence or a direct consequence of CI; their symptoms were found to be indistinguishable. The early stage of CI that might appear as spot leaf were found on the leaves when they arrived to the laboratory or before they were even having any treatments. Therefore these spots could not be used as the indicator of CI.

3.2.1 First Batch (fast cooling and step-down cooling)

The treatments of this Batch were based on the possibility that they will have a very different result in the quality of both basil leaves and the produced pesto. It was suggested that fast cooling and step down cooling will produce a really distinct CI and weight loss level on the leaves (Yang et al., 2003) and hypothetically when the leaves made into pesto, they may also have a different blackening rate. This Batch also aimed to find out the emergence time of CI symptoms after stems had been stored and warmed up to room temperature (20°C) (Wang, Chen, Kong, Li, & Archbold, 2006; Artes & Escriche, 1994).

Fast cooling was achieved by putting 50-60 g of basil stalks into mesh bags, with a logger inside the bags. Mesh bags are loose net-shaped containers made from soft flexible plastic, so basil leaves will not be squeezed when inserted. For fast cooling, some

bags were hung in front of the fans in the TCR until they reached 4 or 6°C. Then, they were stored inside cardboard boxes in 4°C TCR for certain times. Step-down cooling was achieved by 2 steps of slow cooling. Basil stalks weighing 50-60 g were put into mesh bags or plastic bags with 4 holes, with a logger inside the bags, and storedinside cardboard boxes at 10°C for the first day and 4°C for the second day. Loggers were set to measure temperature in 10 second intervals for fast cooling, 15 minute intervals for step-down cooling to 10°C and 5 minute intervals for second day of step-down cooling to 4°C.

All the treatments were arranged as follow:

1. 3 bags of 60 g basil leaves for fast cooling to 4°C + a day of storage at 4°C, with polyliner (FC1)
2. 3 bags of 60 g basil leaves for fast cooling to 4°C + two days of storage at 4°C with polyliner (FC2)
3. 2 bags of 60 g basil leaves for fast cooling to 6°C+ a day of storage in 4°C, without polyliner (FC3)
4. 3 bags of 60 g basil leaves for fast cooling to 6°C + two days of storage in 4°C, without polyliner (FC4).
5. 3 bags of 60 g basil leaves for step down cooling: a day of storage at 10°C and a day at 4°C with polyliner (SDC1)
6. 3 bags of 50 g basil leaves for step down cooling: a day of storage at 10°C and a day at 4°C with polyliner (SDC2). This was an additional treatment to examine the effect of the amount of basil on the cooling rate.

Each of the replications of the treatments was observed for weight loss and rotten leaves. Leaves were then blended into pesto after 1, 2, or 5 days at 20°C. Percentage of rotten leaves was obtained from the percentage of decayed leaves to the total sample within the same bag and percentage of weight loss value gained from the percentage of the weight difference between before and after storage to total amount of initial weight. The same measurement of weight loss and CI was applied for subsequent batches.

3.2.2 Second Batch

This batch aimed at comparing the different durations of 4°C storage (T1, T3, T6); to determine whether 10°C is a non-chilling temperature, and to test whether step-down cooling or step-up warming can technically reduce CI symptoms and improve pesto quality. Basil quality in each of these treatments was assessed after bags were left overnight at 20°C, and pesto was made immediately after this quality assessment. The result will help in the selection of a standard chilling for subsequent batches and narrowed the variability in blackening rate found in Batch 1.

The treatments of Second Batch were arranged as follow:

1. 3 bags of 50 g of basil stored at 4°C for one day + 20°C for one day (T1)
2. 3 bags of 50 g of basil stored at 10°C for one day + 4°C for one day + 20°C for one day (step-down cooling/T2)
3. 3 bags of 50 g of basil stored at 4°C for two days + 20°C for one day (T3)
4. 3 bags of 50 g of basil stored at 10°C for one day + 20°C for one day (T4)
5. 3 bags of 50 g of basil stored at 4°C for one day + 10°C for one day + 20°C for one day (step-up warming/T5)
6. 3 bags of 50 g of basil stored at 4°C for three days + 20°C for one day (T6)

All the samples were stored without polyliners.

3.2.3 Third Batch

The Third Batch experiment was conducted to find the source of inconsistencies in the previous experiments. There were more technical replications (each batch of pesto was assessed using 3-9 samples spread on paper towels); and the same biological replication as before (i.e. three separate batches of pesto made from separate stored bags). There were only three storage durations tested, i.e. fresh, after 3 d at 20°C, and 7 d at 10 C, predicted to be roughly the limit of fresh storage at each temperature. The treatments of Third Batch were arranged as follows:

1. 3 bags of 50 g of fresh basil (un-stored) (C1)
2. 3 bags of 50 g of basil stored at 20°C for three days (C2)
3. 3 bags of 50 g of basil stored at 10°C for seven days (C3).

3.2.4 Fourth Batch

The idea of conducting the Fourth Batch experiment was based on the fact that several findings in previous batches showed high variability in starting colour. 'Odd' colour was the phrase that was used to express the condition when pesto showed a much brighter colour than usual (L value > 50), so that even the bare eyes could see the difference. It was predicted that enzymatic processes related to these differences and blanching basil leaves in hot water might deactivate oxidative enzymes and make the appearance of the pesto more uniform (Barrett et al., 2000; Barrett & Theerakulkait, 1995). However, abusive temperature could change the colour, flavour, texture, and chemical quality of the leaves (Barrett et al., 2000). Therefore, this Batch was meant to test the reaction of basil leaves to blanching. The samples used in this Batch were the most readily available basil in town, which was SuperHerb basil. Basil was separated into two treatments without biological replication:

1. 50 g basil leaves for hot water treatment (HW), ca. 98°C
2. 50 g basil leaves for tap water treatment (TW) as control.

Hot water treatment was conducted by soaking basil leaves in boiled water and tap water treatment was conducted by soaking them in tap water. Each sample was immersed in water for one minute and then shaken with paper towels, blended and placed into 2 separate jars of pesto.

3.2.5 Fifth Batch

Another possible explanation for 'odd' starting colour was sample variability at harvest. It was suspected that the age of the samples was not the same since their stalk's hardness was not in uniformity. Some stalks were harder than others as might happen if they were harvested from older plants that had already been sampled from several times. Therefore, this Batch of experiments was conducted to test whether sample variability influences the results (although preliminary research had shown no significant difference in the blackening rate of pesto made from old or young leaves in basil seedlings from Superherb).

Another aim of this Batch was to compare the results with those of the Fourth Batch (that used Superherb basil). In addition, icy water treatment was applied immediately to blanched leaves to stop the heating process from continuing after blanching time was over.

The treatments for Batch Five were divided into 2 parts. First part (treatment 1 & 2) was assessed on the day basil arrived at the laboratory and the second part was done on the second day (treatment 3 & 4), after storing basil in the original box overnight at room temperature.

1. 3 bags of 50 g of basil leaves from tough stems (‘older’ ones, OL)
2. 3 bags of 50 g of basil leaves from soft green stems (‘younger’ ones, YL)
3. 3 bags of 40 g of basil leaves soaked in tap water for 10 seconds and icy water for one minute (TI)
4. 3 bags of 40 g of basil leaves soaked in hot water (98°C) for 10 seconds and icy water for one minute (HI).

3.2.6 Sixth Batch

There were some inevitable changes to the equipment used in this Batch, resulting from the previous equipment being sent away for maintenance. The first was the use of different type of spectrophotometer, type CM-700d (Figure 4). Both the previous and current spectrophotometer record the same parameters but there were slight differences in operation. Second change was the use of different type of O-ring. A thicker O-ring was required to protect spectrophotometer from staining caused by pesto, and it was initially used from the fourth day of Batch Six measurements. Thus, there were 3 measurement techniques applied in this Batch. However, each replicated the same treatment using only one type of o-ring, never in combination.

Figure 4 Spectrophotometer Konica Minolta CM-700d

(https://www.konicaminolta.eu/en/measuring-instruments/products/colour-measurement/spectrophotometers-portable/cm-700d-cm-600d/introduction.html)

Changing the spectrophotometer and 'O' ring size can affect variables such as starting colour and final colour. Thus, they would not be comparable even among the treatments within the same batch and absolutely could not be compared with those of other Batches. Furthermore, it gave new insight about spectrophotometer usage: the distance between the surface of the sample and the spectrophotometer aperture made a significant difference to the result.

Temperatures of 4°C and 12°C were chosen in this Batch and longer cold storage durations were tested to determine whether storage at 4°C (a known chilling temperature) would cause apparent chilling injury over this longer period. 12°C was chosen for comparison as it may represent a non-chilling temperature. Cold storage duration of this Batch was chosen to cover the predicted day of onset of CI, i.e. 3-6 day.

Batch Number Six was arranged as follows:

1. 3 bags of 50 g of basil leaves stored at 4°C for 3 days + a day in 20°C (TD1)
2. 3 bags of 50 g of basil leaves stored at 4°C for 4 days + a day in 20°C (TD2)
3. 3 bags of 50 g of basil leaves stored at 4°C for 5 days + a day in 20°C (TD3)
4. 3 bags of 50 g of basil leaves stored at 4°C for 6 days + a day in 20°C (TD4)
5. 3 bags of 50 g of basil leaves stored at 12°C for 3 days + a day in 20°C (TD5)
6. 3 bags of 50 g of basil leaves stored at 12°C for 4 days + a day in 20°C (TD6)
7. 3 bags of 50 g of basil leaves stored at 12°C for 5 days + a day in 20°C (TD7)
8. 3 bags of 50 g of basil leaves stored at 12°C for 6 days + a day in 20°C (TD8)
9. 3 bags of 50 g of fresh basil leaves/ without storage (TD9)
10. 3 bags of 50 g of basil leaves stored in the original box at 20°C for 3 days + soaked into icy water for one minute (TDIcy).

The last treatment (TDIcy) was additional. It was conducted to see whether the icy water that creates 'odd' starting colour in previous Batch was replicable, even though the samples were stored for 3 days. After they were stored, they were put in the icy water for about one minute, shaken and made into pesto.

TD9 and TDIcy were measured by using small o-ring (type 1), TD1 and TD5 by side A of the big o-ring (type 2), and the rest were using side B of the big o-ring (type 3). Because of the shape of the 'O' ring, it led to different distances from the sample to the aperture: approximately 3.5 mm for type 1, 13 mm for type 2 and 6.5 mm for type 3.

(1) (2) (3)

Figure 5 Three types of O-ring (1) small size (2) big o-ring side A (3) big o-ring side B

3.2.7 Seventh Batch

Batch Number Seven continued to use all techniques from Batch Six, with side B of big O-ring (type 3) and the new spectrophotometer (CM-700d). The duration was one day shorter than Batch 6 since the result of Batch 6 showed that CI already caused more than 50% of rotten leaves after only 3 days in cold storage. In order to explore the threshold for CI, two different temperatures were used: 7°C and 12°C.

Seventh Batch was arranged as follows:

1. 3 bags of 50 g of basil leaves stored at 7°C for 2 days + a day at 20°C (CK1)
2. 3 bags of 50 g of basil leaves stored at 7°C for 3 days + a day at 20°C (CK2)
3. 3 bags of 50 g of basil leaves stored at 7°C for 4 days + a day at 20°C (CK3)
4. 3 bags of 50 g of basil leaves stored at 12°C for 2 days + a day at 20°C (CK4)
5. 3 bags of 50 g of basil leaves stored at 12°C for 3 days + a day at 20°C (CK5)
6. 3 bags of 50 g of basil leaves stored at 12°C for 4 days + a day at 20°C (CK6)
7. 3 bags of 50 g of fresh basil leaves /without storage (CK7).

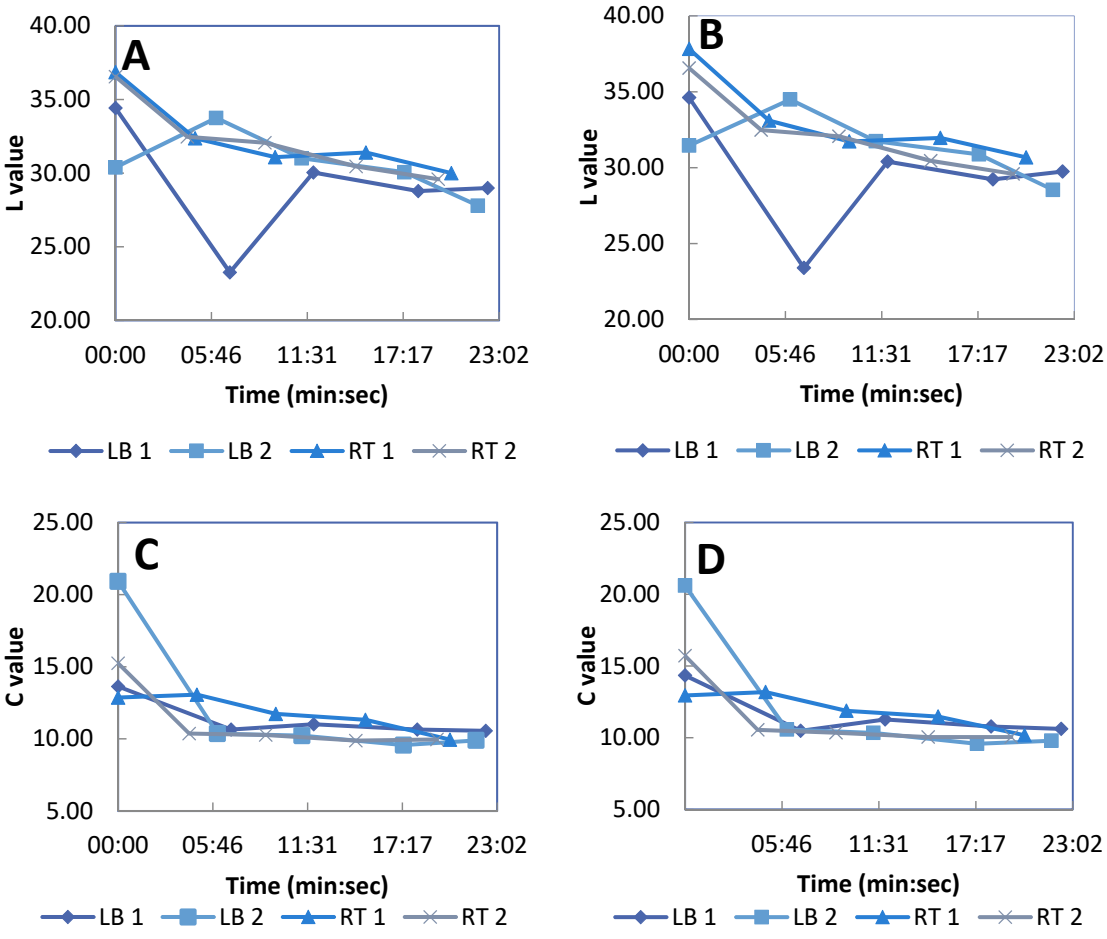
CHAPTER 4. RESULTS AND DISCUSSION

4.1 Preliminary Research

There were two experiment results in preliminary study (first year research) that can be used as the basis for a method for measuring blackening rate of pesto in the second year research: colour proxy testing and plant part testing. Curve fitting developed in year two was used in the processing of preliminary data to provide comparisons between first and second year experiments.

4.1.1 Proxies for describing colour change of pesto

When pesto was prepared from young basil seedlings obtained from 'Superherb', and then spread on paper towels, it began to blacken quickly. Figure 6 shows the result of blackening rate of all samples. The results of all proxies displayed in pairs, the left side (A, C, E, G & I) for specular component excluded (SCE) and the right side (B, D, F, H & J) for specular component included (SCI).



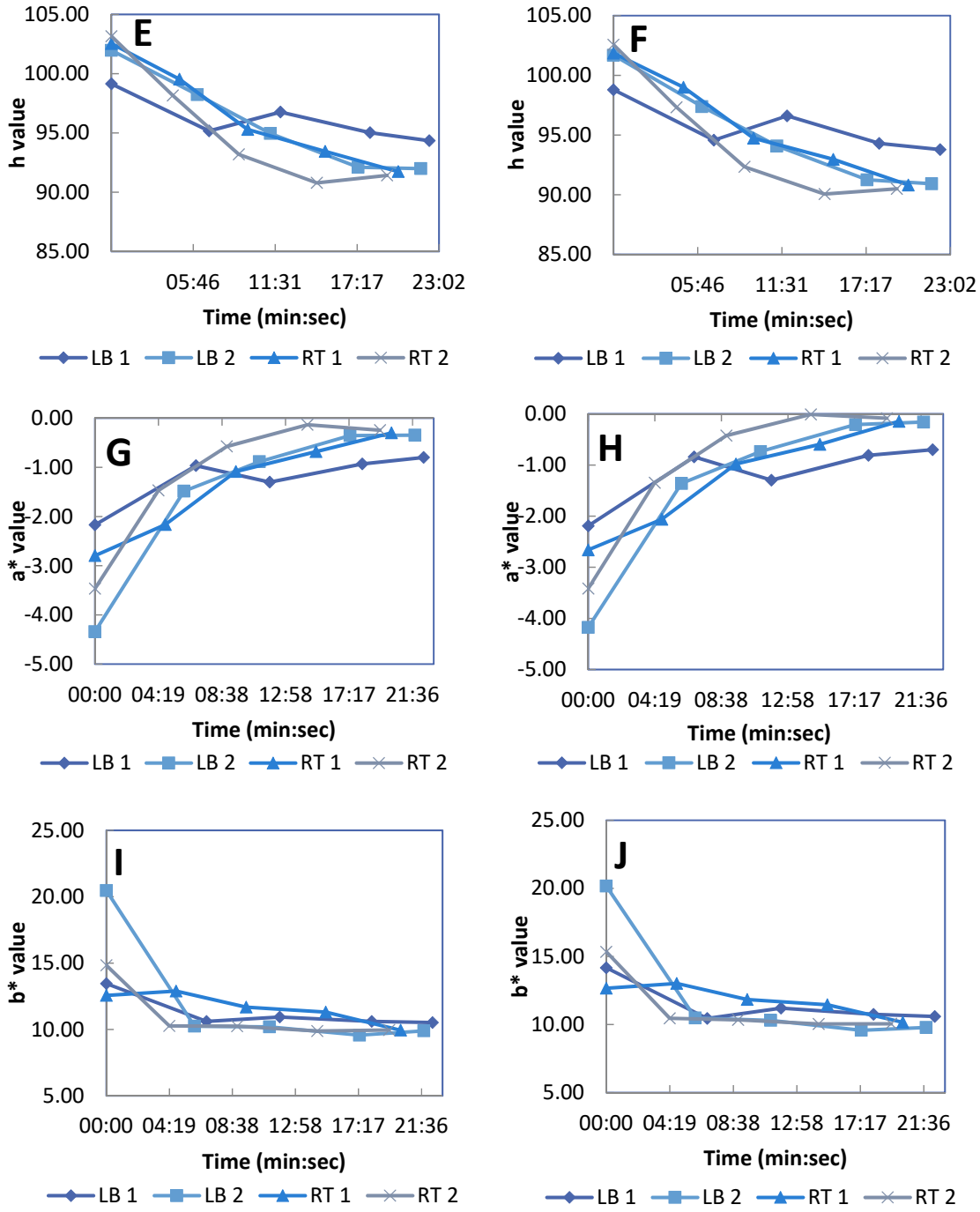


Figure 6 Colour change of pesto in the ten indicators: (A) SCE of L (B) SCI of L (C) SCE of C (D) SCI of C (E) SCE of h (F) SCI of h (G) SCE of a* (H) SCI of a* (I) SCE of b* (J) SCI of b*

Description:

L : Lightness/ luminous intensity of colour
h : hue angle/ colour
C : Chroma/ vividness of colour
a* : Red/green colour scale
b* : Yellow/blue colour scale
SCE : Specular Component Excluded
SCI : Specular Component Included
LB : label for pesto that was placed in the lamp box between measurements (> 20°C)
RT : label for pesto that placed at the room temperature between measurements (20°C).

There was no major difference between the use of SCE or SCI data, as we can see in the Figure 6. All the pairs of graphs display similar pattern. They look identical. Therefore, using either one of the data was acceptable. SCE data were selected and used consistently for all remaining experiments.

All measured proxies (L, C, h, a* and b*) showed changes over time. L value showed that the rate of change in samples that were placed in the lamp box (LB) tend to have more fluctuation than those were placed in the room temperature (RT). It seemed that temperature between measurements played an important role for L values. Therefore, L values would be closer to the nature of blackening rate if samples were placed in the room temperature throughout measurement time.

Fluctuation occurs in the other proxies too but only to one of the replication of each treatment, e.g. h and a* value of LB1. Others, such as C, a* and b* value of LB2 and RT2 only show sharp decrease or increase between the first and second measurement. These led to conclusion that this was not due to the treatment. Overall, h value seemed to change more consistently between measurement times and was also quite consistent among replications. H value was used to determine colour change in the other study of pesto sauce (Severini et al., 2008) and it was known as the best proxy to define special colour distribution (Ihl, Shene, Scheuermann, & Bifani, 1994). Therefore, at this point, h value was selected to describe the nature of the blackening process of pesto (although this result is different from the experiment using Superherb basil).

The occasional sharp fluctuations over time indicated the importance of more replication and close attention to standardised procedures to minimize variability during experiments. Furthermore, colour change begins immediately after pesto was spread on the paper towels; which means it was vital to standardise procedures especially in the first 5 minutes. Therefore, it was not just the interval between measurements that needs to be standardised, but also the time between pesto being laid on the paper towel and first measurement.

4.1.2 Data transformation and fitting curve

Data from spectrophotometer were transformed into negative exponential by ‘what if’ analysis of Microsoft Excel, to create a fitted curve from the 5 minutes measurements. The formula used was:

$L\ calc = L_{final} + (L_{initial} - L_{final}) * e^{-kt}$

Where L calc was the L value at time t, L final was the L value at the asymptote (estimated from the graph after 60 min), L initial was the starting L value, K represented the blackening rate, and T was the time in minutes.

'What if' analysis in Microsoft Excel was used to find the value of K that produces the lowest 'sum of squares' throughout the 60 minutes analysis; that was, the estimated values of L at each time point were subtracted from the observed values, the difference was squared, and Excel varied K until the sum of squares was minimised. This modelling approach allowed reliable comparisons to be made amongst experiments.

In this curve, the K value represented the rate of colour change; higher K values represent faster blackening rates. The initial L value was also found to vary significantly from sample to sample. In general, the curve had flattened close to its final asymptotic value by 60 minutes; hence, the choice of this period for consistent analysis.

Some data cannot be fitted well to a negative exponential. These samples were labelled as 'noisy' data as indicated by an arbitrary sum of squares greater than 10 (SS>10). The lower the SS, the better the fit is. The data with a high SS were usually observed to have a fluctuating curve for unknown reasons.

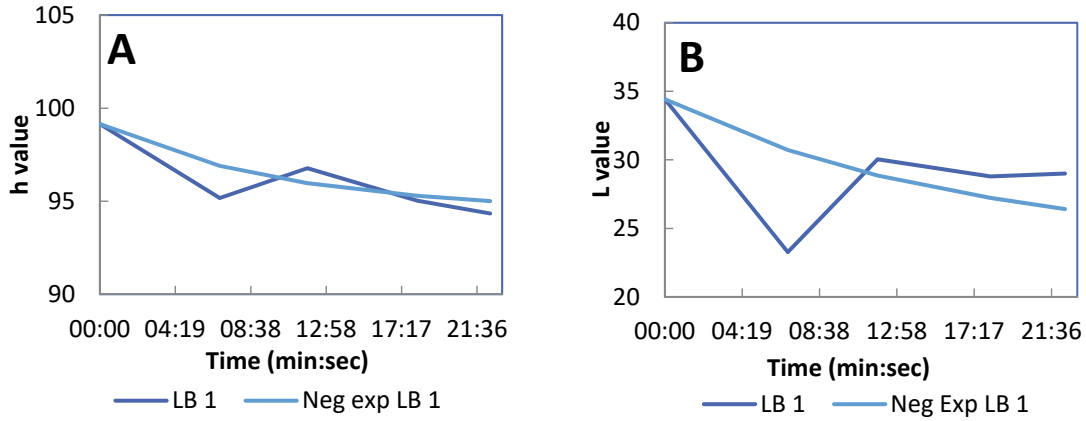


Figure 7 The example of (A) a fitted curve with SS= 4.12 (B) a noisy data curve with SS= 66.13

4.1.3 Plants part testing

Table 1 showed the result of colour change for two different parts of basil plants where old leaves were taken from the leaves on the lower part of basil seedlings and young leaves from upper part, closer to the position of flower buds (but omitting any leaves adjacent to flowers). The data were important since the leaf samples from the

Southernfresh which were used in the seven batches of experiment also probably varied in age. Age is one of the factors that affects chemical amount and composition of basil (Dudai, et al., 2001; Fischer et al., 2011; Fleisher, 1981; Gershenzon, McConkey, & Croteau, 2000; Gupta, 1994; Paton, Harley, & Harley, 1999; Szabo & Bernarth, 2002) and eventually could also affect metabolic processes such as the rate of senescence and discolouring (Alscher et al., 1997; Cantwell & Reid, 1993; Ranwala & Miller, 2000; Viacava et al., 2014).

It is important to note that samples for this experiment were taken from fresh plants. Leaves were separated from the root in the lab and washed by tap water to remove potting mix. They were left at room temperature for not more than 2 hours to dry. Occasionally, there was free surface water on the basil from condensation.

Table 1 Pesto colour of preliminary research

Treatment	Starting colour (L value)	K value	SS	SE
OL	46.14 a	0.054 a	3.91 a	4.75
YL	47.50 a	0.062 a	4.27 a	8.36

The numbers followed by the same letter in each column are not significantly different according to independent samples T test at 95 % level of confidence ($P < 0.05$). OL: Old leaves, YL: Young leaves.

There was no significant difference in all variables between these two ages of leaves according to T test. Standard error between replicates was also very low, although some individual samples appear to have paler starting colours of pesto. It can be seen by the mean of starting colour which was nearly 50 in L value. In this experiment pesto with pale colour was common among young and old leaves.

Old leaves senesce slower than the young leaves although they were not significantly different. This result was totally different from the result of study by Ranwala and Miller (2000) which states that old leaves senesce faster than the younger ones. It occured due to the differences in the content of carbohydrate and anti-oxidant that tend to move upward, from old to young leaves during developmental stage in the field. However, the materials used in the study were different. Ranwala & Miller use Lily leaves for their study and they were taken at the mature flower bud stage, while this study used basil leaves at the early flower stage.

4.1.4 Conclusion of the preliminary research

Some findings of the first-year experiment might be useful to develop the method for measuring pesto quality in the second year research. They were replication number, time for processing and the placement of pesto, fitting curve, and sampling techniques.

Two replicates were not enough to represent a treatment, at least one more replicate was required. The time needed for processing pesto and placing them onto paper towel was very important since the most rapid change occur in the first five minutes of measurement. The difference in just seconds between one pesto to others might create a bias to the result.

Temperature of the pesto storage place in between measurements was also important since temperature above room temperature could cause fluctuation in L value. However, h value showed a more constant change in these differences.

Data transformation was necessary to simplify fluctuations between measurements, established K value and sum of squares. K value was an indicator of blackening rate, while high K value meant fast blackening rate and vice versa. Sum of squares indicates the reliability of fitting curve created by data transformation. Data with SS level more than ten were labelled as 'noisy' data or lack of reliability. An analysis must be carried out to see if include or exclude these data will affect the conclusions.

There were no significant differences between blackening rate of pesto made from the top leaves (young) and bottom (old) of potted seedlings, suggesting leaf age should not lead to different pesto blackening rates of basil. The only problem with this was that the range of ages tested was quite small. Although 'Odd' colour pesto at the start was sometimes found in the preliminary result, this did not contribute significantly to sample variances since the L average of 'normal' pesto was also high.

All the notes from the preliminary study were used in the second year of study and reassessed for any change with different source, condition, and freshness of the samples. The second year of study consisted of 7 batches of experiment which will be further discussed in the next sections.

4.2 First Batch

Temperature logger was used in the First Batch to one of the replicate of every treatment to show the different rate of temperature change between fast cooling (FC) and step-down cooling (SDC). The logger showed the change of temperature inside the bag of the leaves during cooling process (Figure 8). It constantly decreased from around 20°C or room temperature to the temperature that was set (4°C).

Fast cooling showed a rapid temperature decrease on basil leaves, faster than slow cooling. It even started to decrease within a second when logger was switched on (see the starting temperature of graph A, which is lower than B). It only needed less than 6 minutes to make basil leaves cool down to 4°C when held in mesh bags directly in front of the fans of TCR. While in step down cooling, where basil was put in bags inside a box that was moved between temperatures, it needed around 4 hours to equilibrate for each step. Using 7/8 cooling time, fast cooling was achieved within 5 min 10 sec; and slow cooling inside a box takes 3 h 35 min to 3 h 50 minutes. This result showed that even 'slow' cooling was achieved within 4 h. Slow cooling was very simple and was used in all subsequent batches.

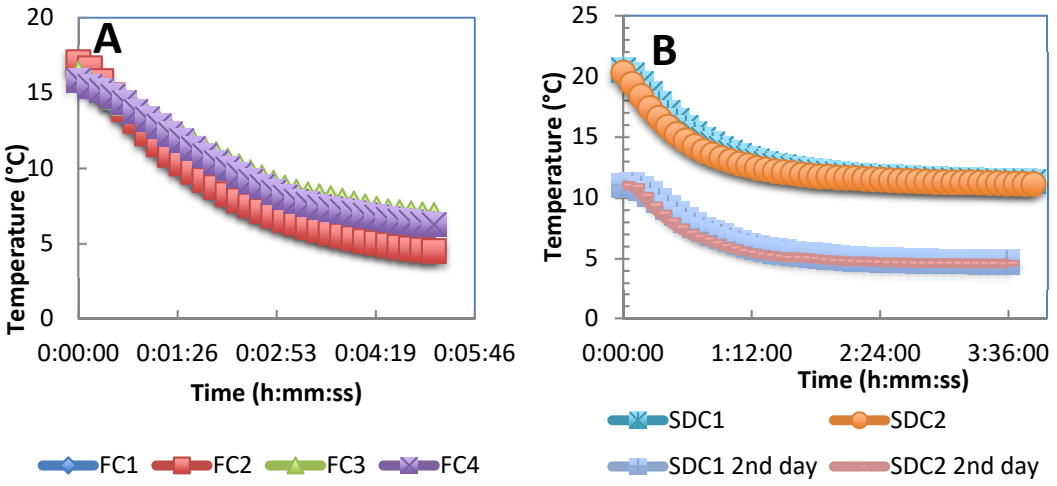
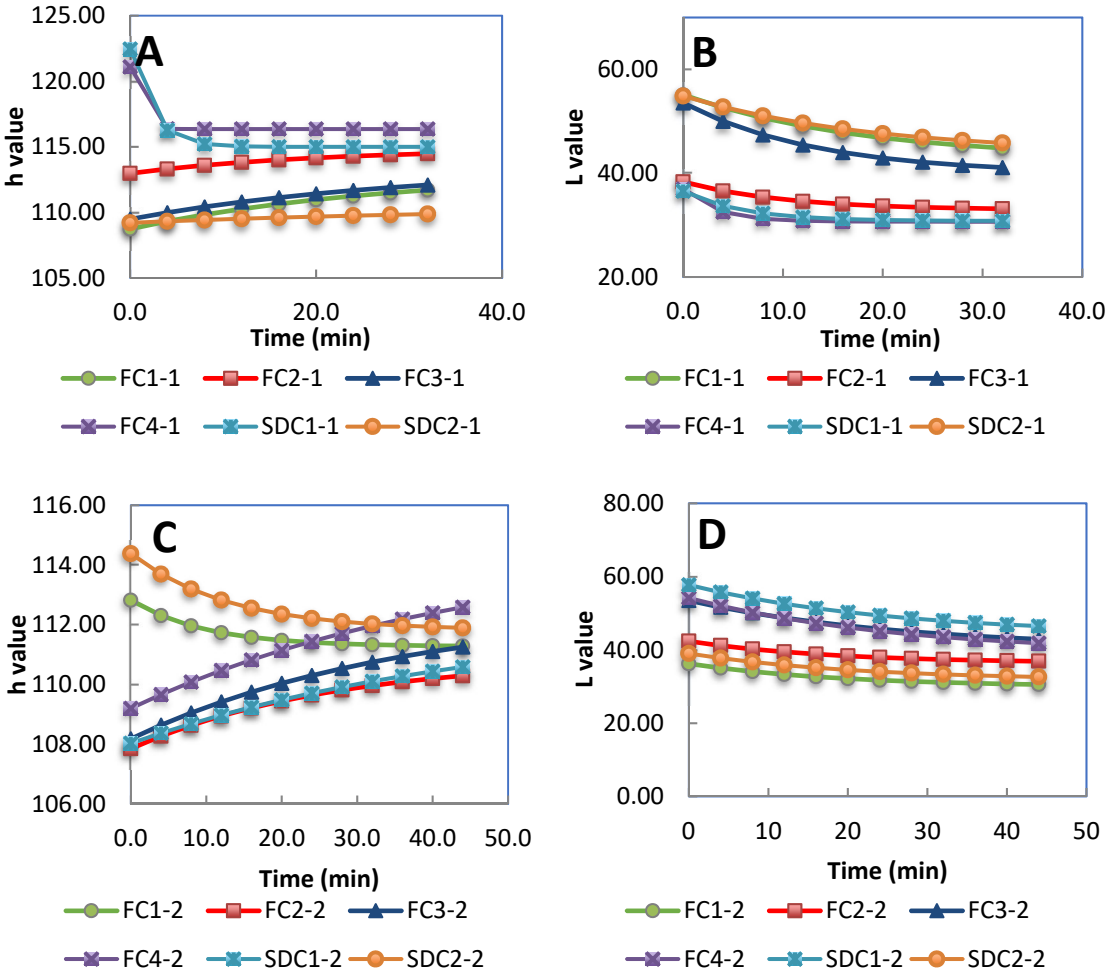


Figure 8 Temperature log of (A) fast cooling and (B) step down cooling. FC1&3: Fast cooling + 1 day at 4°C, FC2&4: Fast cooling + 2 days at 4°C, SDC1&2: Step down cooling 60&50 g

4.2.1 Proxy selection

In the preliminary research, h value appeared to be the most suitable proxy to show the nature of blackening rate. However, these data came from fresh samples with soft slender stems, and pesto was made from both stems and leaves. The samples used

in six of the seven batches in the second year were grown for some months in a green house, then harvested and kept overnight during transport by courier. Some of the stems were tough and pesto was made from leaves only. Furthermore, the average L value of pesto in the second year was lower because all the leaves were darker green than in the first year. L value became a better proxy to show blackening rate than h value in this second year experiment. As a comparison, both h and L were displayed in the Figure 9.



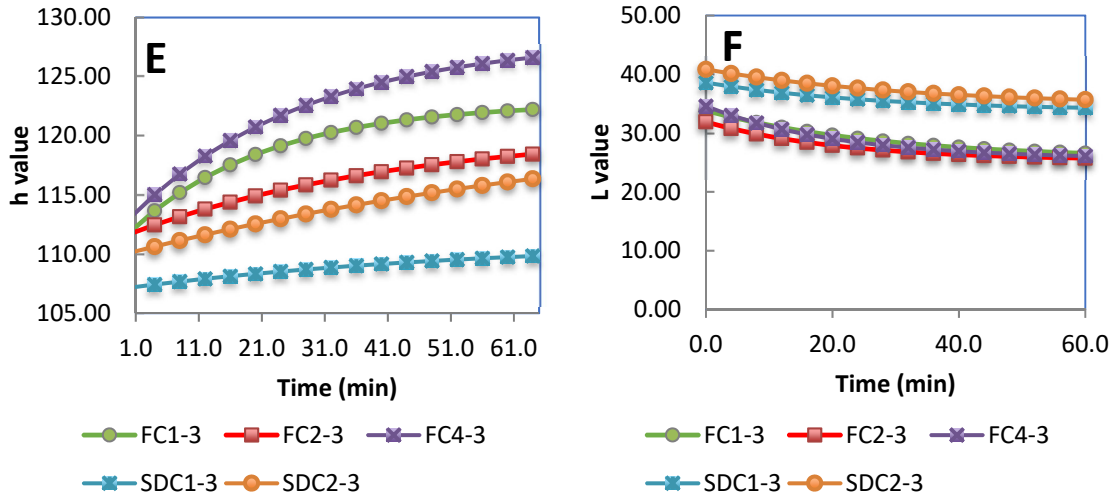
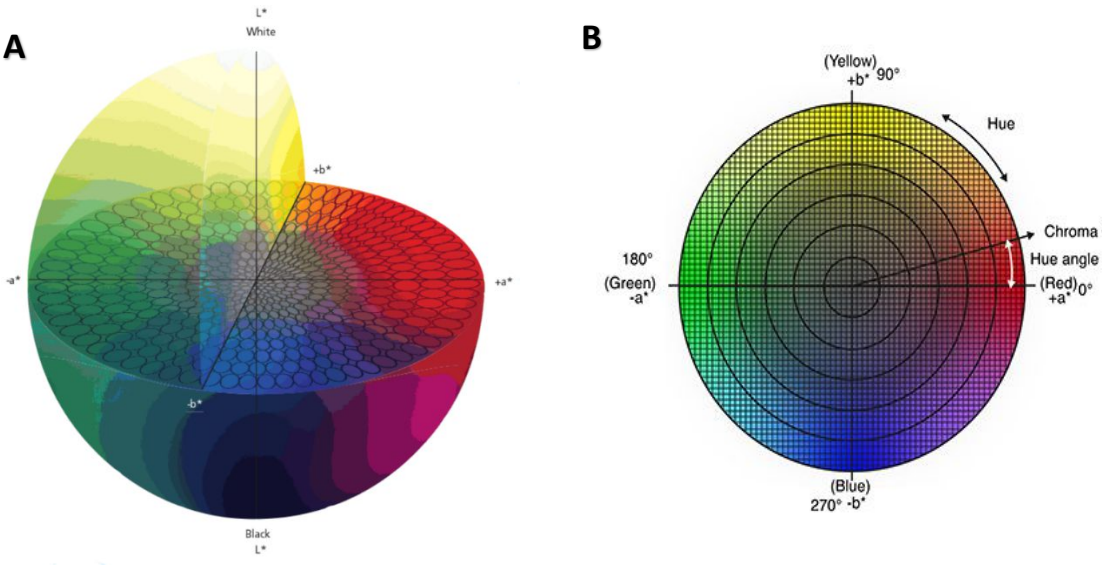


Figure 9 Comparison between h and L: (A) h of the first replicate (B) L of the first replicate (C) h of the second replicate (D) L of the second replicate (E) h of the third replicate (F) L of the third replicate. FC1&3: Fast cooling + 1 day at 4°C, FC2&4: Fast cooling + 2 days at 4°C, SDC1&2: Step down cooling 60&50 g.

h value showed a different trend in each replicate. In the first replication, h value of FC4 and SDC had a decreasing trend like usual blackening rate but it also showed an extreme decrease at the first 5 minutes. In the third replication, this trend turned to reverse and in the second, it even had both trends. This demonstrated that h value was not an appropriate indicator of blackening rate for year two.

The L value of all treatments showed a constant decrease from the beginning to the end of measurement. This meant that blackening rate was displayed better by the change of the L value rather than by the change of the green colour (h). h value is a combination of changes in a* and b*. a* is a proxy that shows the colour tendency from green to red, meanwhile b* from blue to yellow. The angle that is created between a* and b* is h value (Figure 10). h is very useful when there is a clear movement from e.g. green to red (as in tomato ripening); but pesto was changing from green to dark green, as the consequence of the natural blackening rate; and the data show that L was the most consistent descriptor for this change.



(http://blog.xrite.com/tolerancing-in-flexo-and-offset-printing/)

http://sensing.konicaminolta.us/2015/03/understanding-the-cie-lch-color-space/

Figure 10 Three and two-dimensional representation of L, h, a* and b* value

L value only indicated the change of a sample's lightness, and it was not affected by a change of the sample colour. The use of L as a proxy for discolouration was also found to be the most effective colour descriptor for oxidative browning in peach purée and for blanching of broccoli (Avila & Silva, 1999; Tijsken et al., 2001).

4.2.2 Basil quality

The changes in basil quality were marked by the change in the number of rotten leaves and the weight before and after basil leaves were stored in the cold storage and the room temperature. Rotten leaves were first observed at the second day after cold storage in this early batch since the symptom was visible on the surface on that day. There was no sign of the symptoms when basil leaves were removed from the cold storage. However, when the bags of basil leaves were opened the damage was greater towards the bottom, so there was a possibility that the symptoms started from the bottom earlier than 2 days. With this result, duration at 20°C for visible blackening of basil was less than peaches which is 3 days (Wang et al., 2006) and around the same duration as with tomatoes (Artes & Escriche, 1994).

This result may indicate that higher carbon dioxide and lower oxygen concentrations may accelerate blackening of stored basil as it was the case in the storage leaf of *Protea susannae X compacta* (Crick & McConchie, 1999) but gas concentrations

was not measured to confirm this possibility. Another possibility is that decay or CI develops better in the more humid lower parts of the bag as it was suggested to occur to the leaf of *Protea* sp. (Ferreira, 1986).

Percentage of rotten leaves increased over time at 20°C after cold storage for all treatments. All the fast cooling treatment (FC) showed a similar behaviour on the percentage of rotten leaves on day one, two and five (Figure 11). Meanwhile, step-down cooling (SDC) was different from one to another on day two; but overall it seemed that fast cooling leads to higher leaf blackening. This was consistent with the idea that acclimation can reduce chilling sensitivity in plants (Tomashow, 1999; Yang et al., 2003), although in this case, the differences started to show after 2 days at 20°C.

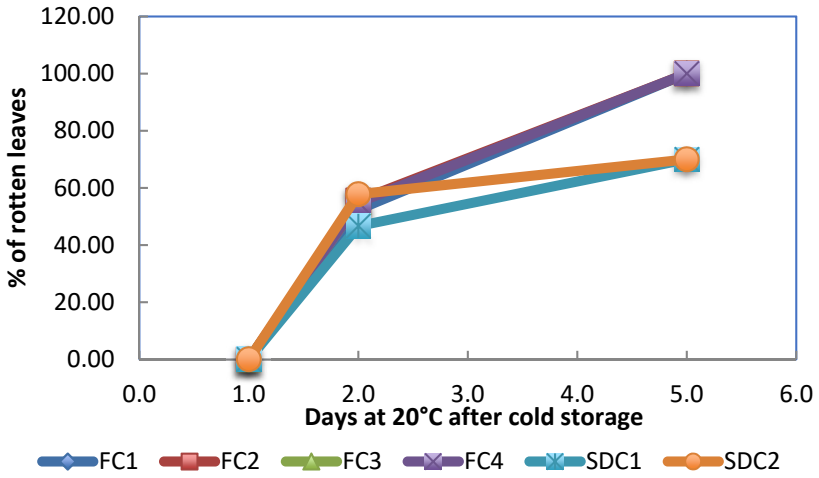


Figure 11 Percentage of rotten leaves Batch 1. FC1&3: Fast cooling + 1 day at 4°C, FC2&4: Fast cooling + 2 days at 4°C, SDC1&2: Step down cooling 60&50 g.

Percentage of weight loss of all treatments was at different rate against the days at 20°C after cold storage (Figure 12). After the second day, FC treatments were divided into two groups. FC1 and FC3 (a day in storage) were in the first group with moderate rate, FC2 and FC4 (2 days in storage) in the second group with a higher rate. Meanwhile, both SDC were at a similar rate before the third day. Although they were ended at the different levels on day 5, both were still below the position of all FC. The initial weight differences between SDC 1 and 2 might not significantly influence the percentage of weight loss and rotten leaves. Overall, SDC reduced percentage of weight loss compared to FC. Weight loss will always be higher in senescent tissue as it had lost chlorophyll, free amino nitrogen, and the ability to control stomatal aperture (Thimann & Satler, 1979).

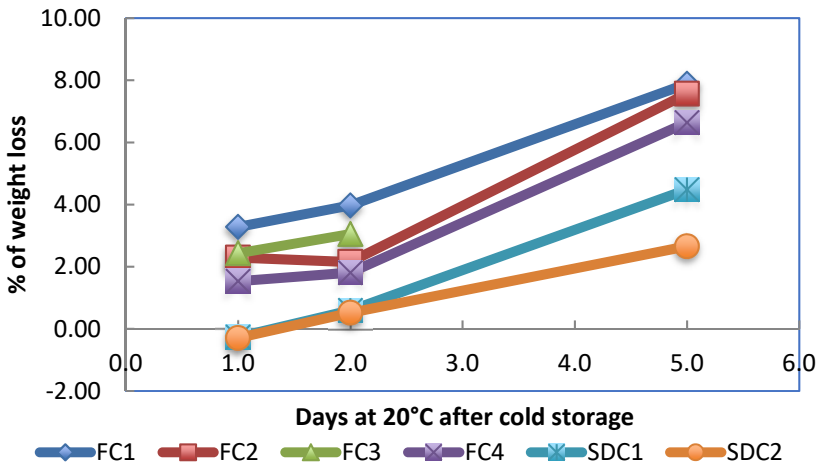


Figure 12 Percentage of weight loss of Batch 1. FC1&3: Fast cooling + 1 day at 4°C, FC2&4: Fast cooling + 2 days at 4°C, SDC1&2: Step down cooling 60&50 g.

As seen in Figure 12, SDC increased in weight, instead of losing weight, on the first day of weight measurement. This could occur since during transpiration process the leaves sometimes absorbed water from their environment instead of lost it (Cantwell & Reid, 1993) and step down cooling possibly accommodates this process on the first day at 20°C after cold storage.

Apart from the possibility that step-down cooling might relate to transpiration process, there was a clear trend that SDC reduced the loss of basil quality. However, the data are not well suited to statistical analysis.

4.2.3 Quality of pesto

The changing in quality of pesto was shown by the blackening rate or K value. K value in this Batch decreased within 2 days at 20°C after cold storage and then became steady after that (Figure 13). It varied between treatments on the first day at 20°C after cold storage and was similar thereafter. It implied that K value differences will be more readily detected among treatments if pesto was made from basil leaves that have been stored in cold storage in certain days and then held at room temperature for only a day, rather than for 2 days and above.

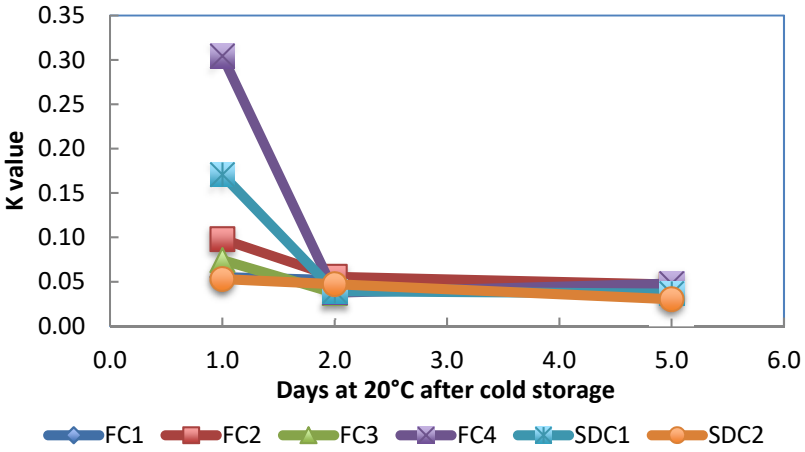


Figure 13 K value throughout days at 20°C after cold storage. FC1&3: Fast cooling + 1 day at 4°C, FC2&4: Fast cooling + 2 days at 4°C, SDC1&2: Step down cooling 60&50 g.

4.2.4 Statistical analysis result

No actual replication was made in the First Batch of experiment since the 3 samples of each treatment were made into pesto in 3 different days. It aimed at testing the correlation between CI and weight loss with the days at 20°C after cold storage. Therefore, the data between treatment cannot be compared by statistical analysis. However, if they were arranged as a group based on the technique of cooling, duration of cold storage and days at 20°C after cold storage, T-test or ANOVA analysis can be conducted to describe the result (Table 2) as well as multivariate analysis for the interaction between those factors (Appendix 9).

Table 2 T test or ANOVA of First Batch (A) cooling technique (B) cold storage duration (C) days at 20°C after cold storage. FC: Fast cooling, SDC: step-down cooling.

(A)

Cooling technic	Rotten leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS
FC	47.07 a	2.34 a	42.77 a	0.077 a	10.17 a
SDC	40.74 a	0.23 b	44.59 a	0.063 a	9.90 a

The numbers followed by the same letter in each column are not significantly different according to independent samples T test at 95 % level of confidence ($P < 0.05$).

(B)

Cold storage duration (day)	Rotten leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS
1	41.46 a	2.94 a	46.43 a	0.051 a	9.25 a
2	46.24 a	1.04 b	42.16 a	0.081 a	10.42 a

The numbers followed by the same letter in each column are not significantly different according to independent samples T test at 95 % level of confidence ($P < 0.05$).

(C)

After cold storage (day)	Rotten leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS
1	0 a	1.25 a	45.90 ab	0.126 b	4.75 a
2	53.70 b	1.74 a	47.16 b	0.045 a	11.88 a
5	88.00 c	1.83 a	35.93 a	0.039 a	14.30 a

The numbers followed by the same letter in each column are not significantly different according to Duncan's ANOVA New Multiple Range Test (DMRT) at 95 % level of confidence ($P < 0.05$).

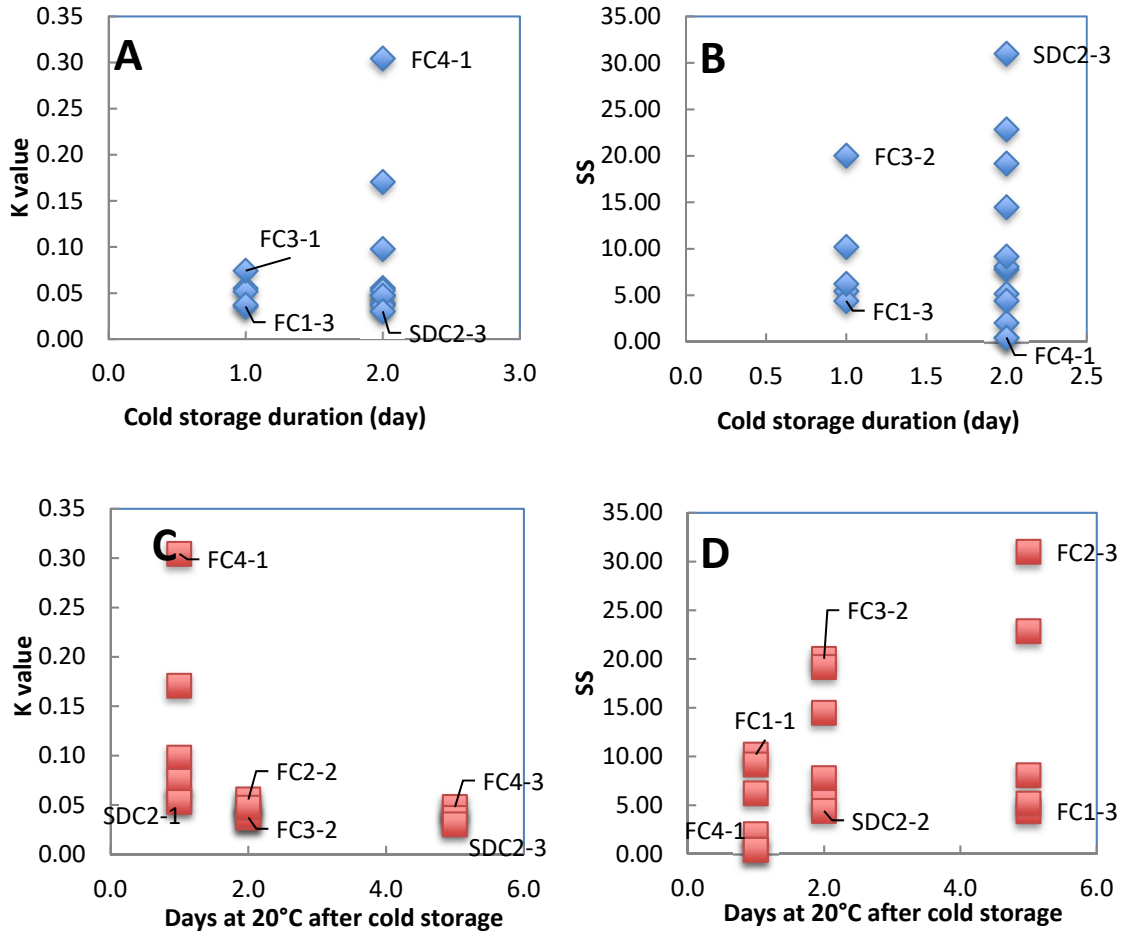
The results indicated that the variation of days at 20°C after cold storage had more influence on the variables of basil and pesto quality than cooling technique and cold storage duration. Cooling technique and cold storage duration created a significant difference only in the percentage of weight loss, while the days at 20°C after cold storage created a significant difference in the percentage of rotten leaves and almost all the variables of pesto quality. A multivariate analysis was then carried out to find whether the independent variables (cooling technique, cold storage duration, and days at 20°C after cold storage) interacted with each other to influence quality variables (appendix 9). The result showed that only cooling technique and number of days at 20°C after cold storage duration had significant interactions to affect percentage of rotten leaves.

The percentage of weight loss was determined by cooling technique and duration of cold storage. However, the interaction between these treatments was not significant. Percentage of weight loss seemed to be affected more by cooling technique since the low weight loss belonged to the samples of SDC and not always to those stored in two days. Significant sign for weight loss in different duration of storage caused by all of SDC samples was in the 2 days group.

4.2.5 Correlation between variables

There seemed to be a connection between K value and SS against duration of cold storage and after cold storage (Fig. 13. A to D). Both tend to be more varied in the second day of cold storage than at the first day and more likely had inverse connection. The treatment resulting in the highest K value had the lowest SS and vice versa. This inverse trend between K value and SS also occurred to their correlation with days at 20°C after cold storage with a slightly different way. K value varies on the first day and then became more uniform at the second and third but for SS this trend was more likely to be reverse. The highest K value had the lowest SS on the first and second day, but not

on the third day. It meant that there might be an inverse correlation between 'noisy' data and blackening rate of the pesto since 'noisy' data only occurs to the pesto that had slow blackening rate (K value < 0.1) as shown in the Figure 14 (E). This was important since SS value might influence the course of other correlations especially the one that became the major aim for this whole research, which was the correlation between quality of basil leaves and the quality of their pesto. The idea of SS relates with K value was supported by statistical analysis which shows that there was a significant negative correlation between both (Table 3).



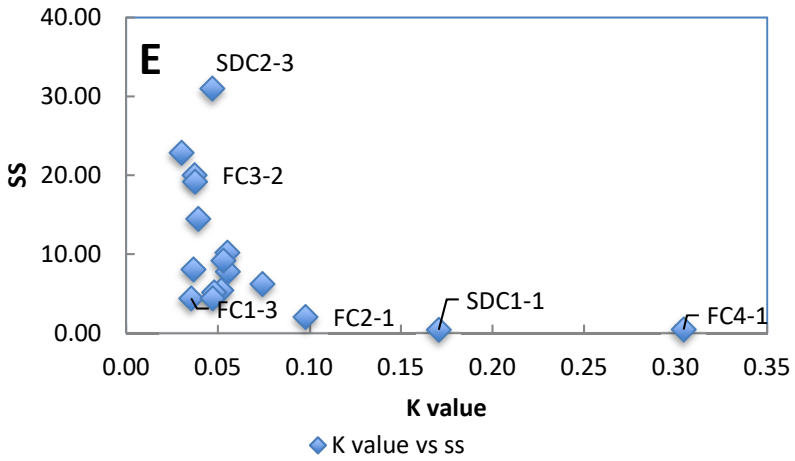


Figure 14 Correlation between (A) Duration of cold storage and K value (B) Duration of cold storage and SS (C) Duration at 20°C after cold storage and K value (D) Duration at 20°C after cold storage and SS (E) K value and SS. FC1&3: Fast cooling + 1 day at 4°C, FC2&4: Fast cooling + 2 days at 4°C, SDC1&2 : Step down cooling 60&50 g

Table 3 Correlation between K value, SS and starting colour

Correlation		Starting Colour	Sum of Squares
K Value	Pearson Correlation	-0.249	-0.485*
	Sig. (2-tailed)	0.334	0.049
	N	17	17

Figure 15 showed the SS impression on the relationship between quality of basil leaves and quality of their pesto. The side-by-side graphs (A vs B and C vs D) were the comparison of the correlation before and after the high SS data were removed. The exclusion of 'noisy' data did not bring significant changes to the course of relationship between K value and rotten leaves and the relationship between K value and weight loss.

K value had more variation when basil leaves weight loss was under 1%. There were two possible causes. First, cold storage in more than 2 days might cause K value to be stable under 0.10 or make the blackening rate slower than those stored for less than 2 days. Although, PPO that influences blackening was known to be inhibited with high temperature, not by cold, as was the case with mate tea leaves at 70-90°C (Ceni et al., 2008). This implied that reduction in blackening rate cannot be explained by heat inactivation of PPO. After all, there are many others factors that related to PPO besides temperature (Broothearts, et al., 2000; Mayer & Eitan, 1979; Mc evely et al., 1992; Viacava et al., 2014). Another possible cause was that the rate of blackening was lower

in senescing basil because PPO may degrade during extensive leaf senescence since PPO is localized on chloroplast thylakoids (Vaughn, Lax, & Duke, 1988).

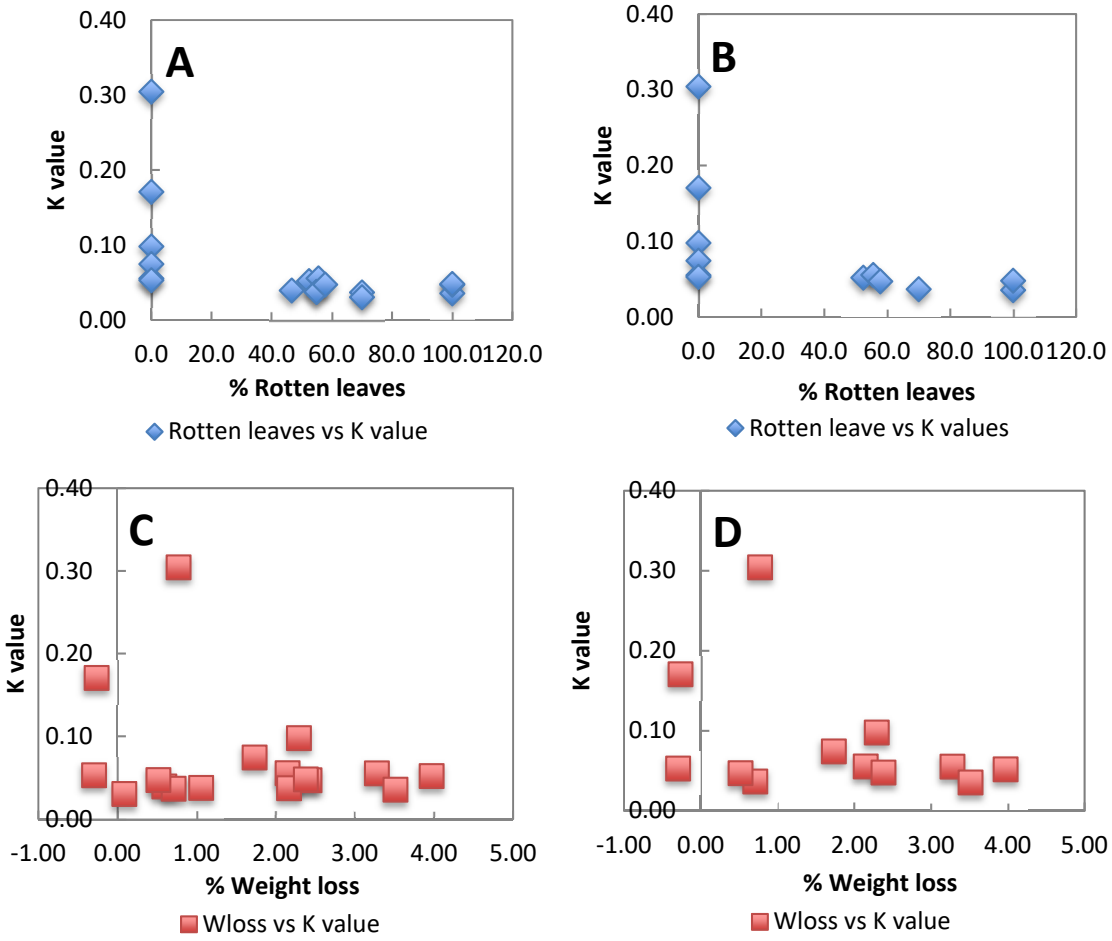


Figure 15 Relationship between the quality of the basil leaves and pesto (A) K value vs rotten leaves that include data with high SS (B) K value vs rotten leaves exclude data with high SS. (C) K value vs weight loss that include data with high SS (D) K value vs weight loss that exclude data with high SS

4.2.6 'Odd' colour of some pesto

Some pesto showed 'odd' starting colour (Figure 16 B) which was marked by the yellow colour as seen in the Table 4. They had a paler colour or higher L value than most pesto, starting colour (>50). However, it seemed that these differences did not have an effect to the K value and SS. Therefore, in this stage (treatments without replications), 'odd' colour did not play an important role in the course of correlation between quality of basil leaves and the quality of their pesto.

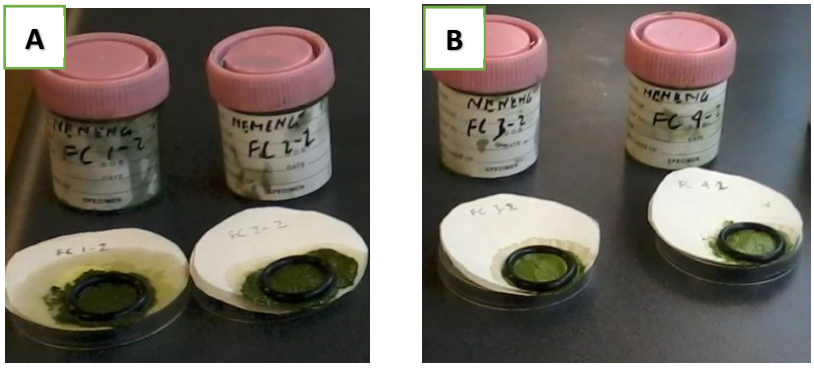


Figure 16 The example of (A) usual pesto colour (B) 'odd' colour

Table 4 Colour of the pesto of Batch 1. FC1&3: Fast cooling + 1 day at 4°C, FC2&4: Fast cooling + 2 days at 4°C, SDC1&2 : Step down cooling 60&50 g

Treatment	Starting colour	K value	SS
FC1-1	55.1	0.055	10.20
FC1-2	36.23	0.052	5.44
FC1-3	33.81	0.035	4.39
FC2-1	38.34	0.098	2.03
FC2-2	42.37	0.056	7.79
FC2-3	31.88	0.047	31.00
FC3-1	53.55	0.074	6.22
FC3-2	53.46	0.037	20.02
FC4-1	36.97	0.304	0.45
FC4-2	54.18	0.038	19.18
FC4-3	34.56	0.048	5.17
SDC1-1	36.55	0.171	0.42
SDC1-2	57.72	0.039	14.48
SDC1-3	38.57	0.037	8.07
SDC2-1	54.89	0.053	9.18
SDC2-2	39.00	0.047	4.41
SDC2-3	40.83	0.030	22.85

4.2.7 Conclusion of Batch 1

Fast cooling and slow cooling can be achieved using a simple way. There were hours differences between duration of FC and SDC. FC needed less than 5 minutes to make basil leaves to be cooling down to 4°C. While slow cooling needed approximately 4 hours.

The best proxy to explain blackening rate was L value since it was more stable than h value and it was not affected by a change of the sample colour that probably changes along the condition of samples.

There were some other outputs that could be drawn from this Batch regarding basil quality, pesto quality and the correlation between variables. Individual treatments cannot be analysed statistically because of the lack of replication. However, as they were collected as a group of cooling techniques, storage and days at 20°C after cold storage, there was enough evidence to conclude some points.

Quality loss of basil leaves, which was indicated by the increase of rotten leaves and weight loss, was determined by the number of days at 20°C after cold storage and can be magnified by the interaction with cooling technique: fast cooling leads to worse leaf blackening. Given that fast cooling caused excessive loss of basil leaves quality, slow cooling (placing the bags of basil into a crate at low temperature) was chosen as the standard process.

The loss of pesto quality which was indicated by the increase of K value or blackening rate was also determined by the number of days at 20°C after cold storage. K value tended to vary between treatments on the first day at 20°C after cold storage and it remained the same after that. Therefore, K value differences will be more readily detected among treatments if pesto was made from basil leaves that held at room temperature for only a day after cold storage, rather than 2 days or above.

There were 2 issues that should be addressed in the next batches, 'noisy' data (SS>10) and 'Odd' starting colour of some pesto (L>50), that seemed to randomize and might disturb the statistical result.

Although SS increased over the period of cold storage and 20°C after cold storage, it did not statistically correlate with any of the independent variables. However, it was absolutely connected with dependent variables such as K value. There was an inverse correlation between 'noisy' data and blackening rate of the pesto. However, the exclusion of 'noisy' data did not influence the course of relationship between basil leaves quality and its pesto.

Statistically, starting colour was only related to the number of days at 20°C after cold storage and there was no significant correlation with K value. Therefore,

hypothetically 'odd' colour would also not play an important role to the course of correlation between quality of basil leaves and the quality of their pesto.

4.3 Batch 2

The result of Batch 2 was presented in Figure 17. All variables of leaves quality and pesto quality of 4°C increased over time of cold storage except for weight loss, which decrease on day 2. On the second day, all variables of step down cooling (T2) and step-up warming (T5) were lower than 4°C except for K value of T2 and percentage of rotten leaves of T5. Meanwhile, the non-chilling temperature of 10°C (T4) seemed to have similar result with T1 except in percentage of rotten leaves which was much higher.

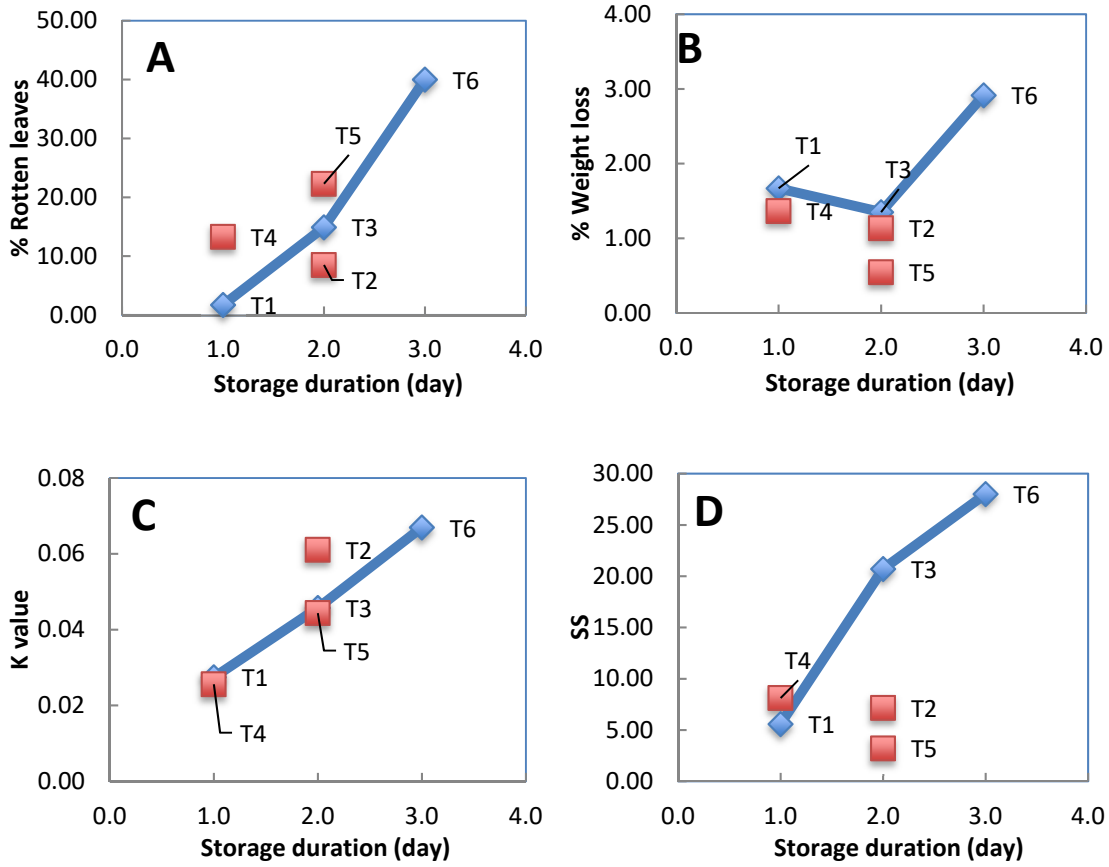


Figure 17 All variables against cold storage time (A) Percentage of rotten leaves (B) weight loss (C) Blackening rate (D) Sum of squares. T1,3,6: 1,2,3 day at 4°C, T2: Step-down cooling, T4: 1 day at 10°C, T5: Step up warming. All plus 1 day at 20°C.

It seemed that the percentage of rotten leaves had an inverse correlation with K value and percentage of weight loss had direct correlation with SS. T4 and T5 were above the treatment of 4°C and T2 was under 4°C in percentage of rotten leaves, but in K value, they were in opposite position. T4 and T5 were under 4°C, and T2 were above 4°C.

Meanwhile, both percentage of weight loss and SS of T2, T4, T5, around in the same position compare to the treatment of 4°C storage.

The result indicated four things. First, there was a big increase in percentage of rotten leaves, K value and SS through duration of 4°C storage. Second, the percentage of rotten leaves seemed to have an inverse relationship with K value and percentage of weight loss had a direct correlation with SS. Third, weight loss decreased on the second day regardless the temperature of the storage. Fourth, reducing fluctuation ('noisy' data/high SS) might be related to the variation of temperature since those samples with 2 levels of storage (T2&T5) had a lower SS compared to those set in single temperature.

Table 5 showed the statistical analysis result. There were no significant differences on pesto quality variables of all treatments but there were some on basil leaves quality. The treatments at 4°C showed a significant difference in percentage of rotten leaves between days of storage but not in percentage of weight loss K value and SS. On the first day, the percentage of rotten leaves was lower than non-chilling (T4) and at second day, both variables of basil quality had no difference between step-down cooling or step-up warming. So was the weight loss between T4 & T1 to T2 & T5. Therefore, the third and the fourth indications that mentioned in the previous paragraph (third indication: weight loss decreased on the second day regardless the temperature of the storage. Fourth indication: graph fluctuation/ high SS might be related to the variation of temperature were not supported by statistical result. The first and second indication will be further discussed in section 4.3.1 and 4.3.5.

Table 5 ANOVA of Batch 2

Treatment	Rotten leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS	SE
T1	1.71 a	1.67 ab	52.48 a	0.027 a	5.59 a	68.83
T2	8.48 ab	1.14 ab	38.29 a	0.061 a	7.17 a	13.38
T3	14.89 bc	1.35 ab	43.67 a	0.046 a	20.71 a	71.68
T4	13.27 bc	1.36 ab	45.72 a	0.026 a	8.13 a	57.84
T5	22.30 c	0.55 a	37.83 a	0.044 a	3.22 a	7.83
T6	40.00 d	2.91 b	53.28 a	0.067 a	28.01 a	81.54

The number followed by the same letter in each column are not significantly different according to Duncan's ANOVA New Multiple Range Test (DMRT) at 95 % level of confidence ($P < 0.05$). T1,3,6: 1,2,3 day at 4°C, T2: Step-down cooling, T4: 1 day at 10°C, T5: Step up warming. All plus 1 day at 20°C.

4.3.1 The quality of basil leaves

Figure 17 A&B showed that the duration of cold storage influenced the percentage of the rotten leaves and weight loss of basil leaves in 4°C storage. The more basil treated in the cold storage, the higher quality loss of leaves will be (Cantwell & Reid, 1993; Meir, Ronen, Lurie, & Philosoph-Hadas, 1997). The data also showed that more than 30 % of basil leaves were blackened when basil leaves were stored for more than 2 days.

Step-down cooling and step-up warming could not prevent quality loss of basil leaves since in the same day of storage, the percentage of rotten leaves and weight loss of T2 and T5 was not significantly different with T3 or steady cooling at 4°C storage in 2 days (Table 5).

The treatment in which the leaves were stored for a day at 4°C (T1) was the best treatment for reducing percentage of rotten leaves which was surprising since in the same duration of cold storage, the leaves that were stored at 10°C (T4) have a significantly higher percentage. The mix between chilling injury and senescence symptoms must have an influence on the result since both symptoms were indistinguishable from one another. This was possible since both share the same gene regulator (Gan & Amasino, 1997; McConchie et al., 1994). T4 probably showed decay and T1 showed chilling injury, and between these 2 symptoms, senescence produced a more damaging result to basil tissues.

4.3.2 The quality of pesto

K value of Batch 2 was generally much lower than k value of a day at 20°C after cold storage of Batch 1. This was because of the differences in treatment between both batches. Most of the data with high K value in Batch 1 were from the samples that had fast cooling treatment, while Batch 2 had slow cooling. It showed that acclimation/slow cooling was not only better than fast cooling in maintaining basil quality, but also in pesto quality.

There were no significant differences in starting colour between the treatments of Batch 2 (Table 5), including between the treatments that used different duration of 4°C storage. However, if the variables of pesto quality were plotted into a graph against duration of cold storage, K value/blackening rate increased throughout the time of storage (Figure 17C).

Storage at 10°C (T4) which represented a non-chilling temperature had a similar blackening rate as T1 and so do step-down cooling (T2) and step-up warming (T5) with T3. It meant that the technique of storage and the level of temperature might have little influence on the quality of pesto.

Blackening rate in all treatments was not significantly different although T2 and T6 seemed to have higher K value. This might be due to high standard error (SE) between replications in some of the treatments. High SE occurs only in steady cooling treatments (T1, T3, T4, and T6) which had one or two replicates with 'odd' colour, while low SE occurs to step-down cooling and step-up warming (T2 and T5) which had no unusual replicates.

'Odd' colour that seemed to be randomized might correlate to a stable temperature that was applied to samples. Thus, treatments with a variation of temperature storage had zero chance of having pesto with 'odd' colour. The less variation of temperature, the higher the chance to having 'odd' ones among their replicates and the higher the SE will be. However, once again, this is not supported by the statistical analysis. Further hypothesis for 'odd' colour will be elaborated in section 4.3.3.

4.3.3 Pesto issues

Two pesto issues in Batch 1 still became an issue in this batch. Some 'noisy' data and 'odd' colour seemed even more random and they were interfering the statistical analysis results. Thus, they might also change the course of the relationship between basil leaf quality and pesto quality. Hypothesis of this phenomenon needed to be addressed to formulate the plan on reducing or maybe eliminating these sources of variability.

There was a possibility that 'odd' colour of pesto was caused by the change of water content within the basil leaves. External water, such as from condensation, can accidentally be added in pesto processing since there were some condensation events in some of the steady cooling replicates. The condensation water seemed to be trapped inside the plastic bag that wrap the basil leaves and probably it was mixed during the process of pesto and created some emulsion. This was apparent from the texture of 'odd' colour pesto which was more creamy than usual. When pesto was mixed with

water, the colour also changed because the change of physical properties of a sample can influence their colour appearance (Lin, 2009).

The change of water content within basil leaves can be explained by the relationship between starting colour and weight loss. However, weight loss is not always about changed in the water content but probably the evaporation of other substrates too, such as EO (Fischer et al., 2011). Therefore, in both batches (1&2) there was no obvious connection between weight loss and starting colour (Figure 18), except that overall pesto samples with 'odd' colour made of basil leaves that have a less variation of weight loss than usual pesto. Most strikingly, there appear to be two 'classes' of starting L value; ca. 40 and ca. 50; the L value data are certainly not normally distributed.

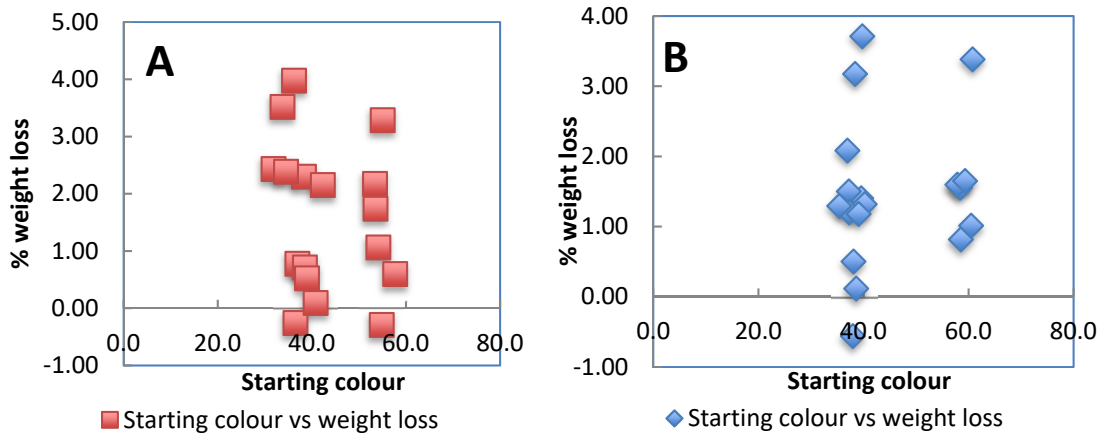


Figure 18 The relationship between starting colour and weight loss of (A) Batch 1 (B) Batch 2. T1,3,6: 1,2,3 day at 4°C, T2: Step-down cooling, T4: 1 day at 10°C, T5: Step up warming. All plus 1 day at 20°C.

It was also possible that 'Odd' colour could related to an enzymatic process within some of the leaves. The enzyme activity in basil affects blackening process (Adams & Brown, 2007) and also determined the EO composition (Lewinsohn et al., 2000), which is the major element in sweet basil. Enzyme that related to blackening is PPO. PPO is reactive to many factors including environmental stresses and biological factors of the samples (refer to section 4.2). Different conditions of the leaves before they arrive can cause differences on their behaviour, perhaps including the appearance of their pesto starting colour. However, this argument would give rise to a full range of starting colours, instead of the bimodal distribution as shown in Figure 18. This 'Odd' colour issue then became the interest of the fourth and Fifth Batch.

Another issue in pesto quality was 'noisy' data. Figure 17D showed that SS as the indicator of 'noisy' data was influenced by the duration of cold storage. It increased over time. However, the data of all treatments were not significantly different although T3 and T6 show much higher number. High SE in some of the treatments might be interfering SS statistical analysis result (Table 5). If the cause of 'odd' colour to the pesto can be identified and minimized or even prevented, and if the result was still the same, then 'noisy' data were more likely caused by the duration of steady storage temperature. T3 and T6 samples were kept in the cold storage for 2 and 3 days respectively in the same temperature, while others were stored for only one day or two days in different level of temperatures.

4.3.4 SS impression to the correlation between basil and pesto quality

SS became an important variable in every table of result since it showed the close resemblance of the transformation data to the actual data. In the First Batch, nothing changed before and after those data with high SS (SS>10) were excluded. However, Batch 2 have a more 'real' replications than Batch 1. Therefore, another test of 'noisy' data exclusion was performed in this Batch.

If neglecting the treatments with high SS (T3 and T6), then the result table of Batch 2 will be presented as Table 6. Before the exclusion, only percentage of rotten leaves and weight loss had some differences between the treatments. After the exclusion there were also some differences between treatments in K value but not in the percentage of weight loss. It seemed that step-down cooling (T2) could reduce rotten leaves better than the other two days treatment (T5), it was even so close with a day treatment at 4°C (T1) and 10°C (T4). However, its ends up with the highest K value or the fastest blackening rate.

Table 6 ANOVA of Batch 2 without T3 and T6

Treatment	Rotten leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS	SE
T1	1.71 a	1.67 a	52.48 a	0.027 a	5.59 a	68.83
T2	8.48 a	1.14 a	38.29 a	0.061 b	7.17 a	13.38
T4	13.27 ab	1.36 a	45.72 a	0.026 a	8.13 a	57.84
T5	22.30 b	0.55 a	37.83 a	0.044 ab	3.22 a	7.83

The number followed by the same letter in each column are not significantly different according to Duncan's ANOVA New Multiple Range Test (DMRT) at 95 % level of confidence ($P < 0.05$). T1: 1 day at 4°C, T2: Step-down cooling, T4: 1 day at 10°C, T5: Step up warming. All plus 1 day at 20°C.

The lowest blackening rate (indicated by the lowest K value) belonged to T1 and T4 which were stored only a day in the cold storage, although both were set in the different temperature (Figure 19B). Next best K value is T5 which was a step-up warming treatment, but it was not significantly different from T2 or step-down cooling treatment. This implied that duration of cold storage might play a more important role in the blackening rate than the level of the temperature and the technique of storage. The longer basil leaves were stored, the faster the blackening rate of their pesto will be, and this pattern did not change before and after the exclusion of the treatments with 'noisy' data (Figure 19 A & B).

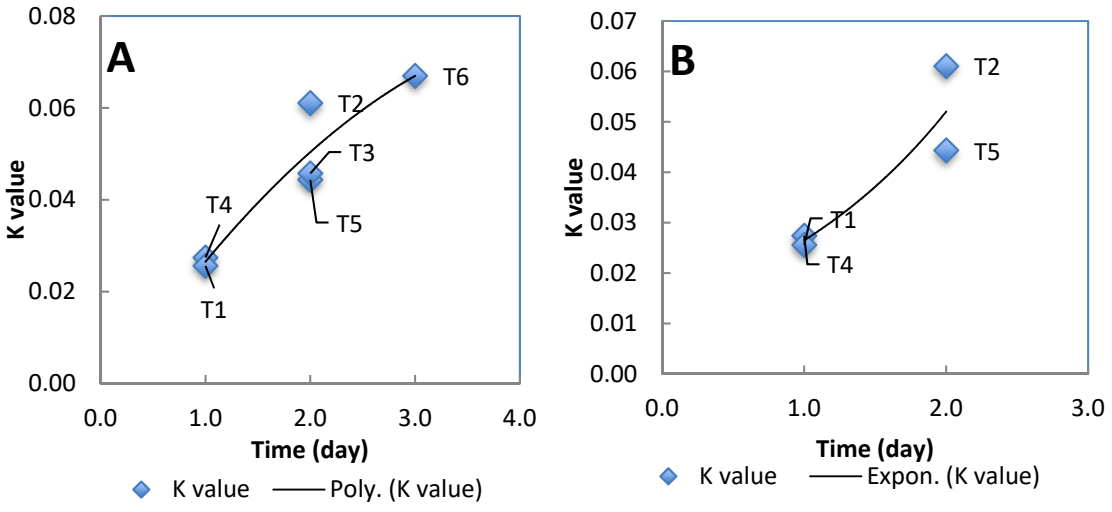


Figure 19 The comparison between trend of K value of Batch 2 (A) include 'noisy' data (B) exclude 'noisy' data. T1: 1 day at 4°C, T2: Step-down cooling, T4: 1 day at 10°C, T5: Step up warming. All plus 1 day at 20°C.

'Noisy' data also did not change the fact that L value data were not normally distributed. There were still two 'classes' of starting L value although all treatments with 'Noisy' data were removed from both Batch 1 and 2, as seen in Figure 20 A & B.

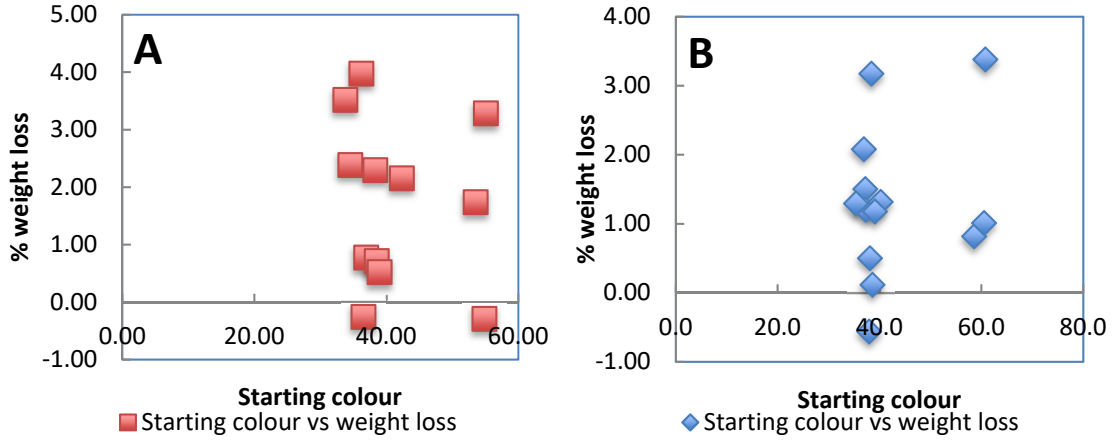


Figure 20 The relationship between starting colour and weight loss of (A) Batch 1 (B) Batch 2 after the exclusion of 'Noisy' data. T1,3,6: 1,2,3 day at 4°C, T2: Step-down cooling, T4: 1 day at 10°C, T5: Step up warming. All plus 1 day at 20°C.

4.3.5 The relationship between basil quality and pesto quality

There was no significant connection between the quality of basil leaves (percentage of rotten leaves and weight loss) and the quality of pesto (K value) with or without 'noisy' data as shown in Table 7. The change was only in how they might relate. If 'noisy' data were excluded from the correlation analysis, it had an inverse connection, but when it was included, it had a direct connection. Either way, the correlation between basil leaves and pesto quality was still not significant. The same with Batch 1, Batch 2 also showed that the quality of basil leaves did not affect the quality of their pesto.

Table 7 Correlation between K value and basil quality

Correlations		With 'noisy' data		Without 'noisy' data	
		RottenL	WeightL	RottenL	WeightL
Kvalue	Pearson Correlation	0.309	0.293	-0.025	-0.278
	Sig. (2-tailed)	0.211	0.238	0.935	0.358
	N	18	18	13	13

*. Correlation is significant at the 0.05 level (2-tailed).

4.3.6 Conclusion of Batch 2

The results of Batch 2 basically reconfirm the result of Batch 1, which were: 2 variables of basil quality always have different reaction to the duration of cold storage, duration of cold storage had more influence on basil quality than pesto quality, and 'noisy' data left no impression to the correlation between basil and pesto quality. The only contradictive result was regarding the 'odd' colour contribution to the result.

The percentage of rotten leaves and weight loss steadily increase during cold storage at 4°C. The percentage of rotten leaves reached more than 30% after basil leaves were stored for more than 2 days. T1 (4°C) was the best treatment for reducing percentage of rotten leaves, better than non chilling temperature/ 10°C (T4) at the same day (1 day storage) since senescence on T4 produced a more damaging result to basil tissues than chilling injury on T1. In the second day of storage, T2 (step-down cooling) had the lowest percentage of rotten leaves and T5 (step-up warming) had the lowest weight loss but neither was significantly different from steady cooling in 2 days (T3).

There were significant differences in basil quality resulting from some treatments but not in the quality of pesto. The 'odd' colour might interfere with the analysis result of pesto quality variables. One of the pesto issues that should be solved in the next batch. Hypothetically, 'odd' colour of pesto related to water content within the basil leaves or to an enzymatic process and 'Noisy' data might be influenced by the duration of cold storage.

The two issues, that did not interfere with the result of Batch 1, turn out to be disturbing the statistical analysis of Batch 2. However, 'noisy' data have no effect on the correlation between basil quality and pesto quality. Overall, this Batch also confirmed that the quality of the leaves showed no effect to the quality of their pesto.

4.4 Batch 3

At the initial stage of fresh basil (C1) measurement, there were 9 technical replications that were prepared for each jar of pesto samples to test the differences among the same samples. The result showed the K value of each of them were different (Figure 21 A), although two 'noisy' data were removed (Figure 21 B). Nine technical replications were probably more reliable for measuring any other type of samples, but pesto samples could become un-reliable after several times of contact with air.

Furthermore, the average of K value for the first three technical replicates were almost the same as nine replicates (Table 8). Therefore, 3 technical replications should be enough and more practical to accomplish rather than nine. This addition to the method then applied to this Batch and after.

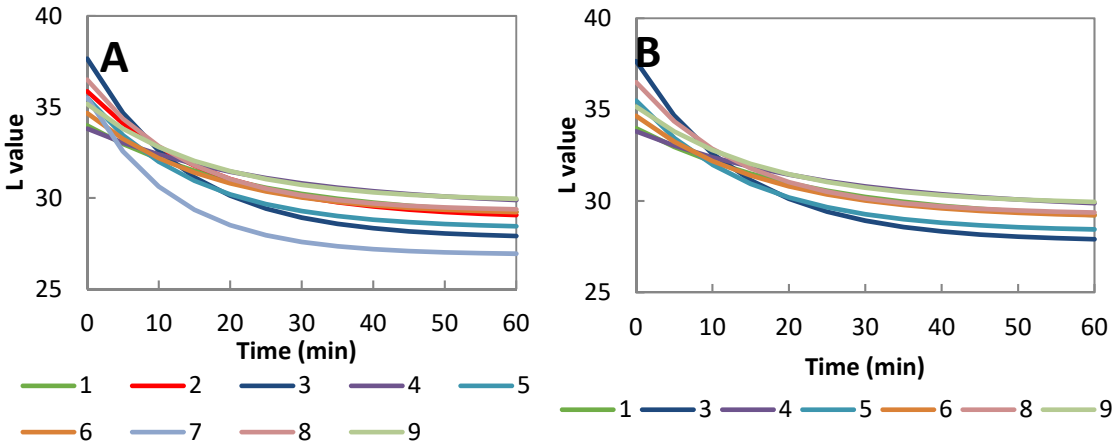


Figure 21 L value of (A) nine technical replicates of C1-1. (B) exclude the 'noisy' data

Table 8 The average of L value for 3 first replicates and 9 replicates of C1-1

Number of measurement	Average of 3 first replicates	Average of all 9 replicates
1	35.72	35.40
2	33.57	33.57
3	32.09	32.26
4	31.06	31.31
5	30.34	30.62
6	29.84	30.12
7	29.49	29.76
8	29.24	29.49
9	29.07	29.29
10	28.95	29.14
11	28.86	29.03
12	28.80	28.95
13	28.76	28.89

Figure 22 presented the results of Batch 3 where A, B, C, and D represented percentage of rotten leaves, percentage of weight loss, K value and SS of all treatments. Percentage of rotten leaves and weight loss of basil and SS yield similar result with previous batches. Percentage of rotten leaves, percentage of weight loss, and SS tended to increase by the duration of the cold storage, regardless of the temperature level. Whereas, K value decreased throughout the time.

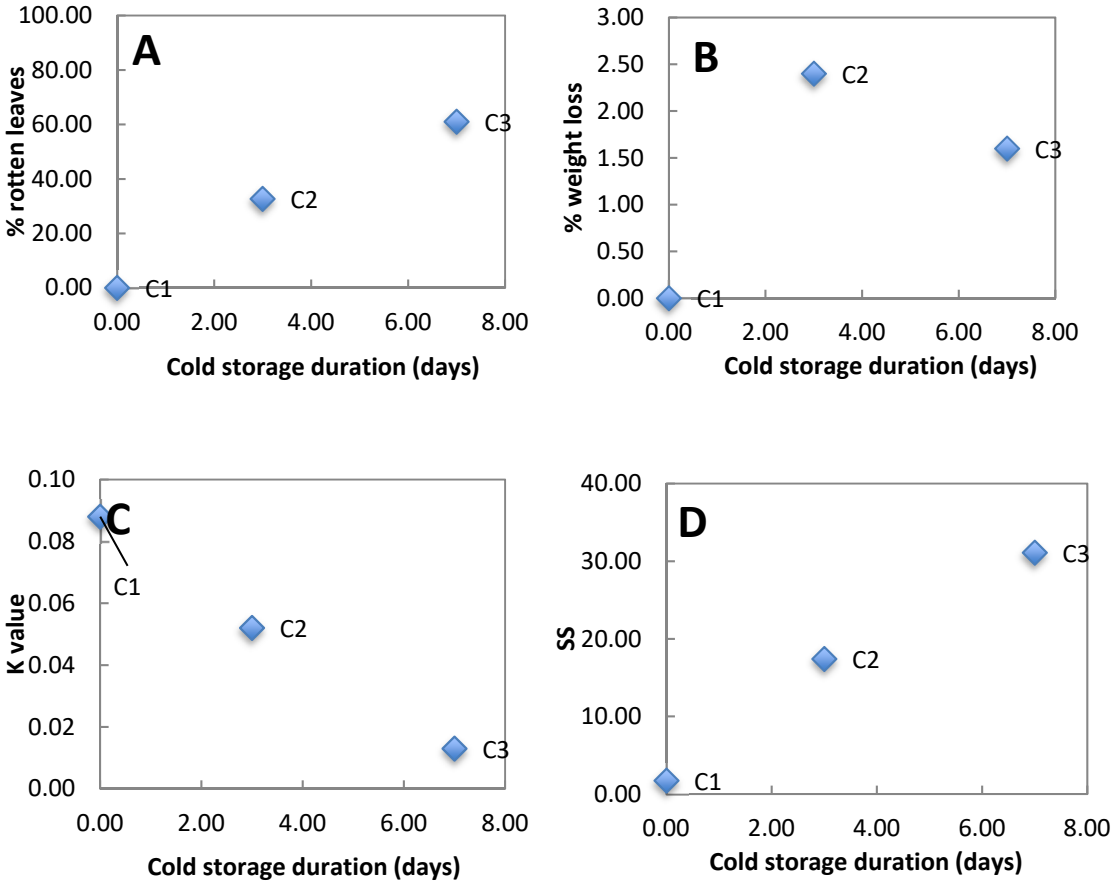


Figure 22 All variables against duration of storage (A) Percentage of rotten leaves (B) Percentage of weight loss (C) K value (D) SS. C1: 0 day, C2: 3 days in 20°C, C3: 7 days in 10°C

There were significant differences in percentage of rotten leaves, percentage of weight loss, and SS of all treatments but still there was no significant difference in variables of pesto quality except in K value between C1-C3 (Table 9). C3 had the lowest K value and it was significantly different from C1 but not significantly different from C2.

Table 9 ANOVA of Batch 3

Treatment	Rotten leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS	SE
C1	0 a	0 a	35.71 a	0.088 b	1.73 a	3.58
C2	32.66 b	2.40 c	44.65 a	0.052 ab	17.40 a	57.47
C3	61.00 c	1.60 b	34.51 a	0.013 a	31.10 a	4.22

The number followed by the same letter in each column are not significantly different according to Duncan's ANOVA New Multiple Range Test (DMRT) at 95 % level of confidence ($P < 0.05$). C1: 0 day, C2: 3 days in 20°C, C3: 7 days in 10°C

4.4.1 The quality of basil

Basil leaves start to lose their quality when it was stored, whether in the warm temperature (C2) or non-chilling temperature (C3), although their percentage of rotten leaves probably caused by two different things. C2 was caused by senescence and C3 by chilling injury. Both also have different percentage of weight loss which was probably more related to senescence than CI. Therefore, C2 which had lower rotten leaves has higher weight loss and C3 with higher rotten leaves has lower weight loss.

4.4.2 The quality of pesto

Blackening rate (K value) of fresh basil (C1) was higher than 10 days at non-chilling temperature (C3) and 3 days at warm temperature (C2). K value fell when blackening occurs and fell even further when it became more severe from long time at low temperature. This suggested that senescence and CI, both lead to a reduction in blackening rate. Variability in pesto blackening may be more of a problem with fresh, un-stored basil; once it had been stored and the leaves were more prone to blackening through senescence or CI, the rate of pesto blackening also lower as a result of a lower starting L value. However, this was not supported by the previous batches. Many samples with high percentage of rotten leaves/blackening also have high K value although their L value were lower. It seemed that it occured only to the basil that was stored at warm/room temperature as it also shown in the Batch 1 (Figure 13 and Table 2C). Therefore, comparing two kinds of storage, warm and cold, might not be suitable to describe the relationship between basil quality and their pesto.

4.4.3 The impression of 'noisy' data

Table 10 shows the analysis result of the correlation between basil quality and pesto quality. K value was significantly related to the percentage of rotten leaves but not to percentage of weight loss and therefore this result was against that of the previous Batch. However, if 'noisy' data were excluded, this correlation turns to be insignificant (Table 10A). The same applies to the correlation between treatments and the quality of basil and pesto (Table 10B). Now, 'noisy' data were becoming more important in this Batch than the previous batches since its existence could change the correlation status between treatments, quality of basil and quality of pesto. This was probably because this Batch compared cold storage and warm storage.

Table 10 Correlation between (A) quality of basil and quality of pesto (B) treatments and the quality of basil and pesto, before and after 'noisy' data excluded

(A)

Correlations		With 'noisy' data		Without 'noisy' data	
		% Rotten Leaves	% Weight Loss	% Rotten Leaves	% Weight Loss
K Value	Pearson Correlation	-0.841**	-0.549	-0.493	-0.493
	Sig.(2-tailed)	0.004	0.126	0.399	0.398
	N	9	9	5	5

**. Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

(B)

Correlations		With 'noisy' data			Without 'noisy' data		
		% Rotten Leaves	% Weight Loss	K Value	% Rotten Leaves	% Weight Loss	K value
Treatments	Pearson Correlation	0.994**	0.645	0.843*	0.998**	0.996**	-0.491
	Sig.(2-tailed)	0.004	0.061	0.004	0.000	0.000	0.402
	N	9	9	9	5	5	5

**. Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

4.4.4 Pesto Issues

The cause of two pesto issues remained unknown up to this Batch except the fact that they seemed to be related and 'odd' colour never occurred in the pesto that was made from fresh basil (un-stored). Duration of cold storage raised the chance of pesto to perform 'noisy' data. However, once again this statement was not supported by the statistics analysis result (Table 8). Those differences were insignificant, perhaps due to the 'odd' colour that was still present in one of the replicates, and this time occurred in C2 treatment. This was the reason to do the next experiments that were

slightly beyond the research objectives, to find the cause of 'odd' colour and prevent it, to eventually have a more reliable data.

4.4.5 The conclusion of Batch 3

The use of 9 technical replicates for each jar of pesto samples was not more reliable than 3 technical replicates. The more pesto contact with air, the more likely to has large differences on their K value. Furthermore, the average of K value for 3 first technical replicates was almost the same as nine replicates.

Basil leaves started to lose their quality when stored, whether in the warm temperature or non-chilling temperature, although both were probably caused by two different things, senescence and chilling injury.

The blackening rate of warm temperature was higher than that of the cold storage treatment but they cannot be compared as long as the relationship between basil quality (rotten leaves) and pesto quality (K value) was concerned since it might go in the opposite directions. For warm temperature basil, K value of their pesto tended to be decreased by the time percentage of rotten leaves increased and for cold temperature basil, it was otherwise.

'Noisy' data became more important in this Batch since they had an impact to the correlation between treatments, and the quality of basil and pesto, although statistical analysis result showed that SS was not significantly different between treatments. This led to other pesto issue, which was 'odd' colour, that needed to be solved in the next batch.

4.5 Batch 4

'Odd' starting colour was predicteed to be a result of enzymatic process inside the leaves during cold storage (Barrett et al., 2000) or water that was accidentally mixed with pesto compound (Lin, 2009). One of the simple methods to stop enzymatic reaction is by blanching (Barrett et al., 2000; Barrett & Theerakulkait, 1995). PPO, which is known as discoloration enzyme, could be deactivated by a high temperature (80°C) (Ceni et al., 2008). However, soft tissues like basil leaves can have a low tolerance to heat treatment (Barrett et al., 2000; Boggia, Zunin, Hysenaj, Bottino, & Comite, 2015). Therefore, in this fourth Batch, a small experiment was conducted to see the reaction of basil leaves after soaked into hot water (HW). Meanwhile, for control and for testing water hypothesis at

once, other basil leaves were soaked into tap water (TW). Theoretically, if blanching can reduce blackening rate, K value of HW will be lower than TW. Furthermore, the starting colour will be similar if blanching did not cause damage to the tissues and water was not the cause of 'odd' colour.

Leaves of HW turn darker after soaked into hot water, but the starting colour of the pesto was as normal as usual treatment which was around 30's (Table 11). It seemed that heat and water were not a good combination to bringing up the starting L value since starting colour of TW was far above HW and categorized as 'odd' one. It was probably because of different sources of sample (this Batch used Superherb basil) or it was really because of contact with room temperature water. The second option met the condition of 'odd' pesto from previous batches since the 'odd' pesto always belonged to the stored basil. The bag of samples probably trapped some condensation water when basil was stored in the cold storage and in some bags, the water evaporates more slowly than others when they warm up in the room temperature. This became the subject of the next batch.

Both treatments have similar K value but different SS (Table 11). SS value of TW was higher although it was made from the fresh basil. It broke the idea that 'noisy' data only occur to the stored basil. Water that was mixed in the pesto may interfere the reflected light from the spectrophotometer to the pesto surface or created an emulsion with olive oils that caused the colour change of pesto.

Table 11 Result of Batch 4

Treatment	Starting colour (L value)	K value	SS
HW	39.73	0.032	2.45
TW	59.95	0.034	11.13

HW: Soaked in hot water, TW: soaked in tap water

In conclusion, hot water treatment (blanching) disrupted basil leaves tissues and create a darker pesto. 'Odd' colour pesto was due to the water that was accidentally mixed during pesto processing. However, it seemed that both did not affect the K value, but they might affect the SS value.

4.6 Batch 5

The uniformity of the samples was tested in this Batch along with a repetition of Batch 4. There are several factors that may cause the leaf samples not to be uniform even though they came from the same factory. Seed contamination is one of the factors since new hybrid could very easily occur in nature for basil (Paton, 1992). Different variety of basil could lead to the variety of chemical amount (Rubiyanto, Sastrohamidjojo, & Anwar, 2015; Simon et al., 1990) and eventually variety of experiment result. However, this will be hard to confirm. Samples that were used in this study had some stalks that were harder than some others. Besides the possibility of contamination, there was also the possibility of leaf sample age varying. Variety in age samples could also lead to the variety of chemical amount (Dudai et al., 2001; Fischer et al., 2011; Gershenzon, McConkey, & Croteau, 2000; Paton, Harley, & Harley, 1999; Szabo & Bernarth, 2002) and eventually cause bias to experiment result. Therefore, basil leaves with hard stalk (suspected from older plants) were separated from those with soft stalk (suspected from younger plant), and then tested them to see whether this difference affected the results.

The L value results that are shown in the Table 12 indicated that there was a significant difference in all variables. The experiment in this Batch was technically error-free, with SE between replicates of the same treatments only around 10 to 13. There was no 'odd' replicate among the normal replicates of the same treatments. 'Odd' colour that used to disrupt the analysis result only occurred in all replicates of tap and icy water treatment (TI). Another evidence that starting colour of pesto was influenced by water.

Table 12 ANOVA of Batch 5

Treatment	Starting colour (L value)	K value	SS	SE
OL	38.37 c	0.024 a	24.95 b	13.07
YL	31.40 a	0.035 ab	3.32 a	10.55
HI	34.91 b	0.058 c	5.20 a	10.14
TI	54.07 d	0.037 ab	14.23 ab	11.06

The number followed by the same letter in each column are not significantly different according to Duncan's ANOVA New Multiple Range Test (DMRT) at 95 % level of confidence ($P < 0.05$). OL: old leaves, YL: young leaves, HI: soaked in hot water + icy water, TI: soaked in tap water + icy water

Blackening rate of OL was lower compared to YL and even the lowest among all the treatments. This result did not match expectations since old leaves usually senesce faster than young leaves. A hormone that relate to senescence, strigolactones, is only produced in old leaves (Yamada et al., 2014), old leaves also had a lower amount of carbohydrate and anti-oxidative activity than younger leaves that make them more susceptible to oxidative stress (Alscher et al., 1997; Ranwala & Miller, 2000). However, since statistical analysis result of this Batch showed that the blackening rate between young and old leaves was not significantly different, all we can say is that leaf age does not seem to be an important variable for pesto blackening rate in basil..

Hot water treatment (HI) had the fastest blackening rate although its starting colour was not the darkest nor the palest. This result showed that instead of hampering the blackening rate of the pesto, blanching basil leaves tended to increase it. Blanching was known to be capable of breaking down membranes of the leaves which allow substrate and enzyme to be mixed or allow enzyme to attack substrate (Tijsken et al., 2001). Therefore, by the time the enzyme inactivated, tissues were already black.

'Noisy' data might depend on the substance that was contained in old leaves only and was probably deactivated by hot water since OL and HI represent highest and lowest SS respectively (young leaves have lower SS than HI, but both numbers statistically show no difference). Yet, it needs a further study to support this suggestion.

In summary, there were some patterns in this Batch which were the case in the previous batches. First, 'odd' colour related to the water in the pesto mixture. Second, 'odd' data can be present in fresh basil if basil leaves had contact with room temperature water or icy water. 'Noisy' data can also be due to the samples that were derived from old leaves. These should be things to ponder in the next batches, in order to maintain uniformity of the samples of pesto, basil leaves should be kept from any contact with water and the number of leaves that are suspected to be older should be equal for all samples.

4.7 Batch 6

This Batch was conducted to test whether duration of cold storage correlates with variables that were measured. The idea was taken from the result of Batch 1 and 2 which suggested that duration of storage might play a more important role than does

the level of temperature in the percentage of rotten leaves, weight loss and K value. By avoiding the causes of 'noisy' and 'odd' data that were suggested by the result of previous batches, this Batch gave more reliable data than Batch 1 and 2.

4.7.1 The quality of basil

Duration of cold storage and the level of temperature still resulted in different pattern of the percentage of rotten leaves and percentage of weight loss. Percentage of rotten leaves and weight loss of basil that were stored at 12°C (Figure 23) increased during the period but at 4°C, it was only the case with the percentage of rotten leaves but not with the weight loss. It increased until day 4 and then decreased in 5-6 days.

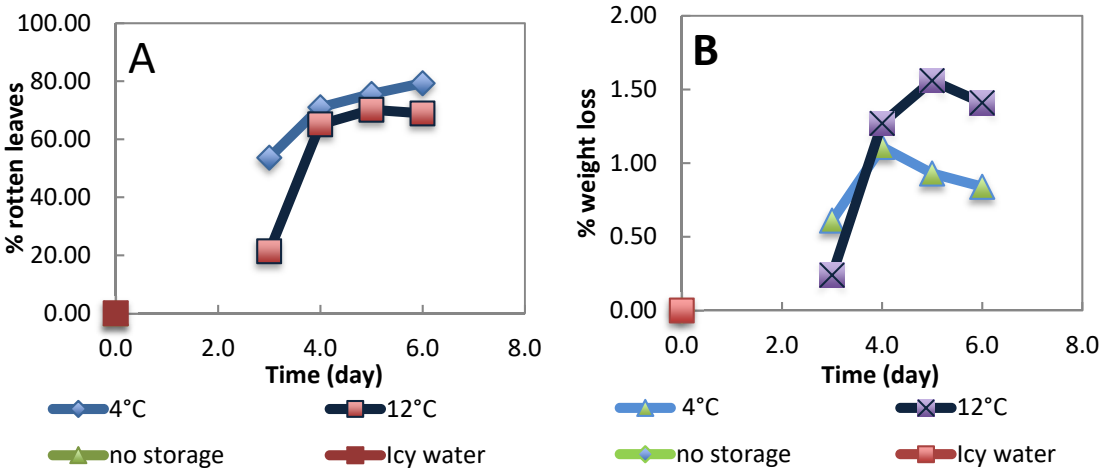


Figure 23 Duration of cold storage against (A) percentage of rotten leaves) (B) percentage of weight loss

Basil leaves that were stored at 12°C had better performance in percentage of rotten leaves but worse in weight loss compared to those in 4°C. After day 4 through 6, percentage of rotten leaves and weight loss at both temperature remained unchanged according to the statistical analysis (Table 13). From day 3 through day 4, there was an increase in both variables at both temperatures. Therefore, CI probably starts between 3-4 days or may be less for chilling temperature (4°C) since the percentage of rotten leaves on the third day was much higher than 12°C. Both temperature was equal in weight loss on day 3 to fresh basil (TD9) and it started to be different on day 4 but for percentage of rotten leaves, they start to be different at day 3. After that, the change was insignificant between days. This became the data that shall be the basis for

determining duration of storage to apply in the last Batch's treatments. Duration of storage for the last Batch should be set at less than 3 days.

Table 13 result for basil quality of Batch 6

Duration (day)	Rotten leaves (%)				Weight loss (%)			
0	TD9	0.00 a	TDIcy	0.00 a	TD9	0.00 a	TDIcy	0.00 a
3	TD1	53.66 c	TD5	21.41 b	TD1	0.61 ab	TD5	0.24 a
4	TD2	71.04 d	TD6	65.13 cd	TD2	1.11 bcd	TD6	1.27 cd
5	TD3	75.63 d	TD7	70.18 d	TD3	0.93 bc	TD7	1.56 d
6	TD4	79.34 d	TD8	68.92 d	TD4	0.84 bc	TD8	1.41 cd

The number followed by the same letter in each column are not significantly different according to Duncan's ANOVA New Multiple Range Test (DMRT) at 95 % level of confidence ($P < 0.05$). TD1-4: stored at 4°C in 3-6 days, TD5-8: stored at 12°C in 3-6 days, TD9: fresh, TDICy: 20°C in 3 days + cold water.

The result of Batch 6 was similar to the result of the study by Rolny et al. (2011) which suggested that the visible symptoms of CI on basil stored at 4-8°C appear in 4 days (Rolny et al., 2011). However, no studies has ever revealed that the symptoms also appear at 12°C within more than 4 days (Lers et al., 2010; Chen et al., 1997; da Silva et al., 2005). It suggested that the lesions resulting from 12°C treatment were caused by senescence, not CI.

4.7.2 The quality of pesto

Treatments at both temperatures showed fluctuation in K value through the duration of time (Figure 24), but the differences were insignificant according to statistical analysis (Table 14). Therefore, they were suggested to be steady through duration of storage time.

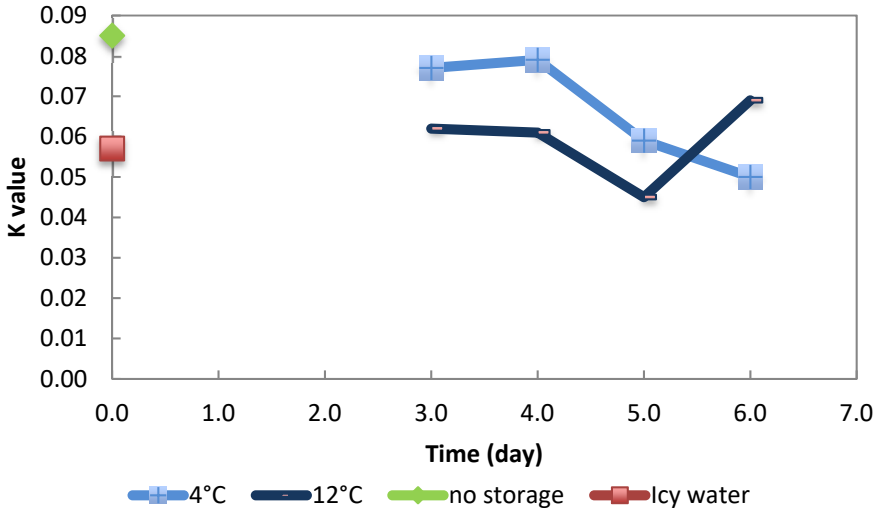


Figure 24 Duration of cold storage against K value

Pesto measuring results of Batch 6 cannot be compared to others due to different type of o-ring used. The different space caused by different type of o-ring that separates spectrophotometer from pesto surface influenced the reading result especially in the L value. Therefore, the data of pesto was classified by the type of o-ring and cannot be compared to one another (Table 14). Type 1 o-ring consist of TD9 and TDIcy, Type 2: TD1 and TD5, both groups were analysed by T test since only two treatments for each type and the rest were type 3, by ANOVA.

There were no 'noisy' data in this Batch, all treatments had SS value that was below 10. SE of TD6 and TD8 were high but still below 40, much lower than those in the Previous Batches that can reach over 50. Some of the treatments had one 'odd' pesto sample among their replicates although not sufficiently different from other 2 replicates. It was important to note that with type 3 of o-ring, those categorized as 'odd' colour pesto shift from L value > 50 to L value > 30.

Table 14 Separated analysis of Batch 6

Duration (day)	Treatment	Starting colour (L value)	K value	SS	SE
0	TD9	32.96 a	0.085 a	1.52 a	8.56
	TDIcy	46.43 b	0.057 a	2.41 a	12.08
3	TD1	19.72 a	0.077 a	5.27 a	26.49
	TD5	16.18 a	0.062 a	2.39 a	3.00
4	TD2	29.09 a	0.079 b	0.99 a	2.84
	TD6	31.76 ab	0.061 ab	3.36 b	37.98
5	TD3	29.09 a	0.059 ab	1.67 ab	20.02
	TD7	36.98 b	0.045 a	2.34 ab	8.04
6	TD4	35.30 ab	0.050 a	1.47 ab	24.16
	TD8	31.05 ab	0.069 ab	1.52 ab	34.97

The number followed by the same letter in each column are not significantly different according to independent-samples T test and Duncan's ANOVA New Multiple Range Test (DMRT) at 95 % level of confidence ($P < 0.05$), comparing only amongst treatments using the same 'O' rings for pesto quality. TD1-4: stored at 4°C in 3-6 days, TD5-8: stored at 12°C in 3-6 days, TD9: fresh, TDICy: 20°C in 3 days + cold water.

Basil washed in icy water (TDIcy), once again, showed that water on basil leaves resulted in a very pale pesto colour. This was indicated by the high initial L values of TDIcy (Table 14); but this colour difference did not affect the K values, which were identical; i.e. pesto blacken at the same rate when made from fresh basil leaves (TD9), with or without surface moisture. These data were generated with type 1 'O' rings, so were also strictly comparable with data from other Batches.

On the 3rd day, both treatments (at 4°C and 12°C) had similar values in all variables of pesto quality according to T test, so was at day 4-6th according to ANOVA. The change between days was also categorized as equal or almost flat. Therefore, the different trend that shown by Figure 24 was not significant according to statistical analysis.

4.7.3 The relationship between basil quality and pesto quality

Day 5 might play an important role to basil leaves that were stored at 12°C since the pattern changed after 5 days in 2 variables: percentage of weight loss and K value (Table 13&14). For weight loss, the pattern increased within 3-5 days and then

decreased on day 6. While K value showed a decreasing trend within 3-5 days and increased in day 6, so close to TD9. Day 4 played an important role to basil leaves that were stored at 4°C since after that day, the pattern changed. Only on day 3-4, both weight loss and K value increased but they decreased within 4-6 days.

Although the percentage of rotten leaves and weight loss for TD1 (stored at 4°C for 3 days) and TD5 (stored at 12°C for 1 day) was significantly different from each other, none of the colour variables show similar result. This confirmed the previous Batch conclusion (Batch 2, cold storage duration ≤ 3 days) that basil quality might not have effect on the pesto quality. However, the result of TD2,3,4,6,7and 8 (stored at 4°C and 12°C for 4,5&6 days) suggested that there might be a connection between basil quality and pesto quality as shown in Figure 25.

K value seemed to be at a maximum rate at around 70% of rotten leaves or 1 % of weight loss and minimize to both direction, lower and higher than 70% of rotten leaves or 1 % of weight loss (Figure 25 A&B). With this result, the relationship between basil and pesto quality can also be related to the time of cold exposure. It started to have connection when the duration of cold storage was about 4 days. However, this was not confirmed by statistical analysis, the change between the day was not significant and the correlation between K value with basil quality was also not real (Table 15), although the treatments which were combination between the level of temperature and duration of storage had a significant correlation to percentage of rotten leaves. Unfortunately, K value between day 3 and the other days could not be statistically tested since the data were unable to be compared.

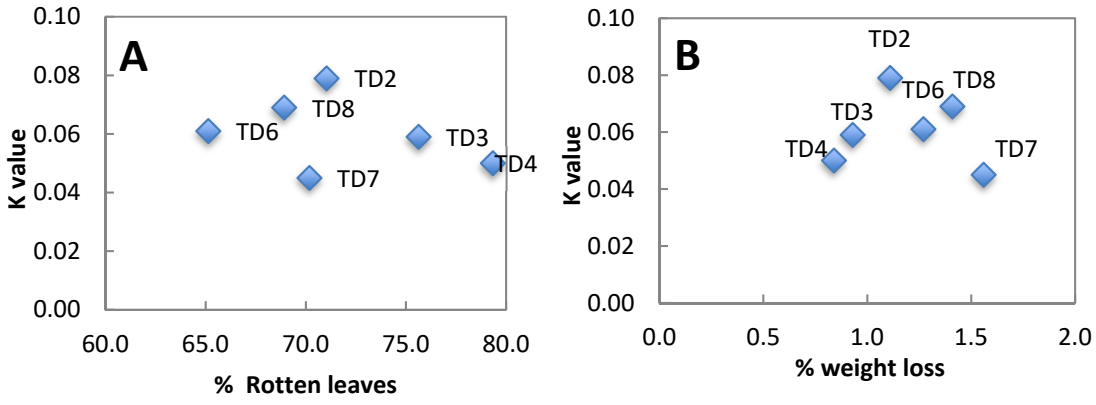


Figure 25 Connection between K value and (A) percentage of rotten leaves (B) percentage of weight loss. TD2-4: stored at 4°C in 4-6 days, TD6-8: stored at 12°C in 4-6 days.

Table 15 Correlation between basil and pesto quality & treatment and basil quality

Correlations		% Rotten Leaves	% Weight Loss
K Value	Pearson Correlation	-0.234	0.146
	Sig. (2-tailed)	0.350	0.563
	N	18	18
Treatment	Pearson Correlation	-0.571**	-0.219
	Sig. (2-tailed)	0.001	0.246
	N	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

4.7.4 Conclusion of Batch 6

Duration of cold storage and the level of temperature had different impacts on the percentage of rotten leaves and percentage of weight loss. These two variables of basil quality tend to increase except for weight loss of basil that were stored at 4°C. It only increased until day 4 and then decreased in the 5-6 days.

Basil leaves that were stored at 12°C have better performance in percentage of rotten leaves but worse in weight loss compared to those stored at 4°C. However, both temperature showed that basil best to be stored not more than 3 days, if intend to have the percentage of rotten leaves less than 30%. CI was probably set less than 3 days of storage at chilling temperature (4°C) since the percentage of rotten leaves on the third day was already half of the samples.

Space created by different types of o-ring that separated the spectrophotometer from pesto influenced the reading result in L value. Therefore, the data of pesto were not comparable with different o-ring.

Basil washed in icy water always caused pesto to have high L value but it had no impact on the K values. K values were more likely to be influenced by the certain days of storage since the pattern change in day 5 for basil leaves that were stored at 12°C and in day 4 for basil leaves that were stored at 4°C.

The relationship between basil and pesto quality can also be influenced by the length of cold storage since it seemed to have connection when the duration of storage was around 4 days. However, statistical analysis shows that the relationship between

basil quality and pesto quality was not significant. Basil quality does not have a consistent relationship with pesto quality.

4.8 Batch 7

This Batch of treatments was conducted based on the result of Batch 6 which predicted that CI start in less than 3 days in cold storage. Therefore, this Batch treatments consist of control (fresh basil) and two levels of temperature within 2,3, and 4 days each. 7°C was used as the replacement of 4°C to explore the threshold for CI.

4.8.1 Basil quality

Unlike previous Batches, both variables of basil quality in this Batch showed a similar pattern throughout the duration of storage (Figure 26). They tend to increased overtime. It was probably because both temperatures were not chilling for basil, although other studies suggest that temperature under 9°C was not suitable for basil (Cantwell & Reid, 1993; Costa et al., 2013; Goto et al., 1993; Lange & Cameron, 1994; Lers et al., 2010; Rolny et al., 2011). However, basil that were stored at 12°C still perform a better quality than those stored at lower temperature. It was a common behaviour for chilling sensitive herb.

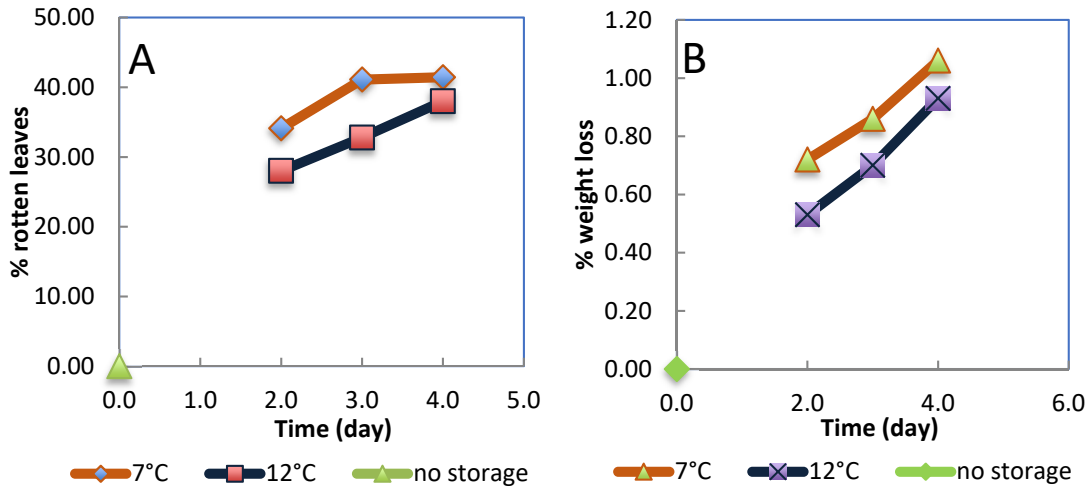


Figure 26 Duration of cold storage against (A) percentage of rotten leaves and (B) percentage of weight loss

Table 16 showed that statistically, there was no difference between those two temperatures on basil quality although it seemed that 12°C could reduce quality loss of basil better than 7°C. The change between days for both variables of basil quality was also not obvious. It was only significant to those stored at 12°C (e.q. day 2 and 4). It

meant that the damage caused by senescence was more rapid than by CI, although it started in a lower level.

Table 16 Result for basil quality of Batch 7

Duration (day)	Rotten leaves (%)				Weight loss (%)			
0	CK7	0.00 a			CK7	0.00 a		
2	CK1	34.11 bc	CK4	28.00 b	CK1	0.72 b	CK4	0.53 ab
3	CK2	41.11 c	CK5	32.78 bc	CK2	0.86 b	CK5	0.70 b
4	CK3	41.44 c	CK6	38.00 c	CK3	1.06 b	CK6	0.93 b

The number followed by the same letter in each column are not significantly different according to Duncan's ANOVA New Multiple Range Test (DMRT) at 95 % level of confidence ($P < 0.05$). CK1-3: stored at 7°C in 2-4 days, CK4-6: stored at 12°C in 2-4 days, CK7: fresh.

4.8.2 Pesto quality

As the case in Batch 6, there were no 'noisy' data in this Batch (Table 17), although SS value of CK7 was different from others, it was still far below 10. SE was still relatively high in this Batch since there were still some 'odd' colours, only that this time, those categorized as 'odd' colours have shifted from L value > 50 to L value > 30 due to the change of o-ring type. Somehow, the interaction between pesto producing and condensation water cannot be avoided.

Table 17 Result for pesto quality of Batch 7

Treatment	Starting colour (L value)	K value	SS	SE
CK1	30.83 a	0.072 a	1.13 a	51.26
CK2	33.61 a	0.058 a	1.64 a	46.62
CK3	33.19 a	0.054 a	1.00 a	40.55
CK4	32.09 a	0.067 a	1.22 a	39.76
CK5	32.73 a	0.057 a	0.91 a	39.99
CK6	29.73 a	0.040 a	0.71 a	26.82
CK7	40.10 a	0.033 a	3.26 b	5.34

The number followed by the same letter in each column are not significantly different according to Duncan's ANOVA New Multiple Range Test (DMRT) at 95 % level of confidence ($P < 0.05$). CK1-3: stored at 7°C in 2-4 days, CK4-6: stored at 12°C in 2-4 days, CK7: fresh.

There were almost no differences in all variables treated at these two temperatures. However, 12°C was still a more suitable temperature for stored basil leaves than lower temperature (e.g. 7°C) regarding reducing blackening rate or K value (Figure 27). This result had a more consistent pattern than that in Batch 6. Perhaps, fluctuation of the variables only occurred to those stored in around 4 days and above (result of Batch 6).

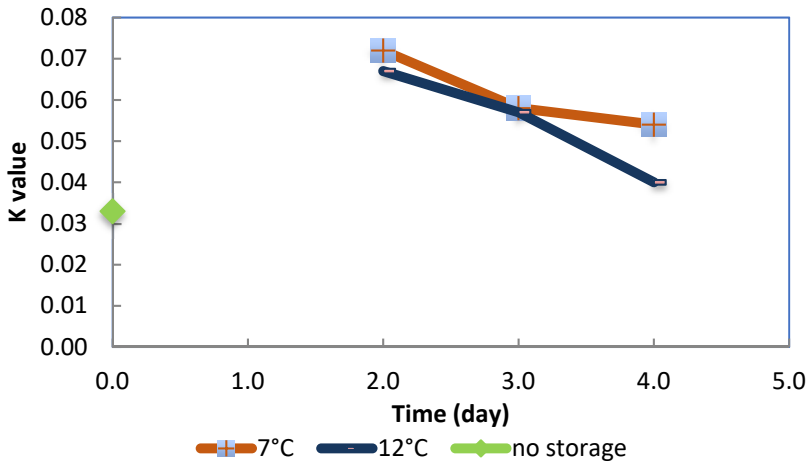


Figure 27 Duration of cold storage against K value

4.8.3 The relationship between basil quality and pesto quality

Like Batch 6, this Batch also showed pattern that indicated a connection between K value and two variables of basil quality (e.g. percentage of rotten leaves and weight loss) (Figure 28). However, in this Batch the pattern was more obvious, K value was at a maximum rate when percentage of rotten leaves was around 30% and weight loss reaches around 0.7 %. This was a feat accomplished in the shortest duration of storage and the lowest temperature (CK1) and the lowest K value was found in the fresh basil and basil leaves that had the longest duration of storage and the highest temperature of storage (CK6).

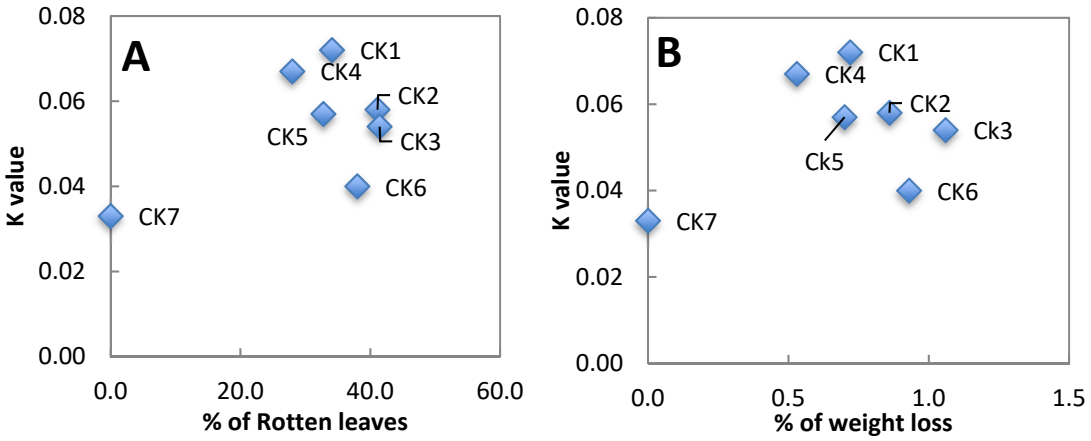


Figure 28 The relationship between K value and (A) percentage of rotten leaves (B) percentage of weight loss. CK1-3: stored at 7°C in 2-4 days, CK4-6: stored at 12°C in 2-4 days, CK7: fresh.

Statistical results showed that the relationship between K value and percentage of rotten leaves or weight loss was not significant. However, K value and percentage of rotten leaves was significantly influenced by the treatments (Table18).

Table 18 Correlation between variables

		% Rotten Leaves	% Weight Loss	K Value
Treatment	Pearson Correlation	-0.598**	-0.413	-0.492*
	Sig. (2-tailed)	0.004	0.063	0.023
	N	21	21	21
KValue	Pearson Correlation	0.388	0.005	
	Sig. (2-tailed)	0.082	0.982	
	N	21	21	

**. Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

4.8.4 Conclusion of Batch 7

Both variables of basil quality in this Batch tended to increase by the duration of storage and statistically, basil that was stored at 12°C did not reduce basil quality and pesto quality loss better than those stored at lower temperature (7°C).

No 'noisy' data was found in this Batch but there existed some 'odd' colours, only that those categorized as 'odd' colour pesto was no longer with L value > 50 but L value > 30 due to the change of o-ring type.

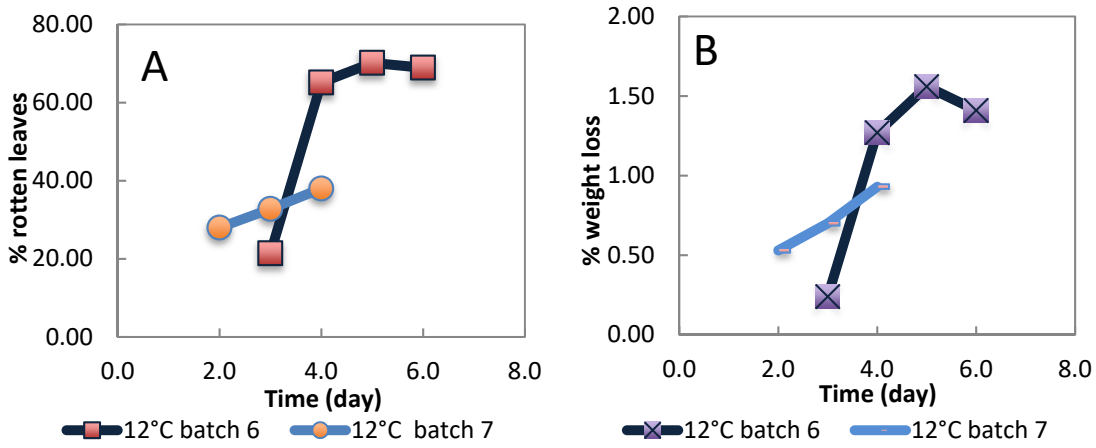
Since the pattern of blackening rate in Batch 7 was more consistent than that of Batch 6, there was a possibility that fluctuation of the basil and pesto quality variables only occur when basil was stored in around 4 days and beyond. However, once again the change between days of storage were not statistically significant.

This Batch also shows that there was no correlation between basil quality and pesto quality, but there was a correlation between treatments and the percentage of rotten leaves and K value.

4.9 Summary of storage at 12°C

Treatments in both Batch 6 and 7 involved 12°C storage, if the results were combined into the same graph, then they can be shown in Figure 29. It seemed that all the variables of basil and pesto quality were in the similar pattern although not exactly in the same level. Both variables of basil quality showed an increasing rate on day 3 to 4. Therefore, there was an indication that quality loss of basil increased sharply during that day and only slowly increased or even remained flat afterward (Figure 29 A&B). It strengthens the presumption that patterns of all variables of basil quality change or fluctuate when the storage time was more than 4 days.

K value for 12°C storage, on the other hand, showed a decreasing trend until day 5 and increasing trend afterward (Figure 29 C). However, the change was not significant from time to time, so it considered to be flat or was not influenced by the duration of storage.



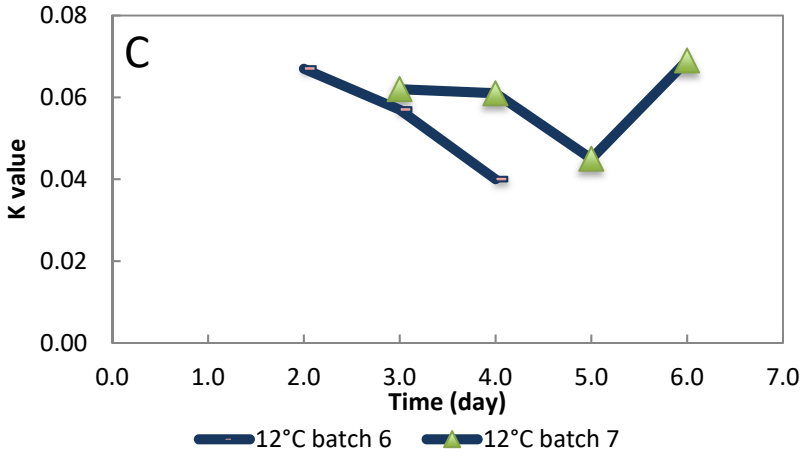


Figure 29 The combined result of Batch 6 and 7 (A) percentage of rotten leaves (B) percentage of weight loss (C) K value

To test whether every level of temperature behaved in the same pattern, summary of 4°C storage was also provided in the next section. This temperature was chosen since it was a chilling temperature, opposite to 12°C, and it was the most abundant data of all other levels of temperature.

4.10 Summary of storage at 4°C

3 batches that used 4°C storage as their method of treatments are Batch 1, 2, and 6. The combined data were presented in Figure 30. The data of 4°C storage in Batch 1 came from the fast cooling treatment and stored in 1 and 2 days, Batch 2 was stored in 1,2, and 3 days, and batch 6 stored in 4, 5, and 6 days. It seemed that in the same day (day 1 to 2) the trend of Batch 1 was similar that in Batch 2, which showed an increasing trend in percentage of rotten leaves and K value, decreasing trend in percentage of weight loss. The trend of Batch 6 seemed to continuing trend of Batch 2, although in the percentage of weight loss, batch 6 started at a lower level than Batch 2.

A more extreme decrease or increase in Batch 1 was probably because of the leaves experienced faster cooling than those in other Batches. However, the changes of the variables between days were only significant in weight loss of Batch 1&6, and in the percentage of rotten leaves of Batch 2&6.

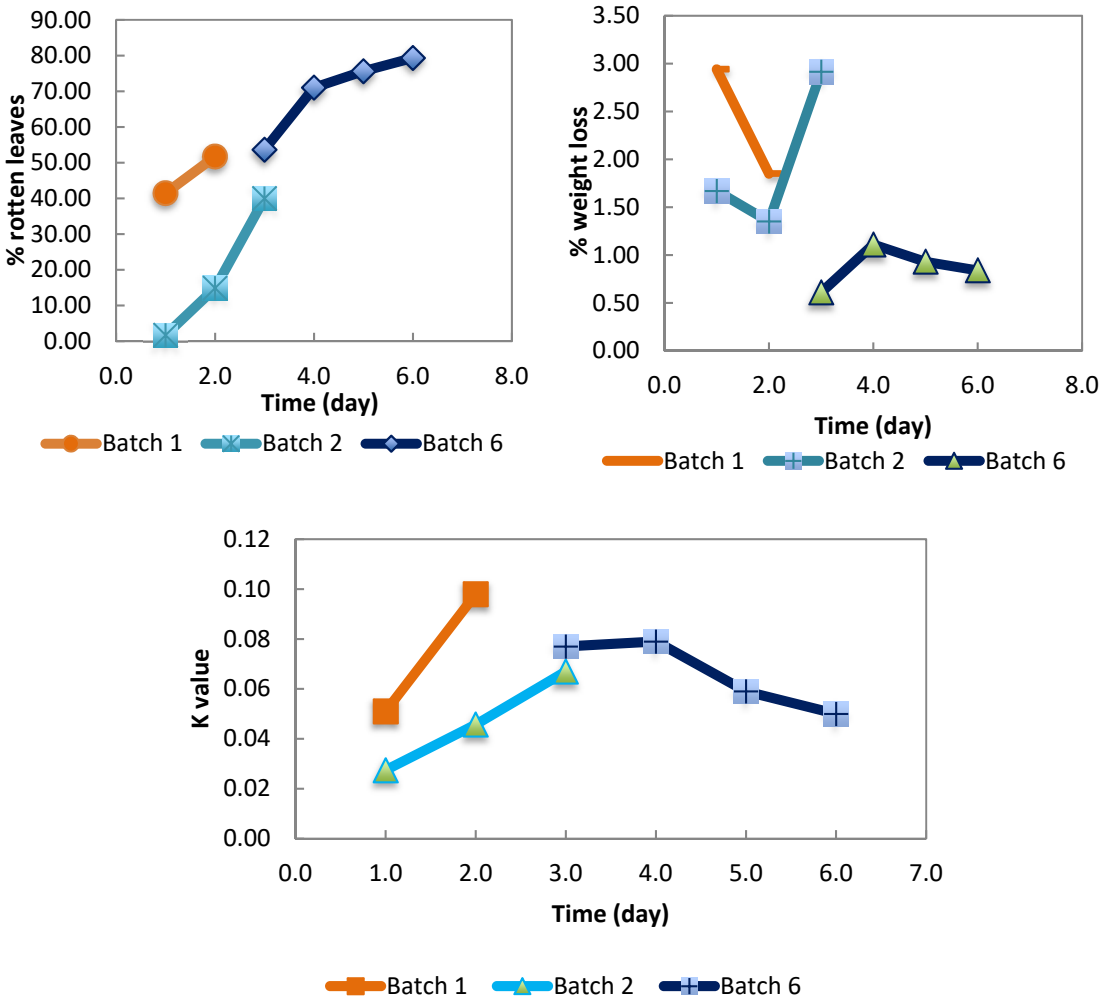


Figure 30 The combined result of Batch 1,2 and 6 (A) percentage of rotten leaves (B) percentage of weight loss (C) K value

In summary, at 4°C storage, there were no significant changes in K value throughout days of duration. Chilling temperature tended to maintain the rate of the pesto blackening but not the rate of the basil damage just like at 12°C storage. There was also a changing of behaviour at day 4, where all variables suddenly changing the trend from increasing to steady or even decreasing

4.11 Summary of water treatment

Water treatment showed a constant result in the colour of pesto especially in starting colour even for basil that was stored in various length of days. It seemed to relate to 'odd' colour since all the basil that were soaked in the water before they were made into pesto always had a brighter colour, although the L value slightly decreased

over time of 20°C storage (Figure 31 A). Therefore, this treatment should have its own separated section.

Those three treatments in Figure 31 were taken from several Batches (Batch 4,5, &6) and coincidentally had been left in the room temperature in varied length of days (0,1, and 3 days). They were not comparable treatments since they were measured from different samples and probably have distinct condition too, plus there was not enough data that could be analysed to support the suspicion. However, it seemed that K value also increased throughout duration of the 20°C storage until day 3 (Figure 31 B), just like pesto that was made from basil stored in chilling temperature (4°C). In the other words, pesto that made from basil that stored at 20°C and contact with water tend to behave similar with those stored at 4°C.

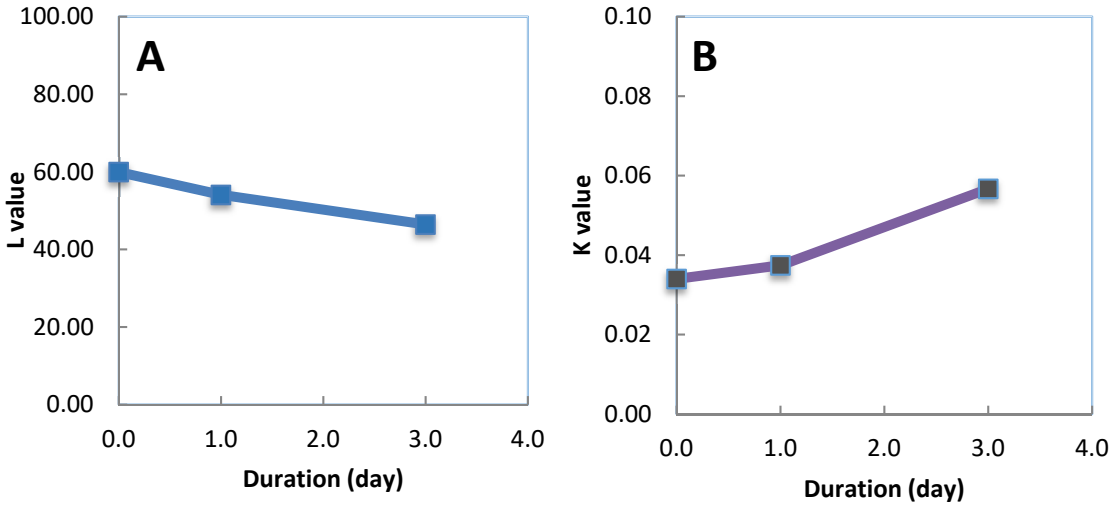


Figure 31 The combined result of water treatment in Batch 4,5, and 6 (A) Starting colour (B) K value

All the summaries above concluded that the best way to keep basil leaves fresh for pesto processing was to store them in the 12°C storage rather than in chilling temperature (4°C) or room temperature (20°C). However, duration of storage was also crucial. After 4 days, basil leaves will lose their quality to over 30 % and the K value of their pesto also will start to rise.

CHAPTER 5. CONCLUSION

A method of cooling technique for basil leaves and measuring blackening rate of pesto was established by several experiments. Cooling down basil leaves can simply be achieved by putting the samples in perforated bags inside cardboard boxes and placing those boxes at different temperatures. It takes about 4 h for basil to equilibrate with the storage environment under these conditions. Basil leaves left at room temperature start to blacken after about a day, presumably due to senescence . The factors such as duration at 20°C after cold storage and cooling technique (fast or slow cooling) can affect basil and pesto quality. Thus, these two factors should remain uniform for all batches.

Both symptoms of cold storage (CI and senescence) cannot be distinguished from each other. However, between those two symptoms senescence could produce a more damaging result to basil tissues than CI in the early stages of storage since at the same duration of storage up to day 2-3, the damage that occurred on basil leaves that were stored in a non-chilling storage were worse than in chilling storage.

Measuring blackening rate of pesto could be done by blending basil leaves with equal amount of olive oil, spreading onto paper towels and recording the colour change by spectrophotometer. The method was effectively tested for measuring blackening rate with some notes regarding replication numbers, time for processing, proxy selection, pesto setting, fitting curve, and sampling techniques.

Three biological replicates instead of two were required for more reliable results and 3 technical replicates were more practical than 9 technical replicates. There should be an equal amount of time for processing and measuring all samples of pesto because the first minutes were important in blackening process.

Initially the h value seemed more consistent in showing blackening rate when different temperatures were applied to pesto, as it was also the case in study by Severini et al (2008) and Ihl et al. (1994). However, in later trials with more mature basil, the L value indicated the blackening rate better than other proxies for the pesto that was made from Superherb basil since it was more stable on dealing with variation of samples condition. This is consistent with blackening of other products such as peach purée and broccoli (Avila & Silva, 1999; Tijsken et al., 2001).

Space between spectrophotometer and pesto surface that created by o-ring influenced the reading results. The more space between them the less the result of L value. Furthermore, fitting curve or transforming data into negative exponential was necessary to simplify fluctuations of L value data, find K value and to detect 'noisy' data.

Two pesto issues, 'odd' colour and 'noisy' data, could affect the correlation between basil quality and pesto quality. 'Odd' colour or pale green pesto related to water. Pesto made from basil that had been in contact with water always had a pale colour, whether they were fresh or left in the room temperature for several days. Water was known as a substrate that could create an emulsion with oil and change the colour appearance of the subject (Lin, 2009).

"Noisy' data might depend on the age of basil leaves since pesto that was made from old leaves was prone to have 'Noisy' data rather than young leaves. The age of leaf was known as one of the factors that influence the chemical and reaction change within the leaf (Adams & Brown, 2007; Alscher et al., 1997; Cantwell & Reid, 1993; Fischer et al., 2011; Ranwala & Miller, 2000; Viacava et al., 2014; Yamada et al., 2014). It was possible that these chemical differences affect to their pesto too and caused random fluctuation. However, these two issues did not appear to affect the K value.

Hot water treatment (blanching) was found to disrupt basil leaf tissues and create a darker pesto, instead of slowing down the blackening rate. The soft tissues of basil leaves had low tolerance to heat treatment (Barrett et al., 2000). Blanching caused membranes to break down that allowed enzymes to react on the substrate to blacken the tissues (Tijsken et al., 2001).

Cold storage had more influence on basil quality than pesto quality. Both non-chilling storage temperatures (e.g. 7 and 12°C) and chilling storage (e.g. 4°C) resulted in similar patterns for each variable of basil and pesto quality. Both variables of basil quality showed an increase over duration of cold storage but no change in K value. It meant that pesto blackening rate was not affected by senescence or CI of the leaves, although duration of storage together with the level of temperature relate to basil and pesto quality.

Basil leaves that were stored at 12°C for less than 4 days were not statistically different from those stored at 4°C, 7°C or 20°C in terms of basil quality and pesto quality.

Finally, this study answered three of its research questions. First, measuring senescence and CI of basil leaves could be simply done by cooling basil leaves with slow cooling technique, storing them in cold storage for certain days, and then holding them at room temperature for a day. However, with this method, CI and senescence symptoms became indistinguishable. Percentage of rotten leaves and weight loss were then used as the indicator of the symptom severity. For measuring blackening rate of pesto, all basil leaf samples should be blended with equal amount of olive oils and equal time in processing, using L value as the proxy of spectrophotometer and transforming the data into negative exponential to find K value. A few issues surrounding pesto measurement could be minimized by avoiding basil leaves from contact with water during pesto processing, using the same tool (e.g. used the same o-ring to separate spectrophotometer from pesto surface), and ensuring that the leaf samples were the same age.

Second, there was no correlation between senescence or CI in basil leaves and blackening rate of pesto. All Batches showed that the treatments did affect CI and blackening rate but CI of basil leaves did not have any influence to the blackening rate of their pesto.

Third, pre-processing factors impacted the basil quality but not the pesto quality. Washing basil leaves before pesto processing allowed water to create emulsion (Lin, 2009) and raised the initial L value of pesto. Blanching caused tissue damage (Barrett et al., 2000; Boggia, Zunin, Hysenaj, Bottino, & Comite, 2015) and created a darker pesto. Storing basil leaves could lead to CI and senescence development. However, these factors never affected the blackening rate of pesto.

CHAPTER 6. FUTURE RECOMMENDATION

There were a few interesting repeating patterns but they have no adequate supporting data for them and therefore should be the subject of subsequent study. The patterns included the correlation between optimum temperature for basil leaves and blackening rate of pesto, and the causes of 'Noisy' data.

It seemed that blackening rate of pesto that was made from basil that was stored outside their optimum temperature was faster throughout time than those stored in the optimum range. However, the result of this thesis showed that blackening rates of pesto did not vary consistently. A study on basil stored at other temperatures and conditions (e.g lower than 4°C, higher than 20°C, or 20°C without water) would be required to test whether this applies through a wider range of treatments.

'Noisy' data could be due to many things although the last two batches showed it was possible to reduce noise with careful handling. It is important to study the cause of 'noisy' data since the reliability of an experiment could depend on how close the raw data are to the transformed data.

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APPENDIX. EXPERIMENTAL RAW DATA

Appendix 1. Raw data of colour change of pesto in ten indicators (section 4.1.1)

Number of Data	Time	Treat-ment	Repli-cation	Group Traits	L*(D65)	C*(D65)	h(D65)	a*(D65)	b*(D65)
1	3:18:01	LB	1	SCI	34.84	14.32	98.85	-2.20	14.15
1	3:18:01	LB	1	SCE	34.67	13.66	99.00	-2.14	13.49
2	3:18:05	LB	1	SCI	34.38	14.36	98.74	-2.18	14.19
2	3:18:05	LB	1	SCE	34.17	13.60	99.29	-2.20	13.42
3	3:19:18	LB	2	SCI	31.58	20.67	101.78	-4.22	20.23
3	3:19:18	LB	2	SCE	30.54	20.92	102.02	-4.36	20.46
4	3:19:22	LB	2	SCI	31.32	20.52	101.60	-4.13	20.11
4	3:19:22	LB	2	SCE	30.24	20.92	101.92	-4.32	20.47
5	3:22:19	RT	1	SCI	37.70	12.98	102.03	-2.70	12.69
5	3:22:19	RT	1	SCE	36.74	12.80	102.63	-2.80	12.49
6	3:22:23	RT	1	SCI	37.94	12.92	101.69	-2.62	12.65
6	3:22:23	RT	1	SCE	36.97	12.94	102.45	-2.79	12.63
7	3:23:28	RT	2	SCI	36.94	15.73	102.68	-3.45	15.35
7	3:23:28	RT	2	SCE	36.54	15.21	103.25	-3.48	14.80
8	3:23:32	RT	2	SCI	37.00	15.69	102.45	-3.38	15.32
8	3:23:32	RT	2	SCE	36.57	15.27	103.07	-3.45	14.87
9	3:24:53	LB	1	SCI	21.75	10.54	94.55	-0.84	10.50
9	3:24:53	LB	1	SCE	21.49	10.79	95.56	-1.05	10.74
10	3:24:57	LB	1	SCI	25.04	10.42	94.60	-0.84	10.39
10	3:24:57	LB	1	SCE	25.04	10.51	94.79	-0.88	10.47
11	3:25:21	LB	2	SCI	34.44	10.63	97.35	-1.36	10.54
11	3:25:21	LB	2	SCE	33.70	10.45	98.28	-1.51	10.34
12	3:25:25	LB	2	SCI	34.54	10.51	97.42	-1.36	10.42
12	3:25:25	LB	2	SCE	33.80	10.27	98.18	-1.46	10.16
13	3:27:07	RT	1	SCI	33.08	13.15	99.06	-2.07	12.99
13	3:27:07	RT	1	SCE	32.37	13.04	99.56	-2.17	12.86
14	3:27:10	RT	1	SCI	33.11	13.21	98.94	-2.05	13.05
14	3:27:10	RT	1	SCE	32.37	13.10	99.51	-2.16	12.92
15	3:27:47	RT	2	SCI	32.99	10.56	97.22	-1.33	10.47
15	3:27:47	RT	2	SCE	32.37	10.40	97.96	-1.44	10.30
16	3:27:51	RT	2	SCI	33.25	10.54	97.44	-1.36	10.45
16	3:27:51	RT	2	SCE	32.57	10.34	98.35	-1.50	10.24
17	3:29:54	LB	1	SCI	30.33	11.22	96.60	-1.29	11.15
17	3:29:54	LB	1	SCE	30.00	11.02	96.31	-1.21	10.95
18	3:29:58	LB	1	SCI	30.47	11.32	96.60	-1.30	11.25
18	3:29:58	LB	1	SCE	30.08	11.00	97.23	-1.39	10.92
19	3:30:30	LB	2	SCI	31.80	10.27	94.19	-0.75	10.24
19	3:30:30	LB	2	SCE	31.07	10.19	95.04	-0.90	10.15
20	3:30:33	LB	2	SCI	31.68	10.40	93.99	-0.72	10.37
20	3:30:33	LB	2	SCE	30.95	10.26	94.87	-0.87	10.23
21	3:31:54	RT	1	SCI	31.77	11.91	94.81	-1.00	11.87
21	3:31:54	RT	1	SCE	31.14	11.72	95.47	-1.12	11.67
22	3:31:58	RT	1	SCI	31.69	11.85	94.67	-0.96	11.81
22	3:31:58	RT	1	SCE	31.03	11.74	95.11	-1.05	11.69

Number of Data	Time	Treat-ment	Repli-cation	Group Traits	L*(D65)	C*(D65)	h(D65)	a*(D65)	b*(D65)
23	3:32:26	RT	2	SCI	32.86	10.34	92.48	-0.45	10.33
23	3:32:26	RT	2	SCE	32.08	10.20	93.18	-0.57	10.18
24	3:32:30	RT	2	SCI	32.81	10.36	92.20	-0.40	10.35
24	3:32:30	RT	2	SCE	32.05	10.33	93.20	-0.58	10.31
25	3:36:13	LB	1	SCI	29.04	10.74	94.35	-0.82	10.71
25	3:36:13	LB	1	SCE	28.64	10.50	95.25	-0.96	10.46
26	3:36:16	LB	1	SCI	29.41	10.82	94.24	-0.80	10.79
26	3:36:16	LB	1	SCE	28.94	10.79	94.80	-0.90	10.75
27	3:36:39	LB	2	SCI	30.82	9.55	91.22	-0.20	9.55
27	3:36:39	LB	2	SCE	30.03	9.56	92.04	-0.34	9.56
28	3:36:43	LB	2	SCI	30.93	9.60	91.28	-0.22	9.60
28	3:36:43	LB	2	SCE	30.11	9.58	92.19	-0.37	9.58
29	3:37:22	RT	1	SCI	31.93	11.44	92.94	-0.59	11.42
29	3:37:22	RT	1	SCE	31.39	11.33	93.41	-0.67	11.31
30	3:37:25	RT	1	SCI	31.99	11.50	92.99	-0.60	11.48
30	3:37:25	RT	1	SCE	31.41	11.32	93.47	-0.69	11.30
31	3:37:55	RT	2	SCI	31.11	10.03	90.09	-0.02	10.03
31	3:37:55	RT	2	SCE	30.46	9.85	90.57	-0.10	9.85
32	3:37:59	RT	2	SCI	31.09	10.05	90.02	0	10.05
32	3:37:59	RT	2	SCE	30.45	9.92	91.01	-0.17	9.92
33	3:40:22	LB	1	SCI	29.77	10.65	93.87	-0.72	10.62
33	3:40:22	LB	1	SCE	29.02	10.53	93.98	-0.73	10.50
34	3:40:26	LB	1	SCI	29.71	10.58	93.68	-0.68	10.56
34	3:40:26	LB	1	SCE	28.97	10.59	94.71	-0.87	10.55
35	3:41:04	LB	2	SCI	28.69	9.83	90.80	-0.14	9.83
35	3:41:04	LB	2	SCE	27.96	9.95	92.14	-0.37	9.94
36	3:41:08	LB	2	SCI	28.38	9.74	91.04	-0.18	9.73
36	3:41:08	LB	2	SCE	27.63	9.84	91.85	-0.32	9.84
37	3:42:30	RT	1	SCI	30.80	10.21	90.94	-0.17	10.20
37	3:42:30	RT	1	SCE	30.13	9.99	91.84	-0.32	9.98
38	3:42:33	RT	1	SCI	30.55	10.13	90.70	-0.12	10.13
38	3:42:33	RT	1	SCE	29.88	9.91	91.64	-0.28	9.91
39	3:42:51	RT	2	SCI	30.42	10.08	90.48	-0.08	10.08
39	3:42:51	RT	2	SCE	29.63	10.08	91.26	-0.22	10.08
40	3:42:55	RT	2	SCI	30.33	10.02	90.52	-0.09	10.02
40	3:42:55	RT	2	SCE	29.55	9.84	91.59	-0.27	9.84

Appendix 2. T-test of pesto colour differences between young and old leaves (Section 4.1.3)

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
StartC	Equal variances assumed	0.167	0.704	-1.582	4	0.189	-1.36333	0.86159	-3.75550	1.02884
	Equal variances not assumed			-1.582	3.855	0.191	-1.36333	0.86159	-3.79135	1.06468
KValue	Equal variances assumed	3.203	0.148	-0.920	4	0.409	-0.008000	0.008692	-0.032134	0.016134
	Equal variances not assumed			-0.920	2.604	0.434	-0.008000	0.008692	-.038205	0.022205
SS	Equal variances assumed	11.390	0.028	-0.173	4	0.871	-0.35333	2.03843	-6.01292	5.30625
	Equal variances not assumed			-0.173	2.084	0.878	-0.35333	2.03843	-8.79361	8.08694

Appendix 3. Raw data of temperature loggers (Section 4.2)

Time	Average		Time	Average	
	FC1	FC2		FC3	FC4
0:00:00	16.425	17.025	22:48:00	17.025	8.213
0:00:10	15.650	16.625	16:48:00	16.625	7.825
0:00:20	15.050	15.775	7:12:00	15.775	7.525
0:00:30	14.350	14.825	16:48:00	14.825	7.175
0:00:40	13.600	13.900	00:00:00	13.900	6.800
0:00:50	12.850	13.100	8:24:00	13.100	6.425
0:01:00	12.100	12.300	15:36:00	12.300	6.050
0:01:10	11.375	11.575	22:48:00	11.575	5.688
0:01:20	10.700	10.875	7:12:00	10.875	5.350
0:01:30	10.075	10.300	15:36:00	10.300	5.038
0:01:40	9.475	9.750	0:00:00	9.750	4.738
0:01:50	8.975	9.200	10:48:00	9.200	4.488
0:02:00	8.475	8.725	21:36:00	8.725	4.238
0:02:10	8.025	8.250	8:24:00	8.250	4.013
0:02:20	7.650	7.850	21:36:00	7.850	3.826
0:02:30	7.300	7.500	10:48:00	7.500	3.651
0:02:40	6.975	7.150	1:12:00	7.150	3.488
0:02:50	6.700	6.825	16:48:00	6.825	3.351
0:03:00	6.425	6.550	8:24:00	6.550	3.214
0:03:10	6.200	6.325	1:12:00	6.325	3.101
0:03:20	6.000	6.075	19:12:00	6.075	3.001
0:03:30	5.775	5.875	12:00:00	5.875	2.889
0:03:40	5.625	5.675	7:12:00	5.675	2.814
0:03:50	5.450	5.500	1:12:00	5.500	2.726
0:04:00	5.325	5.325	21:36:00	5.325	2.663
0:04:10	5.200	5.150	16:48:00	5.150	2.601
0:04:20	5.075	5.025	12:00:00	5.025	2.539
0:04:30	4.975	4.900	9:36:00	4.900	2.489
0:04:40	4.875	4.750	6:00:00	4.750	2.439
0:04:50	4.800	4.650	3:36:00	4.650	2.402
0:05:00	4.700	4.575	0:00:00	4.575	2.352
0:05:10	4.600	4.475	21:36:00	4.475	2.302

Time	Average		Time	Average	
	SDC1	SDC2		SDC1	SDC2
0:00:00	20.525	20.300	12:00:00	20.300	10.262
0:05:00	20.100	19.375	0:00:00	19.375	10.052
0:10:00	19.400	18.350	6:00:00	18.350	9.703
0:15:00	18.575	17.375	9:36:00	17.375	9.293
0:20:00	17.750	16.575	14:24:00	16.575	8.882
0:25:00	17.025	15.825	20:24:00	15.825	8.521
0:30:00	16.350	15.225	4:48:00	15.225	8.185
0:35:00	15.775	14.700	15:36:00	14.700	7.900
0:40:00	15.250	14.225	3:36:00	14.225	7.639
0:45:00	14.800	13.825	16:48:00	13.825	7.416
0:50:00	14.400	13.525	7:12:00	13.525	7.217
0:55:00	14.050	13.250	22:48:00	13.250	7.044
1:00:00	13.725	13.000	15:36:00	13.000	6.883
1:05:00	13.475	12.775	9:36:00	12.775	6.760
1:10:00	13.225	12.625	3:36:00	12.625	6.637
1:15:00	13.000	12.425	22:48:00	12.425	6.526
1:20:00	12.800	12.300	18:00:00	12.300	6.428
1:25:00	12.650	12.150	14:24:00	12.150	6.355
1:30:00	12.525	12.075	12:00:00	12.075	6.294
1:35:00	12.400	12.000	8:24:00	12.000	6.233
1:40:00	12.275	11.875	6:00:00	11.875	6.172
1:45:00	12.175	11.800	3:36:00	11.800	6.124
1:50:00	12.100	11.725	1:12:00	11.725	6.088
1:55:00	12.000	11.700	22:48:00	11.700	6.040
2:00:00	11.925	11.625	21:36:00	11.625	6.004
2:05:00	11.850	11.600	19:12:00	11.600	5.968
2:10:00	11.825	11.600	19:12:00	11.600	5.958
2:15:00	11.775	11.525	18:00:00	11.525	5.934
2:20:00	11.750	11.475	16:48:00	11.475	5.923
2:25:00	11.700	11.450	15:36:00	11.450	5.900
2:30:00	11.675	11.400	15:36:00	11.400	5.890
2:35:00	11.625	11.375	14:24:00	11.375	5.866
2:40:00	11.600	11.350	13:12:00	11.350	5.856
2:45:00	11.550	11.325	12:00:00	11.325	5.832
2:50:00	11.525	11.300	12:00:00	11.300	5.821
2:55:00	11.525	11.250	12:00:00	11.250	5.823
3:00:00	11.500	11.250	10:48:00	11.250	5.813
3:05:00	11.450	11.225	9:36:00	11.225	5.789
3:10:00	11.450	11.225	9:36:00	11.225	5.791
3:15:00	11.425	11.225	8:24:00	11.225	5.780
3:20:00	11.400	11.200	8:24:00	11.200	5.769
3:25:00	11.375	11.175	7:12:00	11.175	5.759
3:30:00	11.375	11.175	7:12:00	11.175	5.760

Time	Average		Time	Average	
	SDC1	SDC2		SDC1	SDC2
3:35:00	11.350	11.125	7:12:00	11.125	5.750
3:40:00	11.350	11.125	7:12:00	11.125	5.751
3:45:00	11.350	11.100	7:12:00	11.100	5.753
3:50:00	11.300	11.100	6:00:00	11.100	5.730
3:55:00	11.300	11.100	6:00:00	11.100	5.732
4:00:00	11.300	11.100	6:00:00	11.100	5.733
4:05:00	11.275	11.100	4:48:00	11.100	5.723
4:10:00	11.275	11.050			
4:15:00	11.250	11.050			
4:20:00	11.250	11.050			

Appendix 4. Raw data of proxy selection (Section 4.2.1)

First Replication			Second Replication			Third Replication		
Data Name	h(D65)	L*(D65)	Data Name	h(D65)	L*(D65)	Data Name	h(D65)	L*(D65)
FC1-1	108.74	55.10	FC1-2	112.82	36.23	FC1-3	112.71	33.81
FC1-1	108.98	51.70	FC1-2	111.73	33.38	FC1-3	111.82	33.46
FC1-1	109.68	50.59	FC1-2	111.81	33.49	FC1-3	114.31	32.41
FC1-1	109.52	49.89	FC1-2	111.57	32.67	FC1-3	116.13	30.29
FC1-1	109.85	49.49	FC1-2	111.67	32.49	FC1-3	117.95	31.03
FC1-1	110.28	47.34	FC1-2	111.27	32.61	FC1-3	118.66	29.57
FC1-1	111.39	46.30	FC1-2	111.63	31.97	FC1-3	120.30	29.05
FC1-1	111.74	44.30	FC1-2	112.05	32.10	FC1-3	121.99	28.64
FC1-1	113.06	42.72	FC1-2	111.72	31.64	FC1-3	122.83	28.57
FC2-1	113.47	38.34	FC1-2	111.78	31.31	FC1-3	120.55	28.37
FC2-1	112.98	35.49	FC1-2	111.75	29.91	FC1-3	122.80	27.87
FC2-1	114.29	35.48	FC1-2	111.74	30.69	FC1-3	121.31	27.36
FC2-1	112.95	34.50	FC2-2	110.78	42.37	FC1-3	120.75	27.24
FC2-1	114.49	34.79	FC2-2	108.57	40.64	FC1-3	119.93	26.43
FC2-1	114.88	34.07	FC2-2	107.85	40.41	FC1-3	117.68	27.03
FC2-1	113.20	33.23	FC2-2	108.08	40.64	FC1-3	120.49	25.63
FC2-1	113.96	33.10	FC2-2	108.57	40.35	FC1-3	119.76	25.59
FC2-1	114.22	32.92	FC2-2	108.69	39.08	FC2-3	111.88	31.88
FC3-1	109.49	53.55	FC2-2	109.67	36.87	FC2-3	111.70	28.46
FC3-1	109.56	50.26	FC2-2	109.73	37.12	FC2-3	111.78	27.28
FC3-1	110.03	46.75	FC2-2	109.51	37.17	FC2-3	112.58	28.06
FC3-1	109.96	46.80	FC2-2	110.54	36.53	FC2-3	114.12	28.27
FC3-1	110.36	44.65	FC2-2	110.49	36.00	FC2-3	113.83	26.78
FC3-1	110.96	43.60	FC2-2	110.87	36.37	FC2-3	116.33	26.95
FC3-1	112.01	41.37	FC3-2	108.49	53.46	FC2-3	115.41	26.44
FC3-1	112.12	40.61	FC3-2	108.18	52.27	FC2-3	116.27	28.76
FC3-1	113.46	39.83	FC3-2	108.29	50.81	FC2-3	115.67	27.10
FC4-1	121.10	36.97	FC3-2	108.55	50.30	FC2-3	116.95	28.62
FC4-1	116.37	32.28	FC3-2	109.17	49.05	FC2-3	117.77	26.99
FC4-1	116.72	31.51	FC3-2	109.37	47.88	FC2-3	118.43	26.52
FC4-1	116.36	31.09	FC3-2	109.70	46.13	FC2-3	118.60	25.57
FC4-1	117.16	30.89	FC3-2	110.13	45.33	FC2-3	120.51	28.17
FC4-1	117.53	31.13	FC3-2	110.78	43.87	FC2-3	118.33	26.05
FC4-1	117.06	30.74	FC3-2	110.39	43.43	FC2-3	117.64	25.33
FC4-1	117.03	30.65	FC3-2	112.72	40.92	FC4-3	119.77	34.56
FC4-1	117.85	30.67	FC3-2	112.53	40.38	FC4-3	113.01	33.06
SDC1-1	122.45	36.55	FC4-2	109.50	54.18	FC4-3	117.22	30.36
SDC1-1	117.16	33.39	FC4-2	109.20	51.98	FC4-3	118.66	29.77
SDC1-1	115.00	32.34	FC4-2	109.80	50.83	FC4-3	119.79	29.64
SDC1-1	114.47	31.87	FC4-2	109.89	49.10	FC4-3	122.06	29.42
SDC1-1	114.18	31.24	FC4-2	109.80	48.59	FC4-3	120.64	28.48
SDC1-1	114.24	30.90	FC4-2	110.40	47.11	FC4-3	126.32	27.93
SDC1-1	114.51	31.22	FC4-2	110.62	46.38	FC4-3	123.43	28.20
SDC1-1	115.00	30.81	FC4-2	111.38	44.72	FC4-3	122.40	27.55
SDC1-1	116.90	31.14	FC4-2	111.88	43.04	FC4-3	128.15	27.91

First Replication			Second Replication			Third Replication		
Data Name	h(D65)	L*(D65)	Data Name	h(D65)	L*(D65)	Data Name	h(D65)	L*(D65)
SDC2-1	109.45	54.89	FC4-2	112.98	41.52	FC4-3	123.63	27.32
SDC2-1	109.21	53.81	FC4-2	114.65	40.44	FC4-3	124.86	26.65
SDC2-1	109.37	52.02	FC4-2	113.61	38.90	FC4-3	124.57	26.27
SDC2-1	108.98	50.98	SDC1-2	108.57	57.72	FC4-3	124.34	26.31
SDC2-1	109.06	49.2	SDC1-2	108.03	55.29	FC4-3	126.64	25.62
SDC2-1	109.58	47.74	SDC1-2	108.36	54.76	FC4-3	123.71	25.59
SDC2-1	109.87	46.79	SDC1-2	108.74	53.76	SDC1-3	107.29	37.94
SDC2-1	109.88	45.38	SDC1-2	109.12	52.30	SDC1-3	107.46	38.57
SDC2-1	110.54	43.8	SDC1-2	109.53	51.23	SDC1-3	107.16	38.32
			SDC1-2	109.78	49.30	SDC1-3	107.16	37.39
			SDC1-2	109.60	49.65	SDC1-3	107.70	37.04
			SDC1-2	109.92	48.64	SDC1-3	107.90	36.93
			SDC1-2	110.42	47.15	SDC1-3	108.12	36.56
			SDC1-2	111.43	45.14	SDC1-3	108.48	35.98
			SDC1-2	112.28	44.07	SDC1-3	108.08	35.57
			SDC2-2	114.38	39.00	SDC1-3	108.69	35.45
			SDC2-2	115.27	37.23	SDC1-3	109.11	34.75
			SDC2-2	112.52	36.46	SDC1-3	108.83	34.85
			SDC2-2	113.33	35.82	SDC1-3	111.13	33.81
			SDC2-2	112.79	34.99	SDC1-3	110.20	33.84
			SDC2-2	111.86	34.39	SDC1-3	109.52	34.14
			SDC2-2	111.81	35.04	SDC1-3	109.93	32.73
			SDC2-2	112.29	34.31	SDC1-3	109.86	33.76
			SDC2-2	111.53	33.65	SDC2-3	112.27	40.83
			SDC2-2	111.88	32.28	SDC2-3	111.78	40.58
			SDC2-2	112.59	31.71	SDC2-3	110.50	38.86
			SDC2-2	111.95	31.77	SDC2-3	112.70	39.91
						SDC2-3	114.48	39.87
						SDC2-3	110.10	36.78
						SDC2-3	111.69	36.95
						SDC2-3	122.40	41.01
						SDC2-3	111.79	36.35
						SDC2-3	113.40	36.54
						SDC2-3	113.88	36.32
						SDC2-3	112.62	35.23
						SDC2-3	113.90	35.90
						SDC2-3	116.66	36.13
						SDC2-3	113.78	34.81
						SDC2-3	118.33	35.95
						SDC2-3	115.79	34.66

Appendix 5. Raw data of Batch 1, percentage of rotten leaves, weight loss, and K value (Section 4.2.2)

Treatment	Days at 20°C	Rotten leaves (%)	Weight loss (%)	K value
FC1-1	1	0	3.281	0.055
FC1-2	2	52.38	3.973	0.052
FC1-3	5	100	7.888	0.035
FC2-1	1	0	2.294	0.098
FC2-2	2	55.56	2.151	0.056
FC2-3	5	100	7.569	0.047
FC3-1	1	0	2.434	0.074
FC3-2	2	54.90	3.052	0.037
FC4-1	1	0	1.536	0.304
FC4-2	2	54.90	1.808	0.038
FC4-3	5	100	6.642	0.048
SDC1-1	1	0	-0.261	0.171
SDC1-2	2	46.67	0.595	0.039
SDC1-3	5	70.00	4.483	0.037
SDC2-1	1	0	-0.294	0.053
SDC2-2	2	57.78	0.519	0.047
SDC2-3	5	70.00	2.657	0.030

Appendix 6. T-test of the differences between fast cooling and step-down cooling of Batch 1 (Section 4.2.4)

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
RottenL	Equal variances assumed	0.526	0.479	0.319	15	0.754	6.32561	19.80726	-35.89256	48.54378
	Equal variances not assumed			0.344	12.796	0.736	6.32561	18.37327	-33.43199	46.08320
WeightL	Equal variances assumed	1.460	0.246	4.993	15	0.000	2.115773	0.423714	1.212648	3.018897
	Equal variances not assumed			6.136	14.780	0.000	2.115773	0.344806	1.379880	2.851665
StartC	Equal variances assumed	0.052	0.822	-0.386	15	0.705	-1.82561	4.72478	-11.89624	8.24503
	Equal variances not assumed			-0.388	10.535	0.706	-1.82561	4.70271	-12.23216	8.58095
KValue	Equal variances assumed	0.113	0.741	0.389	15	0.702	0.013933	0.035786	-0.062342	0.090209
	Equal variances not assumed			0.435	13.922	0.670	0.013933	0.031998	-0.054731	0.082598
SS	Equal variances assumed	0.271	0.611	0.060	15	0.953	0.27124	4.51605	-9.35450	9.89697
	Equal variances not assumed			0.063	12.039	0.951	0.27124	4.28686	-9.06570	9.60817

Appendix 7. T-test of differences between cold storage duration of Batch 1 (section 4.2.4)

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
RottenL	Equal variances assumed	0.048	0.829	-.230	15	0.821	-4.78650	20.80786	-49.13741	39.56441
	Equal variances not assumed			-.219	6.834	0.833	-4.78650	21.85419	-56.71900	47.14600
WeightL	Equal variances assumed	0.077	0.785	3.550	15	0.003	1.897583	0.534517	0.758288	3.036879
	Equal variances not assumed			3.682	8.186	0.006	1.897583	0.515380	0.713798	3.081369
StartC	Equal variances assumed	1.050	0.322	0.880	15	0.393	4.27400	4.85619	-6.07671	14.62471
	Equal variances not assumed			0.807	6.369	0.449	4.27400	5.29815	-8.51018	17.05818
KValue	Equal variances assumed	2.876	0.111	-0.806	15	0.433	-0.029763	0.036930	-0.108479	0.048952
	Equal variances not assumed			-1.226	12.823	0.242	-0.029763	0.024274	-0.082277	0.022751
SS	Equal variances assumed	1.239	0.283	-0.246	15	0.809	-1.16364	4.72751	-11.24009	8.91280
	Equal variances not assumed			-0.292	11.393	0.776	-1.16364	3.99148	-9.91199	7.58471

Appendix 8. Post hoc tests of days at 20°C after cold storage of Batch 1

Rotten Leaves

Duncan[a,b]

Days at 20°C	N	Subset for alpha = 0.05		
		1	2	3
1	6	0.0000		
2	6		53.6983	
5	5			88.0000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.625.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Weight Loss

Duncan[a,b]

Days at 20°C	N	Subset for alpha = 0.05
		1
1	6	1.25467
2	6	1.74467
5	5	1.82560
Sig.		0.522

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.625.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Starting Color

Duncan[a,b]

Days at 20°C	N	Subset for alpha = 0.05	
		1	2
5	5	35.9290	
1	6	45.9000	45.9000
2	6		47.1600
Sig.		0.057	0.797

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.625.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

K value

Duncan[a,b]

Days at 20°C	N	Subset for alpha = 0.05	
		1	2
5	5	0.03946	
2	6	0.04479	
1	6		0.12592
Sig.		0.881	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.625.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Sum of Squares

Duncan[a,b]

Days at 20°C	N	Subset for alpha = 0.05
		1
1	6	4.7494
2	6	11.8849
5	5	14.2953
Sig.		0.079

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.625.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix 9. Interaction test of Batch 1

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	RottenL	22937.542[a]	8	2867.193	352.294	0.000
	WeightL	23.064[b]	8	2.883	4.835	0.019
	StartC	743.103[c]	8	92.888	1.304	0.358
	Kvalue	0.047[f]	8	0.006	1.621	0.255
	SS	475.561[g]	8	59.445	0.668	0.710
Intercept	RottenL	32244.674	1	32244.674	3961.928	0.000
	WeightL	40.630	1	40.630	68.137	0.000
	StartC	27481.618	1	27481.618	385.804	0.000
	Kvalue	0.060	1	0.060	16.617	0.004
	SS	1316.772	1	1316.772	14.787	0.005
Cooling Technique	RottenL	363.110	1	363.110	44.616	0.000
	WeightL	7.891	1	7.891	13.233	0.007
	StartC	71.346	1	71.346	1.002	0.346
	Kvalue	0.004	1	0.004	1.055	0.334
	SS	3.217	1	3.217	0.036	0.854
Storage Duration	RottenL	0.722	1	0.722	0.089	0.773
	WeightL	3.602	1	3.602	6.040	0.039
	StartC	54.609	1	54.609	0.767	0.407
	Kvalue	0.006	1	0.006	1.796	0.217
	SS	15.975	1	15.975	0.179	0.683
Days at 20°C	RottenL	18656.285	2	9328.143	1146.156	0.000
	WeightL	1.816	2	0.908	1.523	0.275
	StartC	359.248	2	179.624	2.522	0.142
	Kvalue	0.017	2	0.008	2.362	0.156
	SS	130.375	2	65.188	0.732	0.511
Cooling technique * Storage duration	RottenL	0.000	0	.	.	.
	WeightL	0.000	0	.	.	.
	StartC	0.000	0	.	.	.
	KValue	0.000	0	.	.	.
	SS	0.000	0	.	.	.
Cooling technique * days at 20°C	RottenL	545.920	2	272.960	33.539	0.000
	WeightL	0.512	2	0.256	0.429	0.665
	StartC	35.696	2	17.848	0.251	0.784
	KValue	0.004	2	0.002	0.611	0.566
	SS	32.635	2	16.317	0.183	0.836

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Storage duration * days at 20°C	RottenL	1.580	2	0.790	0.097	0.909
	WeightL	0.127	2	0.063	0.106	0.900
	StartC	220.216	2	110.108	1.546	0.271
	KValue	0.011	2	0.005	1.484	0.283
	SS	171.047	2	85.523	0.960	0.423
Cooling technique * Storage duration * days at 20°C	RottenL	0.000	0	.	.	.
	WeightL	0.000	0	.	.	.
	StartC	0.000	0	.	.	.
	KValue	0.000	0	.	.	.
	SS	0.000	0	.	.	.
Error	RottenL	65.109	8	8.139		
	WeightL	4.770	8	0.596		
	StartC	569.856	8	71.232		
	KValue	0.029	8	0.004		
	SS	712.418	8	89.052		
Total	RottenL	57175.215	17			
	WeightL	71.112	17			
	StartC	33351.276	17			
	KValue	0.163	17			
	SS	2913.725	17			
Corrected Total	RottenL	23002.651	16			
	WeightL	27.835	16			
	StartC	1312.959	16			
	KValue	0.075	16			
	SS	1187.979	16			

a. R Squared = .997 (Adjusted R Squared = .994)

b. R Squared = .829 (Adjusted R Squared = .657)

c. R Squared = .566 (Adjusted R Squared = .132)

d. R Squared = .610 (Adjusted R Squared = .221)

e. R Squared = .498 (Adjusted R Squared = -.004)

f. R Squared = .618 (Adjusted R Squared = .237)

g. R Squared = .400 (Adjusted R Squared = -.199)

Appendix 10. Correlation between variables of Batch 1 (Section 4.2.5)

Correlations

		KValue	StartC	SS
KValue	Pearson Correlation	1	-0.249	-0.485*
	Sig. (2-tailed)		0.334	0.049
	N	17	17	17
StartC	Pearson Correlation	-0.249	1	0.221
	Sig. (2-tailed)	0.334		0.393
	N	17	17	17
SS	Pearson Correlation	-0.485*	0.221	1
	Sig. (2-tailed)	0.049	0.393	
	N	17	17	17

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 11. Raw data of Batch 2 (section 4.3)

Name of treatment	Rotten leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS
T1-1	0.00	3.176	38.405	0.030	5.406
T1-2	5.13	0.818	58.540	0.031	5.729
T1-3	0.00	1.012	60.495	0.021	5.637
T2-1	13.33	0.502	38.100	0.071	5.557
T2-2	12.12	1.403	39.555	0.042	11.174
T2-3	0.00	1.502	37.205	0.070	4.771
T3-1	11.11	1.202	37.275	0.046	4.789
T3-2	15.38	1.555	58.375	0.028	50.351
T3-3	18.18	1.294	35.370	0.063	7.000
T4-1	12.12	1.319	40.220	0.034	4.922
T4-2	9.52	1.179	39.065	0.020	5.890
T4-3	18.18	1.597	57.870	0.022	13.567
T5-1	15.38	0.118	38.600	0.047	6.805
T5-2	18.18	2.082	36.925	0.044	1.610
T5-3	33.33	-0.560	37.960	0.042	1.249
T6-1	40.00	3.711	39.750	0.143	57.099
T6-2	40.00	1.649	59.325	0.032	17.191
T6-3	40.00	3.382	60.755	0.026	9.737

Appendix 12. Post hoc tests of Batch 2 (Section 4.3)

Rotten Leaves

Duncan[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
T1	3	1.7100			
T2	3	8.4833	8.4833		
T4	3		13.2733	13.2733	
T3	3		14.8900	14.8900	
T5	3			22.2967	
T6	3				40.0000
Sig.		0.165	0.208	0.085	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Weight Loss

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
T5	3	0.54667	
T2	3	1.13567	1.13567
T3	3	1.35033	1.35033
T4	3	1.36500	1.36500
T1	3	1.66867	1.66867
T6	3		2.91400
Sig.		0.203	0.054

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Starting Color

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
T5	3	37.8283
T2	3	38.2867
T3	3	43.6733
T4	3	45.7183
T1	3	52.4800
T6	3	53.2767
Sig.		0.103

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

K Value

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
T4	3	0.02559
T1	3	0.02741
T5	3	0.04434
T3	3	0.04574
T2	3	0.06104
T6	3	0.06697
Sig.		0.138

Appendix 13. Post hoc test of Batch 2 without T3 and T6 (Section 4.3.4)

Rotten Leaves

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
T1	3	1.7100	
T2	3	8.4833	
T4	3	13.2733	13.2733
T5	3		22.2967
Sig.		0.075	0.134

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Weight Loss

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
T5	3	0.54667
T2	3	1.13567
T4	3	1.36500
T1	3	1.66867
Sig.		0.229

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Starting Color

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
T5	3	37.8283
T2	3	38.2867
T4	3	45.7183
T1	3	52.4800
Sig.		0.071

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

K Value

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
T4	3	0.02559	
T1	3	0.02741	
T5	3	0.04434	0.04434
T2	3		0.06104
Sig.		0.052	0.067

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Sum of Squares

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
T5	3	3.2212
T1	3	5.5906
T2	3	7.1675
T4	3	8.1264
Sig.		0.128

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 14. Correlation between basil and pesto quality of Batch 2 (Section 4.3.5)

Correlations

		KValue	RottenL	WeightL
KValue	Pearson Correlation	1	0.309	0.293
	Sig. (2-tailed)		0.211	0.238
	N	18	18	18
RottenL	Pearson Correlation	0.309	1	0.261
	Sig. (2-tailed)	0.211		0.296
	N	18	18	18
WeightL	Pearson Correlation	0.293	0.261	1
	Sig. (2-tailed)	0.238	0.296	
	N	18	18	18

Appendix 15. Raw data of L value from nine technical replications of C1-1 Batch 3 (Section 4.4)

Time (minutes)	C1-1-1	C1-1-2	C1-1-3	C1-1-4	C1-1-5	C1-1-6	C1-1-7	C1-1-8	C1-1-9
0	33.980	35.860	37.650	33.810	35.490	34.660	35.500	36.500	35.160
5	32.965	34.091	34.670	33.034	33.448	33.244	32.563	34.355	33.805
10	32.151	32.768	32.589	32.397	31.987	32.186	30.627	32.846	32.792
15	31.500	31.780	31.137	31.874	30.943	31.395	29.351	31.785	32.034
20	30.979	31.040	30.124	31.445	30.196	30.803	28.509	31.038	31.468
25	30.561	30.488	29.416	31.092	29.662	30.361	27.954	30.514	31.044
30	30.226	30.074	28.922	30.803	29.279	30.030	27.588	30.145	30.728
35	29.958	29.765	28.577	30.566	29.006	29.783	27.347	29.885	30.491
40	29.744	29.534	28.336	30.371	28.811	29.598	27.188	29.703	30.314
45	29.572	29.362	28.168	30.212	28.671	29.459	27.083	29.574	30.182
50	29.434	29.233	28.051	30.080	28.571	29.356	27.014	29.484	30.083
55	29.324	29.136	27.969	29.973	28.500	29.279	26.968	29.420	30.009
60	29.235	29.064	27.912	29.884	28.448	29.221	26.938	29.376	29.954

Appendix 16. Raw data of Batch 3 (Section 4.4)

Name of Data	Rotten Leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS
C1-1	0.00	0.000	35.4	6.43	0.065
C1-2	0.00	0.000	35.38	5.28	0.066
C1-3	0.00	0.000	36.35	5.74	0.134
C2-1	34.09	2.671	58.68	19.25	0.037
C2-2	30.55	2.114	36.85	6.67	0.062
C2-3	33.33	2.422	38.41	6.52	0.057
C3-1	62.60	1.811	34.64	2.19	0.014
C3-2	64.86	1.696	34.27	2.06	0.013
C3-3	55.55	1.303	34.63	1.64	0.011

Appendix 17. Post hoc tests of Batch 3 (Section 4.4)

Rotten Leaves

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
C1	3	0.0000		
C2	3		32.6567	
C3	3			61.0033
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Weight Loss

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
C1	3	0.00000		
C3	3		1.60333	
C2	3			2.40233
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Starting Color

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
C3	3	34.5133
C1	3	35.7100
C2	3	44.6467
Sig.		0.139

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

K Value

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
3	3	0.01278	
2	3	0.05191	0.05191
1	3		0.08828
Sig.		0.093	0.114

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Sum of Squares

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
C1	3	1.7320
C2	3	17.3971
C3	3	31.1013
Sig.		0.076

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 18. Table of correlation between basil and pesto quality of Batch 3 (Section 4.4.3)

Correlations, include the 'noisy' data

		KValue	RottenL	WeightL
KValue	Pearson Correlation	1	-0.841**	-0.549
	Sig. (2-tailed)		0.004	0.126
	N	9	9	9
RottenL	Pearson Correlation	-0.841**	1	0.686*
	Sig. (2-tailed)	0.004		0.041
	N	9	9	9
WeightL	Pearson Correlation	-0.549	0.686*	1
	Sig. (2-tailed)	0.126	0.041	
	N	9	9	9

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations, exclude the 'noisy' data

		KValue	RottenL	WeightL
KValue	Pearson Correlation	1	-0.493	-0.493
	Sig. (2-tailed)		0.399	0.398
	N	5	5	5
RottenL	Pearson Correlation	-0.493	1	1.000**
	Sig. (2-tailed)	0.399		0.000
	N	5	5	5
WeightL	Pearson Correlation	-.493	1.000**	1
	Sig. (2-tailed)	0.398	0.000	
	N	5	5	5

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 19. Table of correlation between treatments, basil quality and pesto quality of Batch 3 (Section 4.4.3)

Correlations, include 'noisy' data

		Treatment	RottenL	WeightL	Kvalue
Treatment	Pearson Correlation	1	0.994**	0.645	0-.843**
	Sig. (2-tailed)		0.000	0.061	0.004
	N	9	9	9	9
RottenL	Pearson Correlation	0.994**	1	0.686*	-0.841**
	Sig. (2-tailed)	0.000		0.041	0.004
	N	9	9	9	9
WeightL	Pearson Correlation	0.645	0.686*	1	-0.549
	Sig. (2-tailed)	0.061	0.041		0.126
	N	9	9	9	9
KValue	Pearson Correlation	-0.843**	-0.841**	-0.549	1
	Sig. (2-tailed)	0.004	0.004	0.126	
	N	9	9	9	9

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Correlations, exclude 'noisy' data

		Treatment	RottenL	WeightL	Kvalue
Treatment	Pearson Correlation	1	0.998**	0.996**	-0.491
	Sig. (2-tailed)		0.000	0.000	0.402
	N	5	5	5	5
RottenL	Pearson Correlation	0.998**	1	1.000**	-0.493
	Sig. (2-tailed)	0.000		0.000	0.399
	N	5	5	5	5
WeightL	Pearson Correlation	0.996**	1.000**	1	-0.493
	Sig. (2-tailed)	0.000	0.000		0.398
	N	5	5	5	5
KValue	Pearson Correlation	-0.491	-0.493	-0.493	1
	Sig. (2-tailed)	0.402	0.399	0.398	
	N	5	5	5	5

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 20. Raw data of L value of Batch 4 (section 4.5)

Time (minutes)	HW1	HW2	HW3	Average	SE	%cv	Neg exp HW	k	SS
0	40.57	38.95	39.67	39.73	0.47	1.18	39.730	0.033	0.000
5	40.12	39.06	39.55	39.58	0.31	0.77	38.963		0.376
10	38.85	38.66	37.35	38.29	0.47	1.23	38.312		0.001
15	38.20	37.51	39.63	38.45	0.62	1.62	37.760		0.472
20	37.89	36.88	38.30	37.69	0.42	1.12	37.290		0.160
25	37.12	36.79	37.80	37.24	0.30	0.80	36.892		0.119
30	37.23	36.31	36.46	36.67	0.28	0.78	36.554		0.013
35	36.22	36.08	37.02	36.44	0.29	0.80	36.266		0.030
40	35.81	35.76	36.06	35.88	0.09	0.26	36.022		0.021
45	36.43	35.17	35.35	35.65	0.39	1.10	35.815		0.027
50	35.37	34.76	35.00	35.04	0.18	0.51	35.639		0.355
55	35.43	34.42	34.09	34.65	0.40	1.16	35.490		0.711
60	34.5	34.05	36.33	34.96	0.70	1.99	35.363		0.163
					4.93		Sum of squares		2.448

Time (minutes)	TW1	TW2	TW3	Average	SE	%cv	Neg exp TW	k	SS
0	57.41	62.39	60.06	59.95	1.44	2.40	59.950	0.034	0.000
5	53.90	61.50	57.23	57.54	2.20	3.82	57.616		0.005
10	52.87	59.54	55.00	55.80	1.97	3.52	55.647		0.024
15	51.70	58.52	53.99	54.74	2.00	3.66	53.987		0.562
20	49.16	56.64	53.04	52.95	2.16	4.08	52.586		0.130
25	50.47	56.42	50.95	52.61	1.91	3.63	51.405		1.461
30	46.81	55.76	50.54	51.04	2.60	5.09	50.408		0.395
35	45.82	55.38	49.30	50.17	2.79	5.57	49.568		0.358
40	45.92	53.31	48.74	49.32	2.15	4.37	48.859		0.215
45	43.58	53.74	47.26	48.19	2.97	6.16	48.261		0.005
50	44.42	51.27	45.08	46.92	2.18	4.65	47.757		0.695
55	42.23	49.11	45.00	45.45	2.00	4.40	47.332		3.553
60	42.15	50.15	42.83	45.04	2.56	5.69	46.973		3.723
					28.93		Sum of squares		11.127

Appendix 21. Raw data of pesto L value of Batch 5 (Section 4.6)

Time (minutes)	OL1-1	OL1-2	OL1-3	Avarage	SE	%cv	Neg Exp OL1	k	SS
0	39.40	38.64	35.90	37.98	1.06	2.79	37.980	0.019	0.000
5	36.74	36.93	34.85	36.17	0.66	1.83	37.131		0.924
10	36.64	36.47	34.00	35.70	0.85	2.39	36.359		0.432
15	36.44	35.53	33.22	35.06	0.96	2.73	35.655		0.352
20	36.12	35.38	32.83	34.77	1.00	2.87	35.015		0.058
25	35.51	35.43	33.25	34.73	0.74	2.13	34.431		0.087
30	35.36	35.81	32.99	34.72	0.88	2.52	33.900		0.667
35	34.29	34.67	32.25	33.74	0.75	2.23	33.416		0.102
40	34.10	36.05	30.94	33.69	1.49	4.42	32.976		0.515
45	34.56	35.25	30.61	33.47	1.44	4.32	32.575		0.805
50	33.97	34.44	30.40	32.93	1.27	3.87	32.209		0.524
55	33.40	33.92	30.28	32.53	1.14	3.49	31.877		0.424
60	29.37	30.61	25.50	28.49	1.54	5.40	31.574		9.491
					13.78		Sum of squares		14.381

Time (minutes)	OL2-1	OL2-2	OL2-3	Avarage	SE	%cv	Neg Exp OL2	k	SS
0	37.09	38.52	38.21	37.94	0.43	1.14	37.940	0.029	0.000
5	34.12	36.50	35.83	35.48	0.71	1.99	36.370		0.792
10	32.74	34.79	34.38	33.97	0.63	1.85	35.012		1.093
15	32.17	33.25	32.95	32.79	0.32	0.98	33.839		1.100
20	31.98	33.49	32.16	32.54	0.48	1.46	32.824		0.082
25	30.99	30.90	32.98	31.62	0.68	2.14	31.947		0.107
30	31.04	31.45	32.20	31.56	0.34	1.08	31.189		0.140
35	30.42	31.47	32.00	31.29	0.47	1.49	30.533		0.578
40	29.89	29.75	32.26	30.63	0.81	2.66	29.967		0.445
45	30.21	30.64	31.12	30.66	0.26	0.85	29.477		1.389
50	25.52	25.85	27.69	26.35	0.68	2.56	29.053		7.288
55	31.38	31.09	30.99	31.15	0.12	0.37	28.687		6.076
60	31.51	26.15	26.22	27.96	1.78	6.35	28.370		0.170
					7.69		Sum of squares		19.259

Time (minutes)	OL3-1	OL3-2	OL3-3	Avarage	SE	%cv	Neg Exp OL3	k	SS
0	39.71	38.95	38.95	39.20	0.25	0.65	39.200	0.025	0.000
5	37.24	36.94	36.34	36.84	0.26	0.71	37.660		0.678
10	36.95	36.24	36.19	36.46	0.24	0.67	36.301		0.024
15	36.18	36.15	36.03	36.12	0.04	0.12	35.101		1.031
20	36.01	35.74	35.25	35.67	0.22	0.62	34.042		2.634
25	35.20	34.94	35.24	35.12	0.09	0.27	33.107		4.058
30	31.31	31.17	31.65	31.38	0.14	0.45	32.282		0.819
35	24.98	25.85	27.40	26.07	0.71	2.71	31.553		30.031
40	30.84	30.77	31.04	30.88	0.08	0.26	30.910		0.001
45	31.11	30.66	30.74	30.83	0.14	0.45	30.343		0.239
50	30.64	30.15	30.33	30.37	0.14	0.48	29.842		0.281
55	31.12	29.78	29.08	29.99	0.60	2.00	29.399		0.351
60	30.25	29.65	30.22	30.04	0.20	0.65	29.009		1.053
					3.13		Sum of squares		41.201

Time (minutes)	YL1-1	YL1-2	YL1-3	Average	SE	%cv	Neg Exp YL1	k	SS
0	31.54	31.79	29.22	30.85	0.82	2.65	30.850	0.025	0.000
5	30.70	31.49	30.17	30.78	0.38	1.25	30.616		0.028
10	29.97	30.08	30.06	30.03	0.03	0.11	30.408		0.141
15	30.57	31.21	28.78	30.18	0.73	2.41	30.225		0.002
20	30.07	30.06	28.82	29.65	0.41	1.40	30.063		0.172
25	30.16	29.81	29.00	29.65	0.34	1.16	29.919		0.071
30	30.09	30.55	29.24	29.96	0.38	1.29	29.792		0.027
35	29.90	29.34	28.63	29.29	0.37	1.26	29.680		0.155
40	31.32	31.10	32.41	31.61	0.40	1.28	29.581		4.112
45	29.51	27.96	29.95	29.14	0.60	2.07	29.493		0.124
50	30.04	28.06	29.79	29.29	0.62	2.13	29.415		0.015
55	28.17	29.11	30.35	29.21	0.63	2.17	29.346		0.020
60	29.37	27.51	29.58	28.82	0.66	2.28	29.286		0.218
					6.39		Sum of squares		5.083

Time (minutes)	YL2-1	YL2-2	YL2-3	Average	SE	%cv	Neg Exp YL2	k	SS
0	34.95	34.76	34.02	34.58	0.28	0.82	34.580	0.062	0.000
5	31.55	33.64	31.11	32.10	0.78	2.43	33.129		1.070
10	31.26	33.08	30.63	31.66	0.73	2.32	32.066		0.167
15	30.85	32.25	30.37	31.15	0.56	1.81	31.285		0.017
20	30.39	30.99	29.68	30.35	0.38	1.25	30.713		0.130
25	30.32	32.04	30.03	30.79	0.63	2.04	30.294		0.250
30	29.96	31.03	29.98	30.32	0.35	1.17	29.986		0.110
35	30.00	32.17	29.33	30.50	0.86	2.81	29.760		0.544
40	29.59	31.40	29.44	30.14	0.63	2.08	29.595		0.299
45	29.28	30.95	29.25	29.83	0.56	1.89	29.474		0.123
50	29.08	30.33	29.06	29.49	0.42	1.42	29.385		0.010
55	28.69	29.97	28.77	29.14	0.41	1.42	29.319		0.032
60	29.00	30.33	28.77	29.36	0.49	1.66	29.272		0.008
					7.09		Sum of squares		2.761

Time (minutes)	YL3-1	YL3-2	YL3-3	Average	SE	%cv	Neg Exp YL3	k	SS
0	29.67	28.78	27.85	28.76	0.52	1.82	28.760	0.016	0.000
5	29.06	29.10	27.54	28.57	0.52	1.80	28.612		0.002
10	29.49	28.56	27.57	28.54	0.55	1.94	28.475		0.004
15	29.41	29.39	27.96	28.92	0.48	1.66	28.349		0.324
20	29.26	28.77	27.48	28.50	0.53	1.86	28.232		0.073
25	28.10	27.15	28.63	27.96	0.43	1.55	28.125		0.028
30	29.36	27.43	29.27	28.68	0.63	2.20	28.025		0.433
35	27.85	26.63	28.29	27.59	0.50	1.80	27.934		0.120
40	27.58	26.80	28.24	27.54	0.42	1.51	27.849		0.098
45	29.51	26.64	27.78	27.97	0.84	2.99	27.771		0.041
50	28.84	27.39	28.31	28.18	0.42	1.50	27.699		0.230
55	26.76	25.54	28.21	26.83	0.77	2.88	27.632		0.641
60	27.90	25.42	28.30	27.20	0.90	3.31	27.570		0.135
					7.51		Sum of squares		2.128

Time (minutes)	HI1-1	HI1-2	HI1-3	Average	SE	%cv	Neg Exp HI-1	k	SS
0	32.77	34.94	35.39	34.37	0.81	2.35	34.370	0.052	0.000
5	29.83	31.52	31.65	31.00	0.59	1.89	31.905		0.825
10	29.26	29.20	29.62	29.36	0.13	0.45	30.003		0.417
15	28.24	28.08	26.93	27.75	0.41	1.48	28.534		0.620
20	27.33	27.39	28.23	27.65	0.29	1.05	27.401		0.062
25	26.92	28.09	26.94	27.31	0.39	1.42	26.527		0.619
30	26.21	26.36	25.90	26.16	0.14	0.52	25.852		0.092
35	25.95	25.81	25.33	25.70	0.19	0.73	25.331		0.132
40	25.47	25.06	25.78	25.44	0.21	0.82	24.929		0.258
45	25.34	24.78	25.71	25.28	0.27	1.07	24.619		0.430
50	25.10	24.47	24.36	24.64	0.23	0.93	24.380		0.069
55	24.44	24.05	22.22	23.57	0.68	2.90	24.195		0.392
60	24.32	24.44	23.02	23.92	0.45	1.90	24.052		0.017
					4.79		Sum of squares		3.934

Time (minutes)	HI2-1	HI2-2	HI2-3	Average	SE	%cv	Neg Exp HI2	k	SS
0	33.97	35.31	34.88	34.72	0.40	1.14	34.720	0.048	0.000
5	31.74	33.29	31.12	32.05	0.64	2.01	32.900		0.728
10	31.03	31.17	29.35	30.51	0.59	1.92	31.469		0.913
15	30.30	30.62	29.20	30.04	0.43	1.44	30.343		0.094
20	29.35	31.46	27.95	29.59	1.02	3.45	29.458		0.017
25	29.15	30.81	28.04	29.33	0.80	2.74	28.762		0.324
30	28.65	29.84	27.26	28.58	0.75	2.61	28.215		0.132
35	27.71	30.17	26.73	28.20	1.02	3.63	27.784		0.175
40	27.72	29.19	26.40	27.77	0.81	2.90	27.446		0.102
45	27.37	28.63	26.85	27.61	0.53	1.91	27.180		0.187
50	25.96	28.54	26.58	27.03	0.78	2.88	26.970		0.003
55	26.96	26.66	25.81	26.48	0.34	1.30	26.806		0.108
60	25.64	27.67	25.29	26.20	0.74	2.83	26.676		0.229
					8.85		Sum of squares		3.012

Time (minutes)	HI3-1	HI3-2	HI3-3	Average	SE	%cv	Neg Exp HI3	k	SS
0	37.38	34.20	35.31	35.63	0.93	2.62	35.630	0.075	0.000
5	32.19	29.46	31.47	31.04	0.82	2.64	33.039		4.008
10	30.80	28.71	31.94	30.48	0.95	3.10	31.260		0.612
15	30.76	28.14	29.98	29.62	0.78	2.63	30.040		0.174
20	31.07	28.55	30.67	30.09	0.78	2.60	29.202		0.794
25	30.51	27.79	30.56	29.62	0.92	3.09	28.627		0.985
30	29.92	27.27	29.70	28.96	0.85	2.93	28.233		0.529
35	28.67	27.75	29.70	28.71	0.56	1.97	27.962		0.552
40	29.65	27.13	28.36	28.38	0.73	2.56	27.776		0.364
45	28.73	26.87	28.30	27.96	0.56	2.00	27.649		0.099
50	28.93	26.90	28.81	28.21	0.66	2.33	27.561		0.421
55	28.82	27.21	27.48	27.83	0.50	1.79	27.501		0.110
60	27.93	26.71	27.49	27.37	0.36	1.30	27.460		0.008
					9.38		Sum of squares		8.654

Time (minutes)	TI1-1	TI1-2	TI13	Average	SE	%cv	Neg Exp TI1	k	SS
0	55.43	54.84	54.91	55.06	0.19	0.34	55.060	0.034	0.000
5	49.29	50.35	50.82	50.15	0.45	0.90	51.999		3.418
10	46.89	48.80	48.79	48.16	0.64	1.32	49.410		1.576
15	46.22	47.70	46.97	46.96	0.43	0.91	47.222		0.068
20	45.79	46.54	45.51	45.94	0.31	0.67	45.371		0.325
25	43.60	44.14	43.99	43.91	0.16	0.37	43.806		0.010
30	43.12	44.47	43.29	43.62	0.42	0.97	42.483		1.300
35	42.82	43.93	42.78	43.17	0.38	0.87	41.365		3.266
40	40.00	41.70	40.54	40.75	0.50	1.23	40.419		0.107
45	40.05	41.40	39.74	40.39	0.51	1.26	39.619		0.600
50	37.91	39.47	37.67	38.35	0.56	1.47	38.942		0.353
55	36.36	39.60	37.59	37.85	0.94	2.49	38.371		0.275
60	34.37	36.77	34.60	35.24	0.76	2.17	37.887		6.990
					6.25		Sum of squares		18.286

Time (minutes)	TI2-1	TI2-2	TI2-3	Average	SE	%cv	Neg Exp TI2	k	SS
0	54.57	54.87	53.90	54.45	0.29	0.53	54.450	0.039	0.000
5	50.03	49.68	48.72	49.47	0.39	0.79	50.814		1.799
10	48.66	47.17	46.74	47.52	0.58	1.23	47.826		0.093
15	48.19	45.11	44.55	45.95	1.13	2.47	45.369		0.335
20	42.64	43.39	43.01	43.01	0.22	0.51	43.350		0.114
25	43.68	41.45	42.19	42.44	0.66	1.55	41.689		0.556
30	43.30	39.92	40.53	41.25	1.04	2.52	40.325		0.850
35	43.84	40.19	39.46	41.16	1.35	3.29	39.203		3.838
40	41.92	38.47	37.90	39.43	1.25	3.18	38.280		1.318
45	38.08	36.03	35.93	36.68	0.70	1.91	37.522		0.709
50	38.74	35.75	34.74	36.41	1.20	3.30	36.899		0.242
55	37.61	33.04	33.73	34.79	1.42	4.09	36.387		2.550
60	36.09	33.71	32.26	34.02	1.12	3.28	35.966		3.798
					11.35		Sum of squares		16.202

Time (minutes)	TI3-1	TI3-2	TI3-3	Average	SE	%cv	Neg Exp TI3	k	SS
0	51.96	52.34	53.79	52.69	0.56	1.06	52.690	0.040	0.000
5	46.43	48.18	49.05	47.88	0.77	1.61	49.263		1.909
10	43.79	46.27	47.08	45.71	0.99	2.16	46.451		0.547
15	41.73	45.67	45.26	44.22	1.25	2.83	44.144		0.005
20	40.59	42.41	44.56	42.52	1.15	2.70	42.251		0.072
25	38.23	42.86	43.90	41.66	1.74	4.19	40.697		0.931
30	37.83	38.80	42.56	39.73	1.44	3.63	39.422		0.093
35	36.54	39.59	41.41	39.18	1.42	3.63	38.376		0.644
40	34.92	38.96	40.08	37.99	1.57	4.13	37.517		0.221
45	34.56	37.09	39.96	37.20	1.56	4.19	36.812		0.150
50	33.03	36.67	38.50	36.07	1.61	4.45	36.234		0.029
55	31.82	35.11	38.39	35.11	1.90	5.41	35.760		0.429
60	31.12	33.44	36.21	33.59	1.47	4.38	35.371		3.183
					17.42		Sum of squares		8.213

Appendix 22. Post hoc tests of L value of Batch 5 (Section 4.6)

Starting Color

Duncan[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
YL	3	31.3967			
HI	3		34.9067		
OL	3			38.3733	
TI	3				54.0667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

K Value

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
OL	3	0.02433	
YL	3	0.03433	0.03433
HI	3	0.03767	0.03767
TI	3		0.05833
Sig.		0.312	0.088

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Sum of Squares

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
YL	3	3.3236	
HI	3	5.1998	
TI	3	14.2334	14.2334
OL	3		24.9470
Sig.		0.139	0.131

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 23. Raw data of Batch 6 (Section 4.7)

Name of Data	Rotten leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS	Type of O-ring
TD1-1	71.19	0.554	16.78	0.091	1.676	2
TD1-2	42.28	0.485	24.49	0.082	0.998	2
TD1-3	47.52	0.780	17.88	0.058	13.151	2
TD2-1	65.74	1.332	28.38	0.073	1.024	3
TD2-2	70.56	1.237	29.50	0.071	1.092	3
TD2-3	76.83	0.767	29.39	0.092	0.843	3
TD3-1	71.37	1.202	27.53	0.063	2.670	3
TD3-2	70.85	0.772	30.70	0.057	1.780	3
TD3-3	84.68	0.801	29.05	0.056	0.568	3
TD4-1	86.38	0.997	29.47	0.043	0.796	3
TD4-2	75.78	0.896	38.12	0.059	1.213	3
TD4-3	75.87	0.616	38.32	0.049	2.408	3
TD5-1	15.24	-0.017	16.85	0.066	3.512	2
TD5-2	30.91	-0.361	15.70	0.052	2.949	2
TD5-3	18.08	1.087	16.00	0.069	0.703	2
TD6-1	62.74	1.510	27.83	0.071	3.212	3
TD6-2	62.01	1.036	39.15	0.057	4.984	3
TD6-3	70.64	1.292	28.31	0.056	1.892	3
TD7-1	62.19	1.642	36.90	0.049	4.085	3
TD7-2	77.49	1.289	37.27	0.045	1.161	3
TD7-3	70.85	1.748	36.77	0.040	1.773	3
TD8-1	54.22	1.775	28.46	0.074	1.203	3
TD8-2	76.65	0.871	36.59	0.041	1.406	3
TD8-3	75.88	1.577	28.09	0.092	1.962	3
TD9-1	-	0.000	32.90	0.075	0.772	1
TD9-2	-	0.000	33.89	0.074	2.083	1
TD9-3	-	0.000	32.09	0.105	1.712	1
TDI-1	-	-	46.74	0.050	3.175	1
TDI-2	-	-	46.24	0.063	3.733	1
TDI-3	-	-	46.32	0.057	0.309	1

Appendix 24. Post hoc tests of basil quality of Batch 6 (Section 4.7.1)

Rotten Leaves

Duncan[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
TD9	3	0.0000			
TDIcy	3	0.0000			
TD5	3		21.4100		
TD1	3			53.6633	
TD6	3			65.1300	65.1300
TD8	3				68.9167
TD7	3				70.1767
TD2	3				71.0433
TD3	3				75.6333
TD4	3				79.3433
Sig.		1.000	1.000	0.104	0.075

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Weight Loss

Duncan[a]

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
TD9	3	0.00000			
TDIcy	3	0.00000			
TD5	3	0.23633			
TD1	3	0.60621	0.60621		
TD4	3		0.83610	0.83610	
TD3	3		0.92500	0.92500	
TD2	3		1.11200	1.11200	1.11200
TD6	3			1.27933	1.27933
TD8	3			1.40767	1.40767
TD7	3				1.55967
Sig.		0.054	0.104	0.074	0.148

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 25. T-tests of TD9 and TDIcy of Batch 6 (Section 4.7.2)

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
StartC	Equal variances assumed	2.110	0.220	-24.809	4	0.000	-13.47333	0.54309	-14.98119	-11.96548
	Equal variances not assumed			-24.809	2.352	0.001	-13.47333	0.54309	-15.50477	-11.44190
FinalC	Equal variances assumed	2.036	0.227	-7.142	4	0.002	-9.17000	1.28387	-12.73459	-5.60541
	Equal variances not assumed			-7.142	3.077	0.005	-9.17000	1.28387	-13.19845	-5.14155
KValue	Equal variances assumed	5.253	0.084	2.582	4	0.061	0.028000	0.010842	-0.002103	0.058103
	Equal variances not assumed			2.582	2.536	0.097	0.028000	0.010842	-0.010370	0.066370
SS	Equal variances assumed	4.594	0.099	-.782	4	0.478	-0.88332	1.13007	-4.02089	2.25425
	Equal variances not assumed			-.782	2.532	0.501	-0.88332	1.13007	-4.88654	3.11989

Appendix 26. T-tests between TD1 and TD5 of Batch 6 (Section 4.7.2)

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
RottenL	Equal variances assumed	2.045	0.226	3.189	4	0.033	32.25333	10.11527	4.16883	60.33784
	Equal variances not assumed			3.189	3.082	0.048	32.25333	10.11527	0.54008	63.96658
WeightL	Equal variances assumed	6.539	0.063	0.830	4	0.453	0.369872	0.445719	-0.867642	1.607387
	Equal variances not assumed			0.830	2.165	0.488	0.369872	0.445719	-1.414237	2.153982
StartC	Equal variances assumed	9.946	0.034	1.453	4	0.220	3.53333	2.43221	-3.21955	10.28622
	Equal variances not assumed			1.453	2.082	0.279	3.53333	2.43221	-6.54723	13.61389
KValue	Equal variances assumed	1.629	0.271	1.315	4	0.259	.014667	0.011155	-0.016306	0.045639
	Equal variances not assumed			1.315	3.048	0.279	.014667	0.011155	-0.020522	0.049855
SS	Equal variances assumed	9.125	0.039	0.715	4	0.514	2.88688	4.03510	-8.31634	14.09011
	Equal variances not assumed			0.715	2.189	0.543	2.88688	4.03510	-13.11404	18.88781

Appendix 27. Post hoc tests between TD2,3,4,6,7 and 8 of Batch 6 (Section 4.7.2)

Starting Color

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
TD2	3	29.0900	
TD3	3	29.0933	
TD8	3	31.0467	31.0467
TD6	3	31.7633	31.7633
TD4	3	35.3033	35.3033
TD7	3		36.9800
Sig.		0.102	0.111

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

K Value

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
TD7	3	0.04467	
TD4	3	0.05033	
TD3	3	0.05867	0.05867
TD6	3	0.06133	0.06133
TD8	3	0.06900	0.06900
TD2	3		0.07867
Sig.		0.054	0.099

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Sum of Squares

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
2	3	0.9862	
4	3	1.4724	1.4724
8	3	1.5238	1.5238
3	3	1.6726	1.6726
7	3	2.3396	2.3396
6	3		3.3628
Sig.		0.179	0.070

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 28. Correlation between treatment, basil quality and pesto quality of type 3 o-ring of Batch 6 (Section 4.7.3)

Correlations

		Treatment	RottenL	WeightL	KValue
Treatment	Pearson Correlation	1	-0.571**	-0.219	-0.115
	Sig. (2-tailed)		0.001	0.246	0.544
	N	30	30	30	30
RottenL	Pearson Correlation	-0.571**	1	0.723**	-0.234
	Sig. (2-tailed)	0.001		0.000	0.213
	N	30	30	30	30
WeightL	Pearson Correlation	-0.219	0.723**	1	-0.139
	Sig. (2-tailed)	0.246	0.000		0.463
	N	30	30	30	30
KValue	Pearson Correlation	-0.115	-0.234	-0.139	1
	Sig. (2-tailed)	0.544	0.213	0.463	
	N	30	30	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix 29. Raw data of Batch 7 (Section 4.8)

Name of Data	Rotten leaves (%)	Weight loss (%)	Starting colour (L value)	K value	SS
CK1-1	31.33	0.245	28.28	0.081	0.813
CK1-2	30.67	1.054	38.37	0.047	1.187
CK1-3	40.33	0.865	25.85	0.088	1.392
CK2-1	41.67	0.830	26.84	0.100	1.394
CK2-2	36.67	0.783	38.03	0.033	2.321
CK2-3	45.00	0.955	35.97	0.042	1.206
CK3-1	38.33	0.739	27.15	0.078	1.240
CK3-2	46.67	1.535	35.16	0.041	0.743
CK3-3	39.33	0.916	37.27	0.044	1.009
CK4-1	37.67	0.218	26.27	0.081	0.523
CK4-2	23.67	1.059	30.67	0.075	1.567
CK4-3	22.67	0.318	39.33	0.045	1.556
CK5-1	28.67	1.065	38.60	0.039	1.172
CK5-2	40.00	0.435	25.14	0.088	0.368
CK5-3	29.67	0.596	34.45	0.043	1.192
CK6-1	36.67	1.032	25.76	0.042	0.312
CK6-2	35.00	0.947	29.74	0.045	0.431
CK6-3	42.33	0.809	33.68	0.033	1.380
CK7-1	0.00	0.000	39.22	0.031	3.421
CK7-2	0.00	0.000	40.02	0.032	3.722
CK7-3	0.00	0.000	41.07	0.035	2.647

Appendix 30. Post hoc tests of Batch 7 (Section 4.8)

Rotten Leaves

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
CK7	3	0.0000		
CK4	3		28.0033	
CK5	3		32.7800	32.7800
CK1	3		34.1100	34.1100
CK6	3			38.0000
CK2	3			41.1133
CK3	3			41.4433
Sig.		1.000	0.196	0.086

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Weight Loss

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
CK7	3	0.00000	
CK4	3	0.53167	0.53167
CK5	3		0.69867
CK1	3		0.72133
CK2	3		0.85600
CK6	3		0.92933
CK3	3		1.06333
Sig.		.057	0.084

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Starting Color

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
CK6	3	29.7267
CK1	3	30.8333
CK4	3	32.0900
CK5	3	32.7300
CK3	3	33.1933
CK2	3	33.6133
CK7	3	40.1033
Sig.		0.061

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

K Value

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
CK7	3	0.03267
CK6	3	0.04000
CK3	3	0.05433
CK5	3	0.05667
CK2	3	0.05833
CK4	3	0.06700
CK1	3	0.07200
Sig.		0.071

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Sum of Squares

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
CK6	3	0.7077	
CK5	3	0.9107	
CK3	3	0.9973	
CK1	3	1.1307	
CK4	3	1.2153	
CK2	3	1.6403	
CK7	3		3.2633
Sig.		0.058	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 31. Correlation between treatment, basil quality and pesto quality of Batch 7 (Section 4.8.3)

Correlations

		Treatment	RottenL	WeightL	KValue
Treatment	Pearson Correlation	1	-0.598**	-0.413	-0.492*
	Sig. (2-tailed)		0.004	0.063	0.023
	N	21	21	21	21
RottenL	Pearson Correlation	-0.598**	1	0.704**	0.388
	Sig. (2-tailed)	0.004		0.000	0.082
	N	21	21	21	21
WeightL	Pearson Correlation	-0.413	0.704**	1	0.005
	Sig. (2-tailed)	0.063	0.000		0.982
	N	21	21	21	21
KValue	Pearson Correlation	-0.492*	0.388	0.005	1
	Sig. (2-tailed)	0.023	0.082	0.982	
	N	21	21	21	21

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).