The Effect of Physical Damage on Carrots and Carrots Respiration

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Abstract

This thesis aims to investigate the influence of physical damage on carrots' respiration rate (RR). The paper comprises eight experiments categorised into two parts. Firstly, research on carrot respiration rate was conducted. To provide a more comprehensive understanding of carrot respiration, factors that may affect carrot respiration; such as the carrot properties and temperature were studied. Linear regression, Monte-Carlo simulation, and other statistical tools, were used to describe how temperature plays a significant role in affecting respiration rate.

This second part of the research investigated the influence of physical damage on the respiration rate of carrots. This work introduced the concepts of respiration rate gap and damage density to reflect the damage-enhanced respiration rate and damage level, respectively. A relationship between damage density and damage-enhanced respiration rate was built, which indicated that both low-density and high-density damage have limited influence on respiration rate.

Keywords: Carrot (Daucus carota L.), respiration, postharvest

1. Introduction.

Carrot (*Daucus carota* L.) is one of the most popular vegetables in the market, and can be consumed directly or offered in fresh-cut formats. Fresh-cut is popular in the market due to its convenience, while just like other fresh-cut products, it has a short shelf life because of carrot deterioration and physiological disorders. Hence there are extended postharvest challenges for fresh-cuts in order to extend shelf life and maintain quality (Fai et al., 2016). This thesis aims to analyse carrots' respiration rate (RR) in the post-harvest stage, focusing especially on the physical damage on the carrot. Respiration is studied as an indicator of carrot metabolism. Respiration results in consumption of oxygen from the environment and production of carbon dioxide, water, and energy. RR is affected by the storage environment and physical damage to the carrot.

The literature review of this thesis consists of the structure illustration and overview of the carrot market, carrot composition, carrot metabolism and respiration, and minimally processed carrot products. The common RR measurement steps are described in the material and method section and include the equipment's details.

The experiments occupy two parts of the paper. The first section is about determining factors related to carrot RR, and the second is about analysing the influence of physical damage on the RR. The first part comprises three sub-experiments, and the discussion of the three experiments has five parts to describe them. The five sub-experiments about physical damage are designed as a whole, and their data and results are discussed to explore the relationship between physical damage and RR.

The last part of the thesis is the final conclusions and recommendations, which concludes all of the experiment results and tries to establish a relationship between physical damage and RR.

2. Literature review.

Food product export is essential to New Zealand's (NZ) economy, contributing to around 10% of NZ GDP and more than 50% of the value of all merchandise exports. Exported food product is dominated by the following categories: dairy, meat, seafood, fruit and vegetables, wine, and specialty food industries (Chen et al., 2015). Carrot is an essential vegetable for NZ vegetable export and food industry. Carrots, as well as fresh juice products, are consumed in the domestic market and for export (Millner et al., 2013). New Zealand carrot cultivation occurs in south Auckland, Manawatu/Ohakune, and Canterbury. However, carrot growth has shown a reduced trend from 1831 hectares in 2002 to 1320 hectares in 2007 (Millner et al., 2013).

The postharvest stage occurs between the farm and final consumption. Postharvest losses can be defined as measurable food loss in the postharvest system (Amentae, 2016; Elik et al., 2019). According to (Gustavsson, 2011), waste can be 50% of the cultivated crops, and the rate will worsen in some developing countries. For carrot, a 2017 study of carrot production in Nepal indicated that carrot loss after the harvest can be 35%, which is made up of 15% at the farm gate, 10% at the collection point, 5% at the wholesale market and 18% at the retail market (Bhattarai et al., 2017). Furthermore, the market for fresh-cut produce has become more popular due to consumer groups' preference for healthy lifestyles, diets, and healthy alternatives in the food industry, as fresh-cut vegetables are more perishable than intact ones.

Respiration is a physiological process which offers the necessary energy for other biological processes within fresh produce. As respiration increases, it also accelerates the quality loss of fresh-cut vegetables as it will accelerate the consumption of nutrients, e.g. starch, sugars, and acids (Iqbal et al., 2008). RR is diverse in individual plants, and different parts of the same crop can have different RRs (Iqbal et al., 2008). In storage conditions, the divergence of RR may be caused by different shapes or cuts of the same product (Iqbal et al., 2008). The study on respiration rate helps extend the product's storage time. Besides, it also can be used to estimate the carrot quality level based on RR measurement because the physiological characteristics of fresh-cut carrots are different from the intact ones, so they require special treatment to store (Iqbal et al., 2009).

2.1. Carrot composition

The carrot is deemed a vitaminised food with moisture, protein, fat, carbohydrates, sugars, and fibre, but it is also rich in carotene and ascorbic acid (Raees-ul & Prasad, 2015). The edible part of a carrot is its root, and the cortex core ratio mainly determines quality, and the index declines with carrot maturity. The carrot nutrients primarily spread on the exterior side of the root (cortex). The composition can be considered into two groups, e.g. dry matter and moisture content (Raees-ul & Prasad, 2015).

Carrot compounds have two principal quality effects: 1) Flavour: Carrot products can offer a characteristic flavour due to terpenoids and polyacetylenes. The two main compounds of terpenoids are mono terpenoids and sesquiterpenoids, and falcarinol compounds form the polyacetylenes (Raees-ul & Prasad, 2015). Nowadays, orange carrots are popular worldwide because of their excellent flavour, which is based on the existence of volatile isoprenoids and sugars. The sugar content within carrots is vital to cover the harsh flavour of carrots, and the hexose, e.g. glucose, is the substrate of carrot respiration, and the isoprenoids are related to carotenoid synthesis and photosynthesis (Klein & Rodriguez-Concepcion, 2015; Raees-ul & Prasad, 2015).

2) Colour: The variance of pigments within carrots contributes to the colour of carrots, and the colour range of a carrot can be red, orange, yellow, purple, black, and white (Raees-ul & Prasad, 2015). Such a colour variety of carrots is caused by different levels and types of carotenoids they synthesised. These pigments not only influence the appearance of carrots but also contribute to their health properties. For instance, the

lutein and carotenoids provide the yellow/orange colour of carrot that is also good for the human's eye functioning, and the colour of carrot changes with the different ratios of lutein, lycopene, carotenoids during its growth period. So, colour can be an indicator of the maturity level of carrots. For example, the young carrots show more pale colours but turn to the characteristic colours with carotenoid accumulation. For mature carrots, there are more β-carotene and lower levels of α-carotene, and carrots are one of the biggest sources of provitamin A in the human diet (Klein & Rodriguez-Concepcion, 2015; Sant'Ana et al., 1998).

2.2. The physiological structure of carrot

The carrot (*Daucus carota* L.) is a biennial herbaceous plant that can be counted as one member of the Apiaceae family. There are two species of cultivated carrot; one is an eastern carrot, and the other one is a western carrot, which can be identified based on pigmentation in the carrot root (Que et al., 2019). The physiological structure of a carrot can be divided into two parts, a flower and a root, as seen in Figure 2.1 (Que et al., 2019).

Figure 2.1 The pictures of a) carrot flower, b) carrot root, c, d) carrot production field (Que et al., 2019)

The flower of carrots is a white flattened umbrella-shaped umbel, and the leaves are compound. The first year of root growth is for nutrient accumulation, so a quantity of carbohydrates is produced and stored in the root for the following year's growth (Que et al., 2019). The carrot root shows excellent storage stability and contains a series of nutrients, e.g., carotenoids, vitamins, and dietary fibre, and is rich in minerals and antioxidants (Que et al., 2019). The top third of the root gets the most dry matter. There are a few factors that may influence the physiological content of carrots; for instance, the change of moisture, temperature, and maturity will affect dry matter content within carrot (Olymbios, 1973). The carrots with high-level dry matter can be obtained from the low-moisture fields.

2.3. Carrot products

Carrots have several nutritional components, e.g., sugar, fat, fibre, minerals, and Vitamin A (β-carotene, α-carotene). Carrot-based products have been developed based on such attributes, including beverages, candy, juice, canned food, powder, and many cutting formats (different damage levels) such as sticks, rounds, diced, etc. (Edelenbos et al., 2020). The carrot juice is a rich beverage resource of α- and β- carotene, which gets extracted by a few processing stages like centrifugal basket, centrifugal pulp-ejecting, twin gear, two-step triturator and hydraulic press, and mastication juice extractors (Raees-ul & Prasad, 2015). One of the essential stages of carrot juice production is extraction. However, the conventional extraction methodologies lead to a low yield rate from carrots because of the complex texture of carrot root, so the application of enzymes and heat processing is introduced to soften carrots, and it also requires a hydraulic press. Otherwise, conventional extraction results in cloudy juice (Raees-ul & Prasad, 2015). The overall procedure of carrot juice production is shown in Figure 2.2.

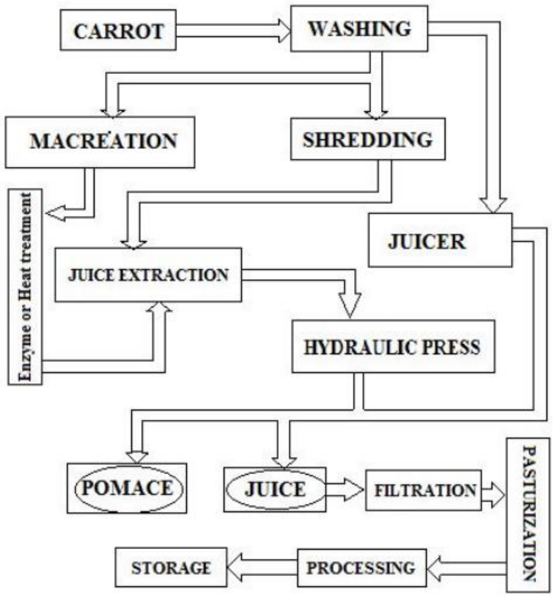


Figure 2.2 The process of carrot juice production (Raees-ul & Prasad, 2015)

Moreover, it finds that the shelf life of fresh-cut carrots cannot be extended via modified atmospheres (MA), which makes it difficult to extend the shelf life of carrot juice products (Raees-ul & Prasad, 2015). Even if the MA cannot extend the shelf life, treatment like post-harvest loss control can reduce loss and maintain quality during storage, which helps to decrease the cost of raw material.

Carrot products with different physical damage levels, including sticks, rounds, diced, slices, and grated, can be considered as minimally processed vegetables. Minimal processing refers to any fresh vegetables that have been processed to increase their functionality without significantly changing their fresh-like properties (Condurso et al., 2020). The quality of minimally processed can be affected by many factors, e.g. raw material, processing conditions, packaging material and storage conditions (Edelenbos et al., 2009). Processing steps like washing, trimming, peeling, cutting, and packaging carrots will also bring physiological changes that need to be controlled in the postharvest environment.

The minimally processed carrot will affect the shelf-life of the carrot via damage-induced RR and microbial growth (Condurso et al., 2020; Iqbal et al., 2008). For example, a previous study indicates that carrot sticks have a higher shrinking extent than other treatments during 25 days of storage. However, the texture (shear resistance) of these carrot sticks remains at the same level as fresh carrots, which is attributed to the application of film that can prevent moisture loss. Colour of carrot sticks also does not show much change (Bruemmer, 1988).

Refrigeration and atmosphere control are two effective methods to assure the quality of minimal processing products because the lower temperature causes a reduction in the RR. Atmosphere control can also achieve this, but there are natural variability and dynamic responses to processing and storage conditions in the raw material (Pilon et al., 2006). The microbial spoilage and surface white blush discolouration are also significant factors that affect the quality and shelf-life of minimally processed carrot products. For instance, the general microbiological populations after processing range should be 3.0–6.0 log CFU g^{-1} (Ragaert et al., 2007). While the carrot slices presented a total bacteria count of 5.90 log CFU g^{-1} at the beginning of the storage, the total microbiological amount rose to 7.95 log CFU g^{-1} on the 9th day (Brizzolara et al., 2020).

2.4. Physiological characteristics of carrots

2.4.1. General physiological and quality change of carrots post-harvest

Metabolism of carrots does not stop once they are harvested (Suojala, 2000). Usually, carrots' general physiological activity, including respiration and transpiration, causes dry matter loss and weight loss, which influences carrot quality (Asgar, 2020). Hence, the storage environment is controlled in order to minimise the rates of change.

Usually, early maturity carrots that were fast-growing at harvest tend to have higher RR because of the higher energy requirement. The carrots show a decline in RR after the carrot recovers from the harvest. Carrots can be considered a sort of nonclimacteric product, being harvested at the best quality (Saltveit, 2019). Under ethylene exposure, the root of the carrot will accumulate bitter-tasting phenolic compounds (Saltveit, 1999). Furthermore, the higher the respiration rate, the greater the response to C_2H_4, and the hence the higher the production of bitter compounds, e.g. isocoumarin. If a mature carrot is exposed to 0.5 μL L^{-1} ethylene at 5 °C for 14 days, that will result in 40 mg/100 g isocoumarin in the carrot that offers an easily tasted bitter flavour (Saltveit, 1999). Immature and fresh carrots never exposed to ethylene show greater ethylene sensitivity (Saltveit, 1999).

Temperature is a significant factor affecting carrot respiration because temperature change affects the activity of enzymes involved in biochemical reactions. Low temperatures can decrease the respiration rate of carrots. Besides, carrots' sweetness can also be enhanced due to the enzymatic limitation from starch to free sugars (Edelenbos et al., 2020). Some enzymes are also involved in carotene oxidation; however, no matter what temperature storage level, the β-carotene content decreases (Asgar, 2020). Furthermore, temperature is related to the textural quality of carrot. Temperature affects the activity of the proto-pectinase enzyme, which hydrolyses insoluble protopectin into soluble pectin in the cell wall leading to the collapse of cell wall cohesion (Asgar, 2020).

Extremely low temperature (< 0 °C) is unsuitable for carrot storage, as they are damaged when frozen. The ice crystals in the cell wall drive the water out of the cell, and the cell is damaged by dehydration (Saltveit, 2019).

Most horticultural products can tolerate high temperatures for a few minutes. High temperatures can be used to purge insects and fungi on the surface. However, long-term exposure to high temperatures (thermal death point) causes respiratory disorders and product tissue collapse (Saltveit, 2019).

For long-term carrot storage, transpiration is the main factor that causes weight loss of carrots, while respiration losses also influence weight loss, but less significantly than transpiration. In contrast, the high RR at high-temperature storage conditions will shorten the shelf life of carrots and accelerate weight loss due to the effect of transpiration and respirational loss (Asgar, 2020). Carrots are without stomata. A few indexes that affect weight loss are carrot surface area, water vapour pressure, air velocity, and relative humidity (RH) of the storage environment (Apeland & Baugerød, 1969). The weight loss shows an inverse ratio with surface area, water vapour pressure, and air velocity but minimises the weight loss with high RH (98%–100%) (Apeland & Baugerød, 1969; Edelenbos et al., 2020). In practice, 8% weight loss of carrots can be acceptable (Asgar, 2020).

As a result of carrot respiration, dry matter loss, e.g. glucose and starch occur. The rate of dry matter loss can be determined by periodical dehydrating via a dehydrator, but such an operation is destructive toward samples from carrots. Alternatively, the dry matter loss can be estimated with an equation being: the weight of CO_2 lost multipled by 0.68. The factor 0.68 is the ratio of the ratio of the molecular weight of glucose (180) divided by the weight of the lost CO_2 ($6\times44 = 264$) (Saltveit, 2019).

2.4.2. The factors cause carrot loss and respiration characteristics

After carrots get harvested, a few factors cause loss (Edelenbos et al., 2020).

i) Moisture loss and desiccation
ii) Spoilage caused by fungi and bacteria
iii) Rooting and sprouting
iv) Bitterness, harsh flavour, and lack of sweetness

Controlling respiration rate aids in controlling carrot losses. However, respiration metabolism is made up of complex biochemical reactions. Hence, the respiration rate is introduced as a representation of the rate of respiration metabolism, and both O_2

consumption and CO_2 production can indicate RR. Carrot respiration has the following characteristics.

2.4.3. The respiration metabolism of carrot

Respiration is one part of carrot metabolism that supports basic physiological activities, such as carrot survival and growth. However, respiration offers the energy that drives metabolic reactions while supplying raw materials as substrates for respiration reactions. (Saltveit, 2019). The procedure for carrot respiration is shown in Figure 2.3.

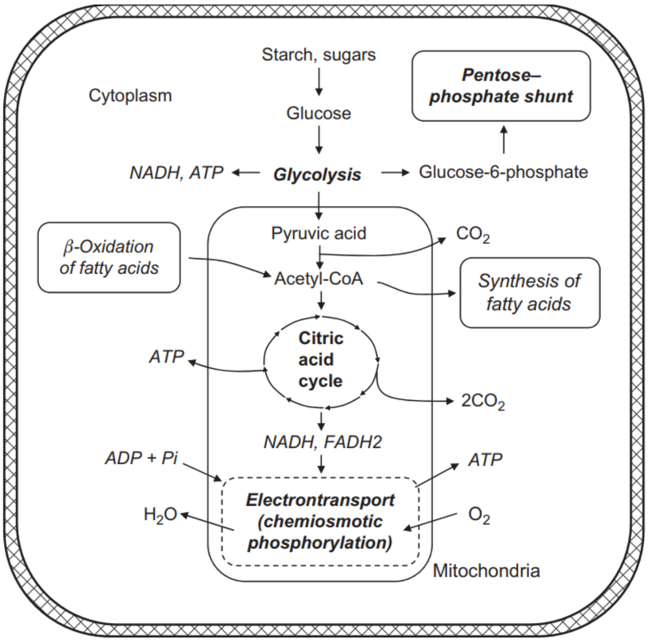


Figure 2.3 The overview of four respiratory metabolism pathways (Saltveit, 2019)

The carrot respiration process comprises four parts:

1) Glycolysis, the first stage of respiration. Glycolysis begins with an isomerase reaction of a 6-carbon molecule from glucose structure to fructose. Then, the fructose will receive phosphate from ATP to form 1,6-fructose diphosphate, which can be split into two 3-carbon molecules that undergo an isomerisation to form two 3-carbon molecules of pyruvate after five additional enzyme-coupled reactions. Finally, such a process will consume two ATPs but produce 4 and 2 NADHs. Besides, there is no O_2

consumption and CO_2 production. Around 20% of the energy of glucose can be captured from glycolysis.

2) Citric Acid Cycle (Krebs Cycle): Before the 3-carbon pyruvate enters into the cycle, it needs to be decarboxylated to form an acetate fragment (CH_3CO-) of the molecule, which can be transformed into acetyl-CoA via coenzyme A (Saltveit, 2019). In many biochemical reactions, such a compound can deliver a 2-carbon acetate group to other compounds. The Citric Acid Cycle (CAC) starts from the addition of the 2-carbon acetyl group from acetyl-CoA to the 4-carbon oxaloacetate to form the 6-carbon, 3-carboxyl molecule citric acid, which is also the end of the cycle.

3) Chemiosmotic Phosphorylation: After the CAC, a few compounds contain energy like NADH and $FADH_2$, but the energy of these compounds may be over the requirement for physiological needs. Hence, compounds with less energy need to be formed in this stage. The O_2 is an acceptor with high-energy electrons that form H_2O with enzymatic reactions. Still, these reactions do not produce ATP directly, creating a proton gradient across the inner mitochondrial membrane (Saltveit, 2019). The ATP is made when the ADP and phosphate as the protons flow from the intermembrane space back to the matrix through an ATPase complex (Saltveit, 2019). The processes above can be simplified as equation (2.1) assuming that hexose is chosen as the substrate.

$$C_6H_{12}O_6 + 6O_2 + 38ADP + 38P_i \rightarrow 6CO_2 + 6H_2O + 38ATP + 686kcal \quad (2.1)$$

As per the equation above, carrots consume O_2 during respiration. As such O_2 consumption can be chosen as one of the measures of carrot respiration. Furthermore, CO_2 production can also be used as a measure of carrot respiration. However, compared with O_2 production, CO_2 production is a better parameter to be measured as there is 21% O_2 and 0.04% CO_2 in the natural atmosphere. Hence measures of O_2 are proportionally small in comparison to the existing atmosphere, and hence it is more likely for there to be errors in measurement in comparison to measuring CO_2 concentration change. As an example, if a carrot is placed into a limited-volume container, it can reduce the O_2 concentration from 21.0% to 20.9% (a 0.5% change) while the CO_2 concentration should simultaneously change from 0.04% to 0.14% (a 250% change). Hence the change in concentration of CO_2 is proportionally far more significant, which means which means it can be more easily measured with confidence of less error. As a result, in the following experiments in this thesis, CO_2 production is used to represent respiration rate.

During the storage period of carrots, the environmental O_2 is an essential factor influencing the anaerobic and aerobic respiration of carrots. Carrot storage is extended in low O_2 concentrations (lower than 8%). Still, the concentration must not be so low as to cause anaerobic respiration, which may accumulate unwanted compounds within carrots. Hence, the optimal O_2 is located between 4% and 8% to avoid anaerobic respiration but extend storage time (Leshuk & Saltveit Jr, 1991). Besides, the pH condition of carrots also plays a significant role in balancing the aerobic and anaerobic respiration of carrots (Leshuk & Saltveit Jr, 1991). In addition, the respiration of potential extra microbes from the environment attached to the surface of carrots is considered insignificant in affecting the CO_2 production rate (Saltveit, 2019; Seljåsen et al., 2001).

2.5. The carrot storage

For some vegetable and fruits like lettuce, kiwifruit, and apples, storage technologies of MA (modified atmosphere) and CA (controlled atmosphere) storage can be applied. The differences between these two technologies are their scope and facilities. CA storage is a built physical facility where a control system is used to monitor and change the concentration of CO_2 and O_2 (Brizzolara et al., 2020). The application of MA focuses on CO_2 and O_2 changes in small individual units (packages). The resulting atmosphere is a function of several factors such as RR, cultivar, temperature and packaging composition (Brizzolara et al., 2020). No matter which scheme is applied, both aim to reduce temperature, and oxygen concentration while controlling the carbon dioxide concentration in order to manage weight loss, and RR and ultimately extend the shelf life of carrots. Figure 2.4 shows that combining high RH management and MAP can minimise carrots' weight loss in long-term storage. However, high RH will promote microbial growth and spoilage (Edelenbos et al., 2020).

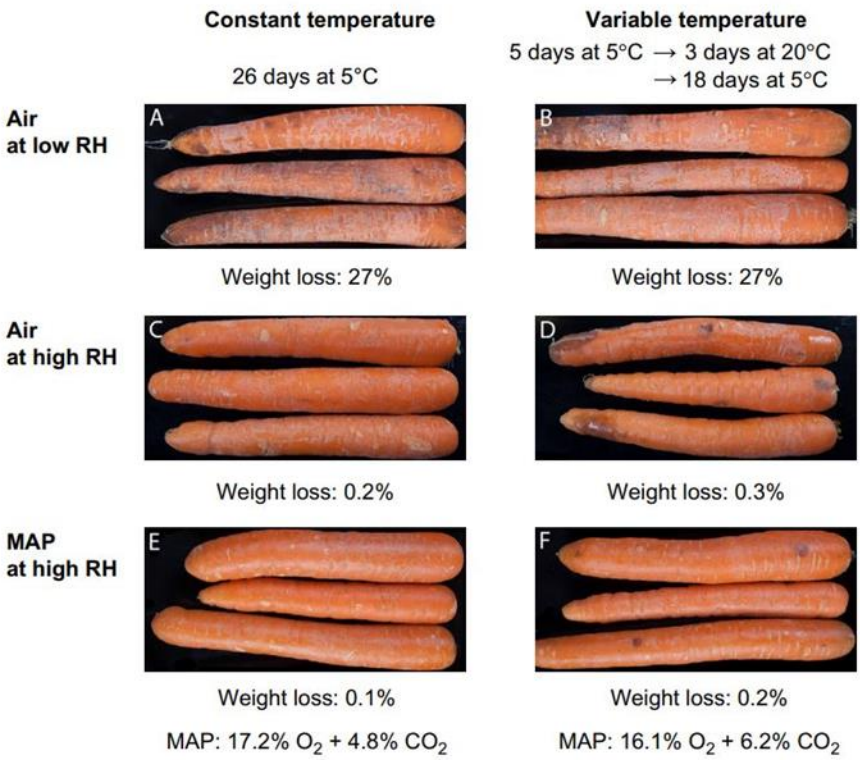


Figure 2.4 The weight loss of carrots under different storage conditions (Edelenbos et al., 2020)

As for respiratory rate response, Simões et al., (2011) illustrates that the RR of baby carrots under four CA conditions (air, 2 kPa O_2 + 15 kPa CO_2, 5 kPa O_2 + 5 kPa CO_2 and 10 kPa O_2 + 10 kPa CO_2) in Figure 2.5. Carrots stored with the lowest O_2 content of the four CA schemes had the lowest RR. However, the lowest O_2 is not optimal for carrot preservation because if the carrots need long-period storage, the low O_2 content may lead to anaerobic respiration or fermentation. (Simões et al., 2011). So, the MAP must also incorporate temperature management to limit the RR together.

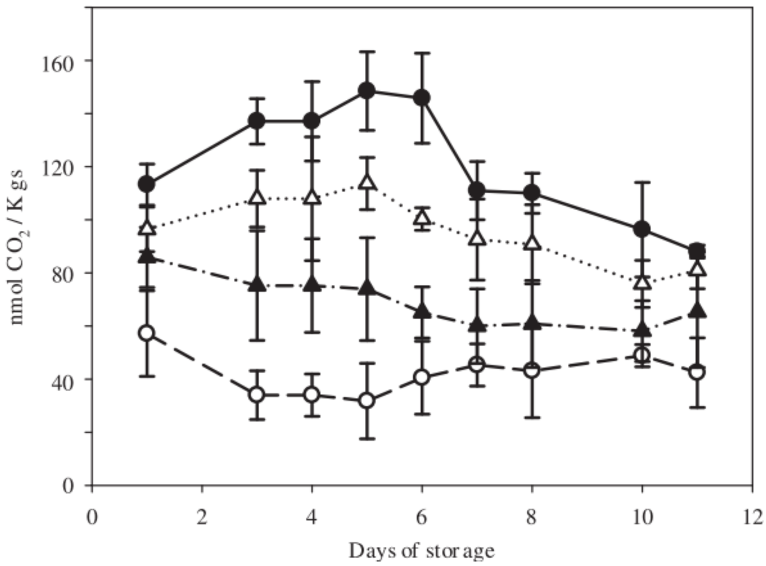


Figure 2.5 The RR of carrots placed under four CA conditions, e.g. Air (●) and controlled atmospheres (2kPa O_2 + 5kPa CO_2) (○); 5kPa O_2 + 5kPa CO_2 (▲); 10kPa O_2 + 10kPa CO_2 (△) (Simões et al., 2011)

Carrot browning is a significant problem related to carrot storage caused by microbe growth or phenolic compound degradation. The carrot root is rich in phenolic compounds, especially chlorogenic acid, which can be substrates of enzymatic browning (Zhang et al., 2005). The phenolic compounds within carrots are oxidised into quinones by chelation with copper ions in the presence of polyphenol oxidase (Zhang et al., 2005). Such a phenomenon is not unique to carrots but is also being reported for other fruits and vegetables, such as apples, apricots, peaches, and lettuce (Zhang et al., 2005). In most cases, carrot browning starts after a period of cold treatment, and tends to spread on the surface because the phenolic content of carrots is highest near the tissue surface and decreases with depth of tissue (Zhang et al., 2005). Carrot browning is a complex process, so a few factors influence the browning extent: including substrate levels, enzyme activity, the presence of ascorbic acid. Carrots with physical damage more easily turn brown due to the physical interaction facilitating the mixing of the enzyme and substrate (Zhang et al., 2005). In addition, fungal growth on the surface of carrots may cover the carrot with brown spots if the carrots are stored in high RH environment.

2.6. Wounded carrots

2.6.1. The physiological characteristics of wounded carrots

After the carrot gets physical damage, a series of physiological reactions will launch to repair the damage within the carrot (Surjadinata & Cisneros-Zevallos, 2003). The short-term physiological activity is the production of signal compounds in the adjacent and distant tissues to launch a series of physiological and biochemical responses. These responses within carrots lead to some common damage-related reactions like respiration enhancement, ethylene production, quality changes, synthesis, and loss of phytochemicals (Surjadinata & Cisneros-Zevallos, 2003). Therefore, the RR plays an essential role in indicating the shelf-life of carrots, as product with a higher RR value tends to have less storage time as it is consuming its resources faster. The specific extent of RR increase will be affected by several factors, e.g. tissue type, temperature, atmosphere, and degree of wounding (Surjadinata & Cisneros-Zevallos, 2003; Watada et al., 1996). For minimal processing conditions, fresh-cut (damaged) carrots can be divided into four levels: whole, sliced, batons, and shredded (Iqbal et al., 2008).

2.6.2. The mechanism of damage-induced RR enhancement

The physical damage on carrots will lead to higher RR because the damage is related to enzyme synthesis, which will also influence enzymes within the respiration process. For instance, ATP-dependent phosphofructokinase that is involved in carbohydrate breakdown to produce pyruvate, cytochrome oxidase located on the membrane of mitochondria helps the production of H_2O, and it changes the number and structure of mitochondria (Surjadinata & Cisneros-Zevallos, 2003). However, the existence time of these enzymes will not be long due to the regulation effect of the tissue inactivation system that can control the level of phenylalanine-ammonia-lyase (PAL) and other enzymes within carrots. The high activity of PAL will launch the phenolic accumulation within carrots induced by physical damage (Simões et al., 2011; Toivonen & De Ell, 2002). The influence of bodily damage on the phenolic metabolism has two aspects: 1) the oxidation of endogenous phenolics, which leads to the collapse of the cell membrane and mixing of phenolics with oxidative enzymes. 2) The phenolic production launches through adjacent cells around wounds to repair the damage (i.e. lignification) (Toivonen & De Ell, 2002). The production and inactivation of these RR-related enzymes lead to RR fluctuation. The increase in enzyme activity is related to previously inactive enzymes or novo synthesis (Surjadinata & Cisneros - Zevallos, 2003). Furthermore, enzyme synthesis is not the only factor that drives RR enhancement; the α-oxidation of long-chain fatty acids from membrane deteriorative processes also contributes to the RR enhancement and ethylene production; besides, the oxidation effect on carrots also accelerates carrot browning (Surjadinata & Cisneros-Zevallos, 2003).

The study about physical damage is necessary because crop harvest is associated with mechanical stress/damage; once the plant is separated from the parent, it loses supplies like water, nutrients, and energy (Watkins, 2017). Moreover, physical damage stress stimulates metabolism to heal the damaged tissues and activates mechanisms that prevent further damage. A previous study also indicates that increased wounding intensity and higher storage temperature promoted the generation of reactive oxygen species (ROS) and enhanced phenolic accumulation in wounded carrots (Han et al., 2017). The ROS induce cell death by promoting the intrinsic apoptotic pathway (Marchi et al., 2012).

So, the harvested crops can dynamically fit the conditions of the external environment (Watkins, 2017). Besides, the damage that was caused during carrot harvesting will lead to postharvest physiological disorders, which can be defined as those disorders that occur in fresh crops after harvest resulting from altered metabolism in response to the imposition of stresses, and typically, these are manifested as visible symptoms of cell death in the susceptible plant part (Watkins, 2017). There are two appearances of physiological disorders observed during the experimental period: 1) water spot, 2) surface browning (Villeneuve & Geoffriau, 2020).

The water spot is formed due to cell breakdown and longitudinal cracks with irregular contours, which pathogens will attack (Villeneuve & Geoffriau, 2020). The emergence of water spots requires a hypoxic environment and high moisture, so such a phenomenon can be observed when the carrots are stored in plastic bags.

The surface browning of carrots is caused by the oxidation of polyphenols that are spreading on the surface of the carrot, and the enzyme polyphenol oxidase typically induces oxidative browning (Villeneuve & Geoffriau, 2020). That can be seen as one of the carrot decaying symptoms.

3. Materials and Methods

3.1. Respiration Rate Measurement

This work features respiration rate (RR) measurement of carrots under different scenarios. RR indicates the carrot's physiological status. In this work, the static method for measuring respiration rate was used. In the static method, samples are sealed in a closed jar (1000 mL), in which gas samples (1 mL) are removed upon sealing (t_1) and after a known period (t_2).

A CO_2 analyser (Citicell C/S type, City Technology Ltd), coupled with an integrator (3396A, Hewlett–Packard), was used to measure the CO_2 concentration of each sample. The analyser was first calibrated with 0.534% CO_2 β-standard (BOC). The RR (nmol/kg/s) is calculated using equation 3.1 where V_c is the volume of container (1000 mL); W_{carrot} is the mass of carrot (g), ρ_{carrot} the density of carrot (g/mL); CO_{2i} is the initial CO_2 concentration (%), CO_{2f} the final CO_2 concentration (%), P_{Lab} the atmosphere pressure of lab (Pa), R is the universal gas constant (8.314 m^3 Pa/mol K), T is temperature (°C), and time difference between t_2 and t_1 (s).

$$\mathrm{RR} = \frac{\left(V_{container} - \frac{W_{carrot}}{\rho_{carrot}}\right)(CO_{2f} - CO_{2i})P_{lab}(0.00001)}{\frac{(R(T+273.15)W_{carrot})(t_2 - t_1)}{0.000000001}} \quad (3.1)$$

Usually, the respiration rate is measured at a constant temperature. In these cases, carrots were shifted to the desired temperature the day before measurement to enable an assumed physiological equilibration to the desired temperature. Lab atmospheric pressure was estimated using equation (3.2), where lab altitude (h) is 46.5 m. M is the molar mass of dry air, equal to 0.02896968 kg/mol; g is the gravitational acceleration, equal to 9.81 m/s; T is the temperature at the altitude h in Kelvins (K).

$$P_{Lab} = P_{sea-level} \times e^{\left(\frac{-gMh}{RT}\right)} \quad (3.2)$$

3.2. Statistical Analysis

3.2.1. Linear regression

Linear regression is a useful tool to predict a quantitative response given a known value. In simple linear regression, a quantitative response Y is predicted based on a single predictor variable X, assuming a linear relationship. To predict the Y based on the value of X, the residual sum of squares (RSS) is minimised. This work uses simple linear regression via Excel to determine relationships between carrot properties and RR. Outputs from a linear regression include S and R^2. S represents the standard error of the regression that describes the average distance of observed values to the regression line. The R^2 measures the proportion of variability in Y that can be explained using X (James et al., 2023). An R^2 statistic close to 1 indicates that the regression explains a good regression-fitting response (James et al., 2023).

3.2.2. Students T-test

A student's T-test is useful for testing the hypothesis by comparing means between two groups. However, The T-test can only be used to compare the means between two groups, and also requires the tested variable (dependent variable) to be on a continuous scale and approximately normally distributed (Connelly, 2021; De Winter, 2019). In experiment 4, the T-test is used to test the significance of the difference between the control group and the cut group. The P value of two tails represents the two-way hypothesis, which fits the T-test hypothesis of experiment 4.

4. Experimental Method Validation and Development

4.1. Introduction

The chapter of the thesis comprises three experiments created to explore the respiration of regular carrots under variable but controlled scenarios. The first experiment is the basis of the following experiments that explore the factors that may affect respiration and the relationship between carrot properties and RR. The second experiment aims to understand the influence of temperature and dry matter on carrot respiration. The carrots are placed under different temperatures. The third experiment investigates the homeostasis of carrots, where the carrots are placed into an environment with dynamic temperature change in order to measure the RR change of carrots in these changing environments.

4.2. Experiment 1 (Checking Methodology and Assumptions)

4.2.1. Introduction

A well-designed experiment carefully considers actions and values before settling on a final design and analysis procedure. In particular, considering the number of replicate measures to estimate a population's typical mean behaviour is prudent and reasonable. Understanding the key factors that tend to influence the measurement results is also helpful in ensuring what aspects of data collection should be of focus during experimentation. In particular, understanding if numerical values or responses are unrelated to other properties helps understand the co-variate relationships that may influence sample means or research outcomes. Validating assumed numerical values used in the analysis is also good practice.

Consequently, this experiment had multiple aims, such as developing and confirming methodology for the remainder of the thesis. Firstly, the experiment aimed to determine a suitable number of carrots to measure in the future by understanding the likely natural variability in respiration rate in a population. Secondly, a sensitivity analysis of the likely contribution to measurement error from each component of the experimental methods was determined. This analysis enabled future work to be optimised, enabling focus on accuracy for parts of the measurement that are likely to produce error and (potentially) simplifying other components of the experiment by making assumptions on factors that have little influence. Thirdly, the density of the carrots used was determined to determine the value used in the consequent analysis that may represent the carrots used in the experimental work. Finally, correlations were conducted between properties measured to identify potential relationships of respiration rate to measurable carrot properties (weight, density, and mass).

4.2.2. Methods

The respiration rate (RR) was determined at 20°C, as described in section 3.1. Twenty carrots sourced from a local supermarket were used. Besides, some tools like drainage method, sensitive analysis, and Monte-Carlo simulation are used to determine the error level of parameters.

4.2.2.1. Density Determination

Measurement was required to determine carrot density (ρ_{carrot}) and volume (V_{carrot}). The drainage method uses a 500 mL measuring cylinder and an electric balance (UP1023X, SHIMADZU). Volume and density were then determined from equations 4.1 - 4.3. $W_{cylinder}$, W_{water}, and W_{carrot} are the mass (g) of cylinder, water, and carrot, respectively. V_{carrot} and V_{water} represent the volume (mL) of carrot and the initial volume of water before the carrot is placed into a cylinder. The W_2 value needs to be directly determined by the electric balance.

$$W_{cylinder} + W_{carrot} = W_1 \quad (4.1)$$
$$W_{cylinder} + W_{carrot} + W_{water} = W_2 \quad (4.2)$$
$$Vol_{carrot} = Vol_{water} - (W_2 - W_1) \quad (4.3)$$
$$\rho_{carrot} = \frac{W_{carrot}}{V_{carrot}} \quad (4.4)$$

4.2.2.2. Data Analysis

The results of Exp 1 are made of CO_2 concentration, so the specific 20 RR values need to be calculated based on equation 3.1. At the same time, a few parameters are used for RR calculation, so their error will affect the reliability of the final result. Moreover, as 20 carrots were chosen as experiment materials; the group size may also lead to errors. Therefore, the error level of group size and RR calculation parameters can be analysed based on sensitive analysis and Monte-Carlo simulation, respectively. However, the group size is 20, so the normal distribution assumption of data needs to be checked with Shapiro-Wilks test. Moreover, the factors that may cause errors, such as the volume measurement and the CO_2 analyser calibration are quantified.

4.2.2.2.1. Monte-Carlo simulation

A Monte-Carlo simulation is a computer simulation in which a population of results are generated from a known variable population of inputs. Monte-Carlo simulations help determine likely range outcomes in given scenarios where inputs are of a variable known population. The parameters of Monte-Carlo are not fixed values, but are treated as stochastic or random variables. The introduction of Monte-Carlo simulation estimates mathematical functions and mimics complex systems' operations via random sampling and statistical models (Harrison, 2010). Moreover, the Monte-Carlo simulations have the following features: (1) they model a system as a probability density function (PDFs); (2) they repeatedly sample from the PDFs, and (3) they tally/compute the statistics of interest (Harrison, 2010).

Excel can be used to run a Monte-Carlo simulation. The 20 original RR data can be summarised to get the mean and standard deviation. However, the 20 data may not be enough to be a normal distribution, so the Shapiro-Wilkes test is used to test the data is indeed a normal distribution. The random value function within Excel is used to produce random values as “Probability” of the function NORM.INV and the “Inverse of the normal cumulative distribution” values are produced based on probability (random values), mean and standard deviation. Then, the deviation standard and mean of each single group are calculated to get the results of the simulated means and standard deviations of each group. The average of simulated means of each group is the “mean of means”, and the average of simulated standard deviations is the “STANDARD DEVIATION of means”. The division between “STANDARD DEVIATION of means” and “mean of means” represents the error level of the group size. The simulation was run for groups sized between 5 to 100 individual carrots.

4.2.3. Results and Discussion

4.2.3.1. Determination of Respiration Rate Variability in a Population

The distribution of carrot weight is shown in Figure 4.1. The graph divides the weight of 20 carrots into four ranges and indicates their range and frequency. The most common weight is between 66.575 and 93.575 g, and only one carrot has a high weight value between 120.575 and 147.575 g. Furthermore, the distribution of carrot weight is close to a normal distribution.

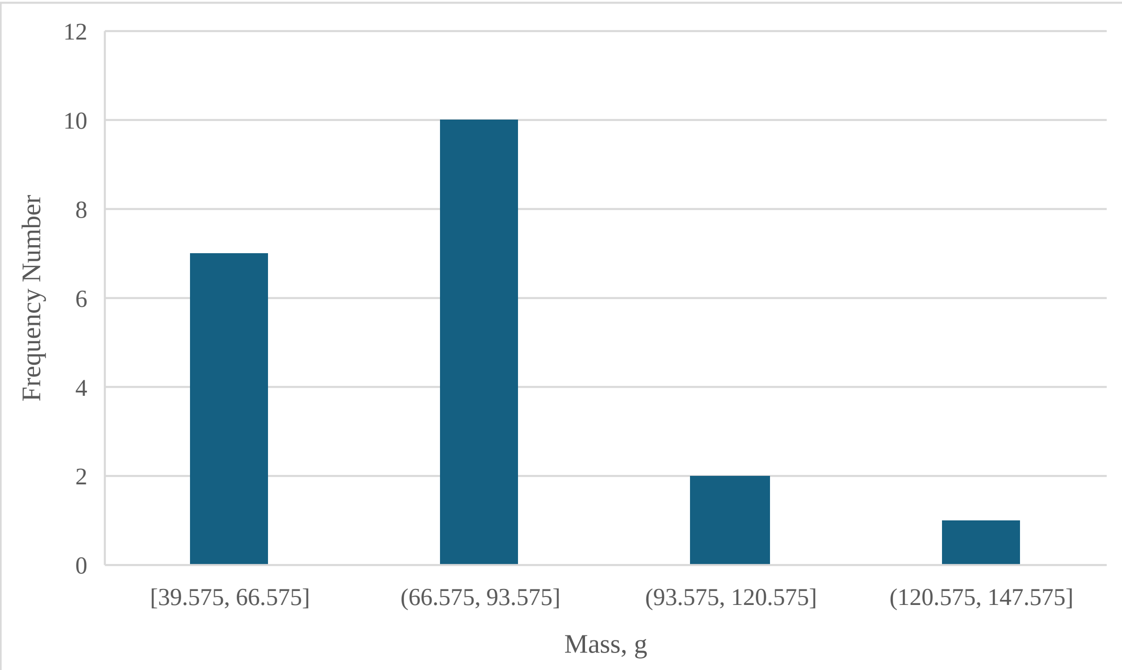


Figure 4.1 The frequency distribution of 20 carrot weight

The density data is from the calculation results of the drainage method. The density of one carrot was unknown due to the errors caused by the carrot floating. In equation (4.3), the difference between W_2 and W_1 is caused by the displaced water of the carrot when it is placed into a cylinder. The distribution of carrot density skews towards the smaller end, with most carrots having a density within the range of 0.836 to 0.996 g/cm^3

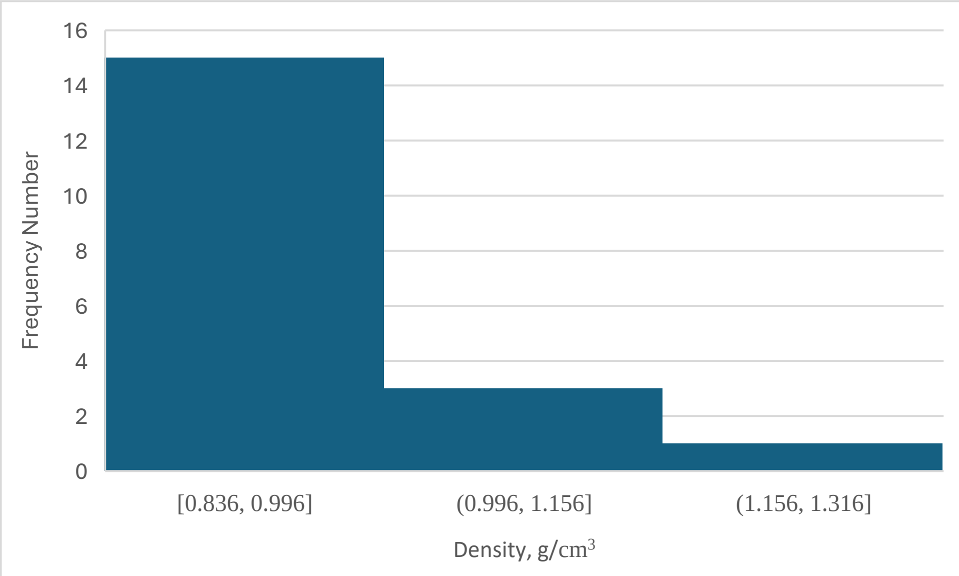


Figure 4.2 The density distribution of 20 carrots

The RR values of 20 carrots are the calculation results of equation (3.1). The RR and carrot density have a similar trend, which deviates to the lower end. The most common RR range of carrots was between 256.164 and 426.164 nmol/kg/s, which means that even though the RRs of carrots are highly diverse, they will group around a particular range. As Figure 4.3 illustrates, the RR results of 20 carrots do not fit the normal distribution well, and the total number of RR data is less than 30, so it cannot assume normality directly.

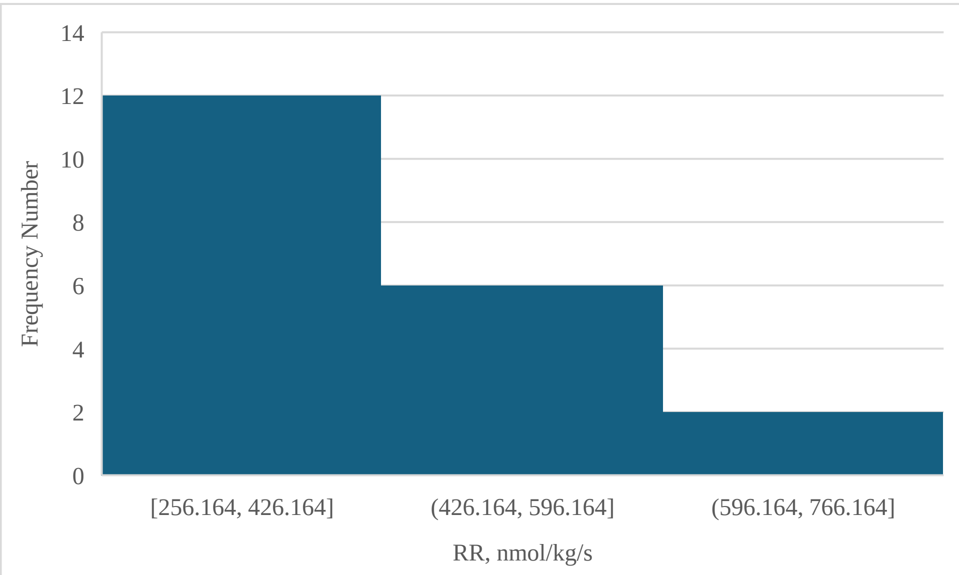


Figure 4.3 The RR value distribution of 20 carrots

Conveniently, the 20 data can be checked through the Shapiro-Wilkes test to determine whether the data set can be assumed normality. The Shapiro-Wilkes test is one of the most popular static tools for proving normality because it has the best power for a given significance (Razali & Wah, 2011). As the content of Table 4.1 finds, the W value based on the original RR data is 0.876, which is less than the reference value of 0.905 (Shapiro & Wilk, 1965). The W value is less than the reference value, indicating the P value of the Shapiro-Wilkes test below 0.05. The assumption (H_a) is significant, which means the samples cannot be recognised as normal distribution. In such a situation, there are two options: 1) Transform the dependent variable and 2) non-parametric test. The first methodology is chosen, and after taking the square root of the original data, the W value of Shapiro-Wilkes increases to 0.918, which indicates that the samples can be assumed to have a normal distribution. Based on this result, the 20 RR data can be assumed to obey normal distribution, and the Monte-Carlo simulation can be run based on these samples.

Table 4.1 Result of the Shapiro-Wilkes test

	Original RR data	The square root of the original RR
W value	0.876	0.918
Reference value	0.905 (0.05 point with 20 group size)	
P value	$P < 0.05$	$P > 0.05$
H_o (null hypothesis)	The samples belong to the normal distribution	
H_a	The samples do not belong to the normal distribution.	

In Figure 4.4, the X-axis represents the population size (n), while the Y-axis represents the resulting expected standard deviation of means. Data is produced from 20 carrots and normal distribution, running a Monte-Carlo simulation 100 times, from group size 5 to 100. As expected, as the group size enlarges, the error level decreases. The curve indicates that the error becomes stable after the group size is over 45, around 0.05. However, when the group size is 25, the error is at the same level (close to 0.05). For experiment convenience, the group size error of 25 was deemed acceptable.

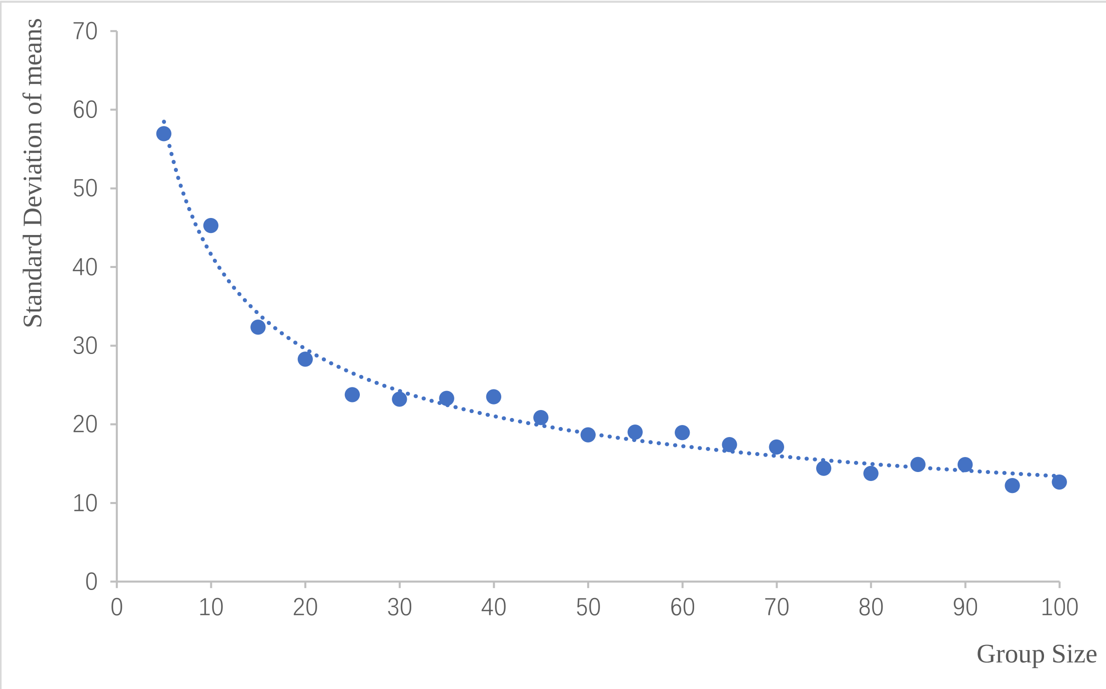


Figure 4.4 Error Standard deviation corresponding to different group numbers (Based on Monte-Carlo simulation)

4.2.3.2. Sensitivity of Respiration Rate Determination to Estimated Values

The investigation was built upon previous research of the RR of carrots. There are a number of sources of error when measuring RR including equipment error, calibration error, weather changes affecting the lab pressure, and temperature changes of the lab. In order to quantify how the extent of the parameters used to calculate RR influence calculation results, a sensitive analysis was utilised. this analysis indicates which parameters tend to influence the calculation result most, and is helpful in ensuring what aspects of data collection should be of focus during experimentation. Estimated errors for each parameter and the resulting impact on RR error are provided in Table 4.2. The estimated errors were from a few aspects; for example, the likely measurement error of V_i is ± 20 cm^3, which is the manufacturing tolerances of the jar, and the error ± 0.01g of M is the value reading fluctuation of electric balance. The V represents the volume of carrots, which is determined via the drainage method; the likely measurement error is analysed based on the referred density value (1.02 kg/L), which is the gap between measured volume calculated volume data based on the preferred density. The time error can be ± 20s because 20 jars and carrots are used in the experiment; there will be a 20s sampling time error between the first and last jar. The error of P is caused by the temperature fluctuation during the experiment. Moreover, the primary error for CO_2 concentration measurement is the CO_2 analyser calibration operation.

The error of mass (M), volume (V), time (t), and atmosphere pressure (P) showed less effect on carrot RR (less than 1%)**,** so it assumes that the value of these parameters equals its average data. Except for the second CO_2 concentration measurement, the error of most RR parameters remains low (below 5%), and the calibration causes the high-level error of the second CO_2 measurement. Hence, it is clear that accurate measurement of the CO_2 is the critical factor in influencing the accuracy of the estimated RR.

Table 4.2 The error-sensitive analysis of parameters for respiration rate calculation

	Approximate Average	Likely measurement error	%Error	Error for RR (nmol/kg/s)	%error for RR
V_j	1000 cm^3	±20 cm^3	2%	±9.3678	2.2%
M	74.827g	±0.01 g	0.013%	±0.07	0.02%
V_p	80.029 cm^3	±6.67 cm^3	8.3%	±3.07	0.71%
t	2580s	±20 s	0.78%	±3.30	0.77%
P	100936	±6 Pa	0.006%	±0.025	0.006%
$C[co_{2i}]$	0.145	-0.01	6.9%	21.603	5%
T	20℃	±1.4℃	7%	±2.068	0.48%
$C[co_{2f}]$	0.357	0.018	5%	-38.885	9%
△C ($C[co_{2f}]$-$C[co_{2i}]$)	0.212	0.028	13.2%	8.472	2%

4.2.3.3. Carrot Density Determination

The average carrot density was found to be 0.935 g/cm^3. Previously others have reported carrot density to be 1.02 kg/L, 1.04 g/cm^3, and 1.001 ± 0.017 g/cm^3 (Jahanbakhshi et al., 2018; Rohwer, 2021; Seljåsen et al., 2001). Figure 4.5 illustrates that the linear regression line fits the observed points well, and the S is small enough. R^2 of the fitted line is over 0.8, which means the weight and volume of the carrot have a significant linear relationship. The slope of the fitted line indicates the carrot density should be 0.9902 g/cm^3. It was observed that some carrots suspended on the water surface within a cylinder that caused volume fluctuation, which led to a lower density estimation. Consequently, the preferred density closer to the measured density of 0.9902 g/cm^3 was chosen in the experiment, and the referred density (1.02 kg/L) will be used as the assumption value in the following experiments.

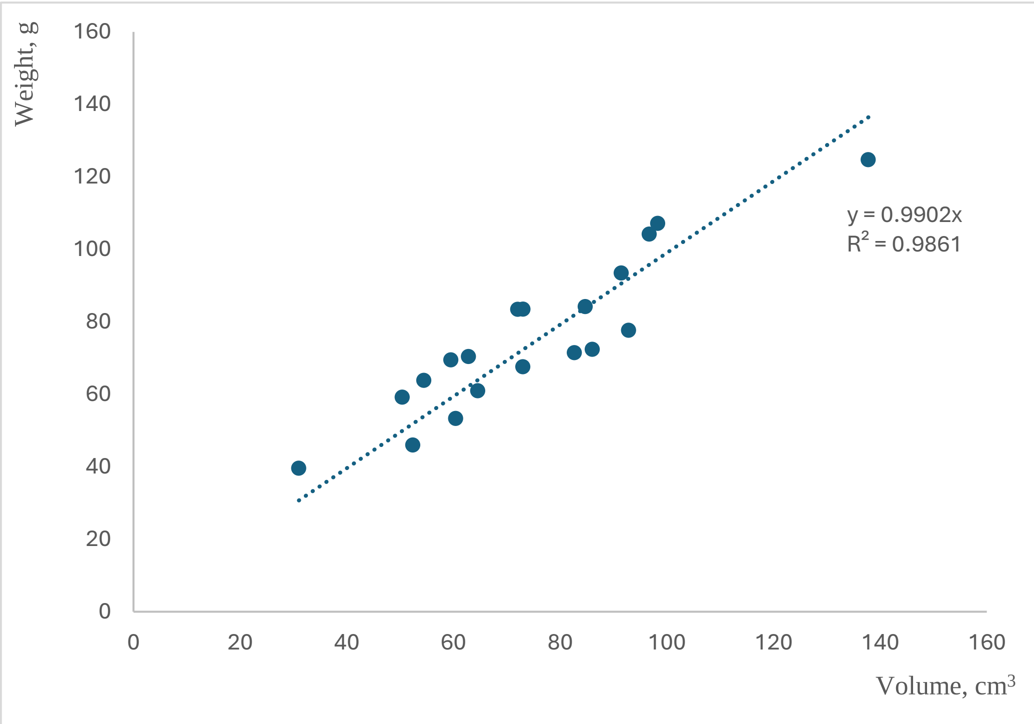


Figure 4.5 Weight (g) versus Volume (cm^3)

4.2.3.4. Relationship of Carrot Properties to Physical Attributes

4.2.3.4.1. Relationship between RR and Weight

Figure 4.6 demonstrates that most of the dots are spread widely on the graph. The value of $R^2 < 10\%$, which means the weight of carrots does not have a strong linear relationship with RR. Carrot weight does not have a large influence in determining the RR.

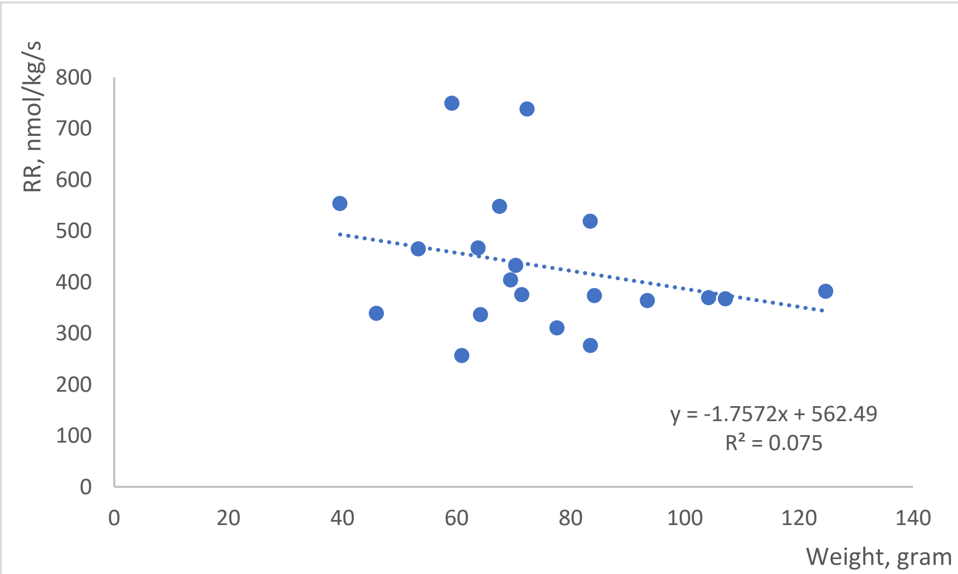


Figure 4.6 Regression analysis respiration rate (nmol/kg/s) versus weight (g)

4.2.3.4.2. Relationship between RR and Density

Density is also a carrot property. A linear regression with RR was used to analyse if there was any influence of carrot density on RR. Figure 4.7 demonstrates the linear relationship between RRs and carrot density. However, the carrot density and RR do not show much linear relationship. The R^2 is very low. It would seem that the density of carrots, does not affect the RR.

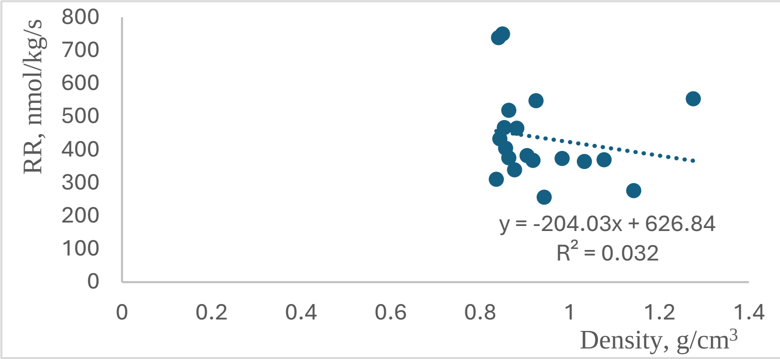


Figure 4.7 Regression analysis respiration rate (nmol/kg/s) versus density (g/cm^3)

4.2.4. Conclusions from Experiment 1

This experiment provides a basis for the following experiments in that it determines the error level of parameters, carrot density, and the relationship between the weight and RR. While twenty (20) carrots were chosen as experiment materials to determine the error level in the RR measurement results from the Monte-Carlo simulation found that a carrot sample size around 25 carrots would be appropriate for future experiments, as it offers around a 5% deviation error.

According to the sensitive analysis, most parameters provide limited error toward RR calculation that locates between 0.001% and 5%, except the second CO_2 concentration measurement offers higher error because of CO_2 analyser calibration.

The fitted line of carrot weight and volume shows a strong linear relationship for carrot density. However, the carrot volume determination of the experiment was somewhat error because the drainage method operation is influenced by the carrot floating in the cylinder, so the volume data 1.02 g/cm^3 is used in the following experiments.

The linear relationship analysis between carrot properties and RR indicated that the RR did not have much relationship between the weight and density of carrots. Hence while the carrot weight is an important parameter required to calculate RR values, it does not affect the RR.

4.3. Experiment 2 (Influence of temperature and dry matter on respiration rate)

4.3.1. Introduction

Temperature is an essential factor for carrot respiration, significantly influencing the physiological activity of carrots. Most physical, biochemical, microbiological and physiological reactions contributing to the deterioration of produce quality are largely dependent on temperature (Tano et al., 2007). A few studies report the RR of carrots under variable temperatures. As Figure 4.8 demonstrates, the RR of shredded carrots rises with increasing temperature. The relationship of RR to temperature can be described by equation 4.5, which is created based on the Arrhenius-type equation (Iqbal et al., 2009).

$$RR = R_{ref} \times e^{\left[-\frac{Ea}{Rc} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]} \quad (4.5)$$

Where RR is the product respiration rate [ml/(kg hr)], R_{ref} is the reference respiration rate [ml/(kg hr)], E_a is the activation energy (kJ/mol), R_c is the universal gas constant [0.008314 kJ/(mol K)], T is the temperature (K), and T_{ref} is the reference temperature (the average temperature in the range tested is 283.15 K for ambient air and 285.15 K for other atmospheres).

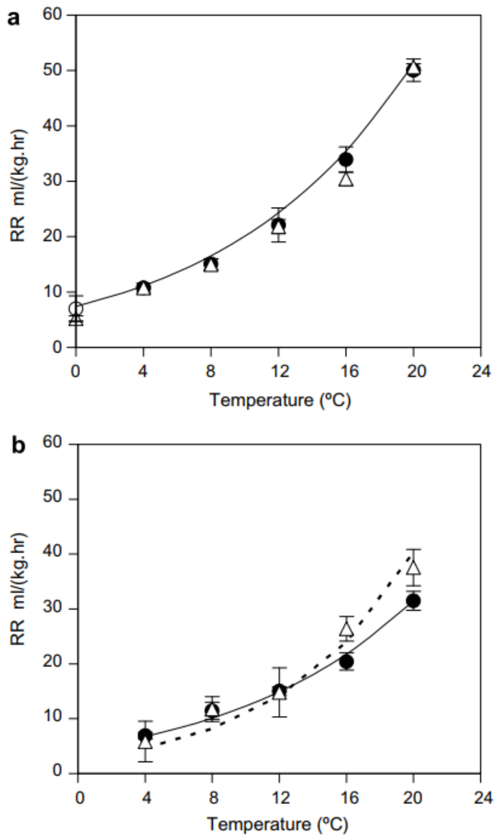


Figure 4.8 The RR change of shredded carrots under variable temperature tiers and atmosphere composition a) Respiration rate at ambient atmosphere b) Respiration rate at low O_2 and high CO_2 gas atmosphere (O_2: 2%, CO_2:16%) (Iqbal et al., 2009)

Another study on carrot growth reports that carrots' dry matter may be consumed due to carrot growth and maintenance respiration (Saltveit, 2019).

This experiment is designed to determine the influence of temperature on the respiration rate of carrots. However, the carrot will also lose weight during the experiment because of transpiration and respiration consumption on dry matter, so it requires weight loss management (plastic bags with bunch holes) to limit the carrot transpiration in the long-term experimental procedure (Apeland & Baugerød, 1969; Correa et al., 2010). The parameters of plastic bags for weight loss management used were 200 × 305 mm with 50 μm thickness.

Transpiration rate (TR) is proportional to the superficial area and influenced by the water vapour pressure and air velocity (Correa et al., 2010). Furthermore, previous studies reports that storage time, relative humidity (RH), and temperature also influence the TR (Singla et al., 2021). Weight loss under variable RH and temperature versus time is illustrated in Figure 4.9, while the TR data versus RH and temperature are shown in Figure 4.10.

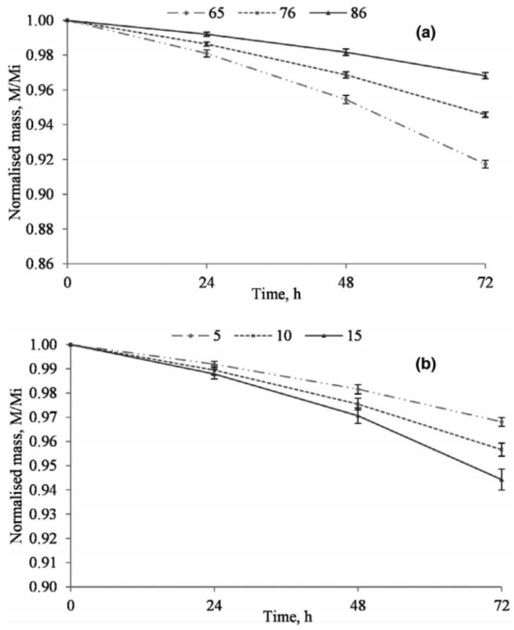


Figure 4.9 Weight loss versus time under variable RH and temperature tiers a) Weight loss variation of black carrots at 5°C, RH impact b) Weight loss variation of black carrots at 86% RH, temperature impact (Singla et al., 2021)

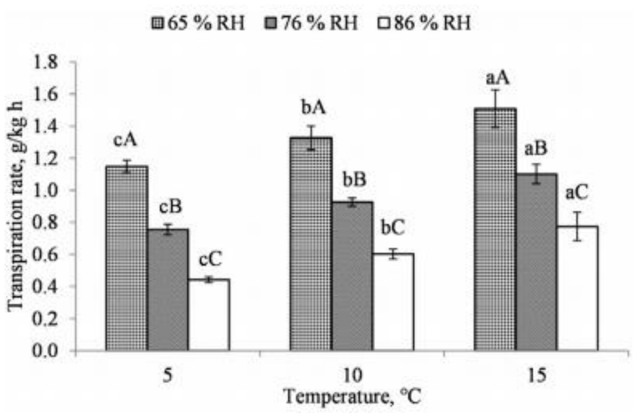


Figure 4.10 The TR change versus variable temperature and RH tiers (Singla et al., 2021)

To model the influence of RH and temperature on the TR, an Arrhenius relationship-based model can be used as equation 4.6 (Singla et al., 2021).

$$TR = k_{ref} \times exp^{\left[-\frac{Ea_{TR}}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]} \quad (4.6)$$

Where K_{ref} is a frequency factor at reference temperature (g ▪ kg ▪ hr^{-1}); Ea_{TR} is the activation energy (kJ/mol); R is the universal gas constant (kJ mol K^{-1}); T is the storage temperature (K); T_{ref} is the reference temperature (mean of the storage temperatures, 283.15 K). The referred model parameters (K_{ref} and Ea_{TR}) of equation (4.6) are listed in Table 4.3.

Table 4.3 Model parameters (K_{ref} and Ea_{TR}) of the equation 4.6 (Singla et al., 2021)

RH (%)	K_{ref} (g kg hr^{-1})	Ea_{TR} (kJ/mol)	R^2
65	1.32 (±0.14)	18.17 (±1.3)	.97
76	0.92 (±0.07)	25.07 (±2.1)	.95
86	0.59 (±0.05)	37.14 (±2.4)	.98

4.3.2 Methods

The experimental carrots were sourced from the supermarket, and all of the carrots are packaged in plastic bags with gas (unknown composition) and in a low-temperature environment. The temperature parameter must be adjusted during the experiment, so the carrots are also placed into plastic bags and stored in the TCR (temperature-controlled room) for different periods at different temperatures. The RR measurement is similar to Exp 1. However, after the syringes are taken out from TCR at a lower temperature than the lab temperature (20°C), they need to be placed on the lab table for 8 minutes to adjust the temperature of the syringes to avoid CO_2 concentration measurement error.

4.3.2.1. Measurement of storage conditions (temperature and RH)

Temperature and RH were measured with (I-buttons) to monitor the storage conditions of the carrots during the experiment. The four I-buttons need to be prepared ahead of time, and their data and settings need to be initialised via "OneWireViewer" to set the recording frequency to logging every 10 minutes and delete previous data. The two I-buttons were sealed with carrots into plastic bags and another two were placed into TCR to record the environment temperature. After a long storage period, the temperature data of I-buttons turns to the same level. However, the two I-buttons inside plastic bags tend to be in a higher RH due to the respiration effect of carrots, which can produce water.

4.3.2.2. Weight loss measurement

The weight difference over time determines the carrots weight loss. The experiment runs for an extended period, so carrot weight management is also required. All carrots were placed into plastic bags with five punch holes during the storage period in the TCR to manage their weight loss. The ventilation system and five holes in the plastic bags accelerate the atmosphere exchange and maintain the bioactivity of carrots.

The experiment's initial weight (W_1) is determined before the carrots are placed in the TCR. The end weight (W_2) is measured when the CO_2 measurement on the last temperature tier is finished. Because the carrots were placed in the TCR where the temperature was lower than the lab, after the carrots were taken out from the TCR, they needed to be air-dried to avoid weighing errors caused by condensate water on the surface of carrots. So, the weight loss can be calculated based on equation 4.7.

$$\text{Weight loss} = W_1 - W_2 \quad (4.7)$$

4.3.2.3. Dry matter measurement

At the end of the experiment, all carrots were sliced into slices of 3 ± 1 mm thickness as fresh materials, and their weight was determined via electric balance (PG503-S, METTLER TOLEDO) and trays. W_3 and W_5 represent the weight of trays and the total weight of fresh trays. After that, these fresh materials were labelled, and placed in a dehydrator overnight. After dehydrating, the weight of dried materials with trays was determined (W_4). The dehydrating step removes the moisture from within the carrot slices. Combining the data of RR and dry matter content illustrates the potential influence of dry matter content on carrot respiration. The dry matter content can be calculated via equation 4.8.

$$\text{Dry matter content} = \frac{W_4 - W_3}{W_5 - W_3} \quad (4.8)$$

For the operation, two factors contributed to the error of the experiment: chilling time and freshness of carrots. The chilling time of carrots should be as long as possible to reduce the internal and surface temperature of carrots at the same level when the RR measurement starts. Due to the long-term experiment, carrot freshness is essential. Otherwise, the carrot deterioration may lead to high RR, which causes dry matter loss, and the deterioration extent shows great individual diversity, which leads to undetectable error for the experiment result.

4.3.3. Results and Discussion

4.3.3.1. Respiration Rate as a function of temperature

Low temperature restricts carrot respiration rate as Figure 4.11 illustrates. However, the figure also shows that the increase of RR is not a linear relationship with temperature. The RR increases between 12 °C and 20 °C is more significant than other groups on lower temperature tiers. A similar result is observed on other plants in Figure 4.12 (Kurimoto et al., 2004).

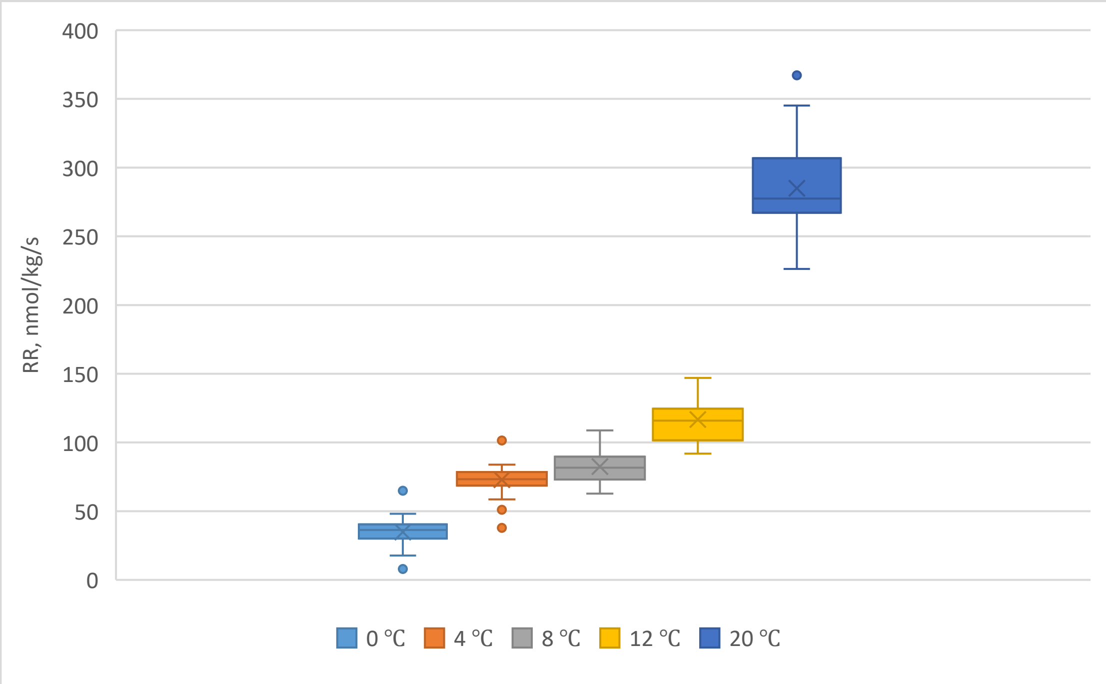


Figure 4.11 The RR range of 25 carrots under five temperature tiers

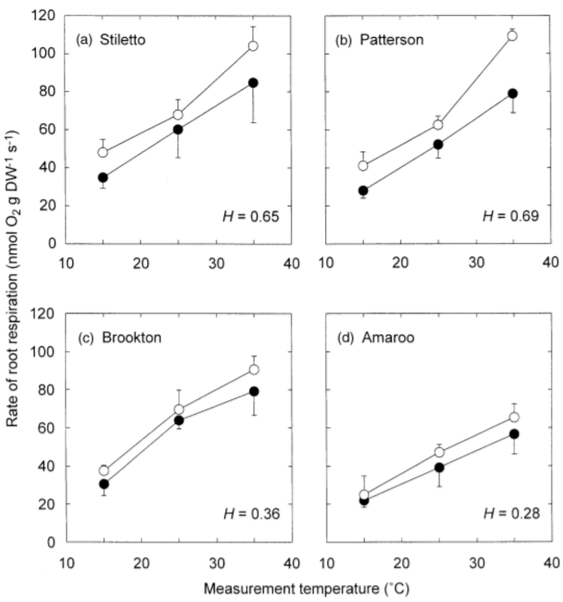


Figure 4.12 Rate of root respiration at 15, 25, and 35 ℃ of four kinds of cultivar grown at 25 ℃ (●) and 15 ℃ (○) (Kurimoto et al., 2004)

To quantify the RR change, the Q_{10} value can be used, which is defined as a value that measures the rate of a biochemical process changes over a 10 ℃ temperature change (Aisami et al., 2017). Nevertheless, the experiment's temperature data does not have a gap of 10 ℃. Hence, the average RR value at 10 ℃ gets estimated based on the RR data above and equation (4.5), which is around 90.00 nmol/kg/s. The Q_{10} value from 0 to 10 °C is 2.58, and the 10 to 20 °C is 3.17. These Q10 values demonstrate that the temperature-induced RR increase is uneven. Mostly, the Q_{10} value should be between 2 and 3. For example, when the value is 2, this means for every 10 °C rise in temperature, the rates double (Aisami et al., 2017).

The results found in this work show a similar trend to that observed in other experiments, (Figure 4.12). As Figure 4.13 illustrates, no matter what format carrots are treated, the temperature is a significant factor affecting RR, and its increasing extent is uneven among different temperature ranges. Interestingly, the temperature change induced RR increase on shredded carrots is the most dramatic.

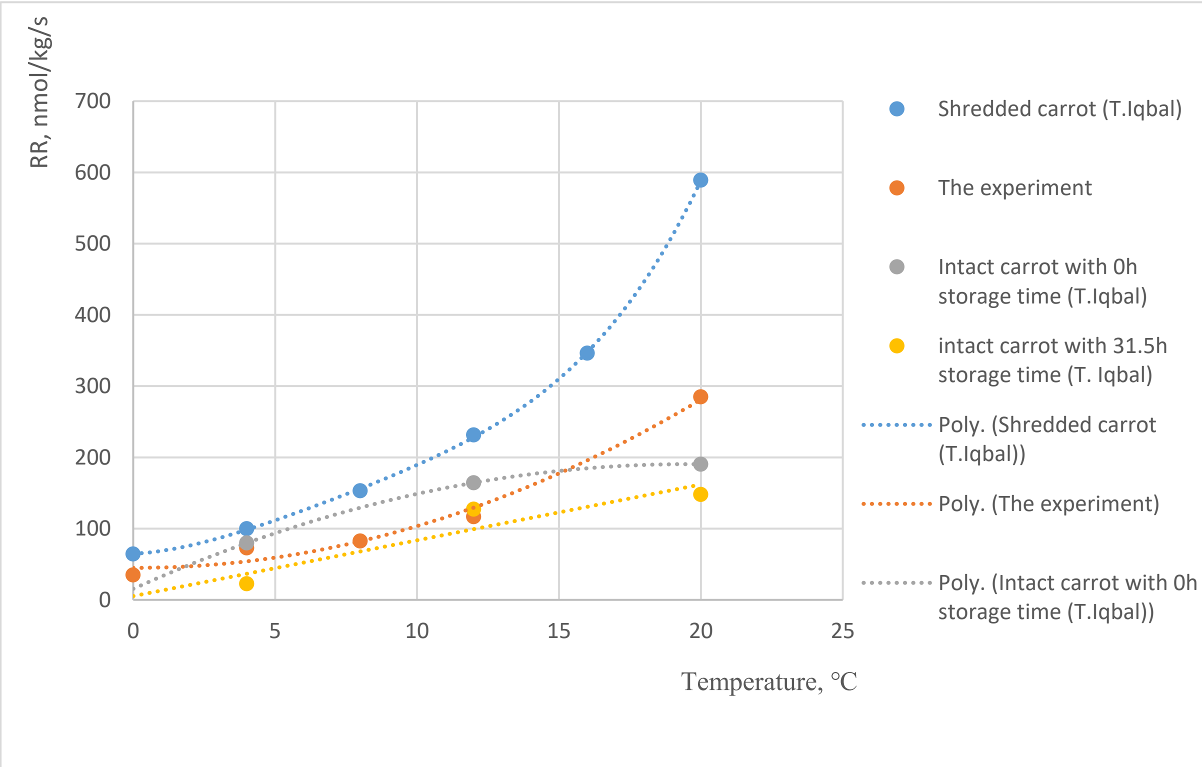


Figure 4.13 The RR data comparison between carrots with variable treatments (The experiment line is created based on the average RR value of five temperature tiers)

4.3.3.2. Correlation of respiration rate to dry matter content

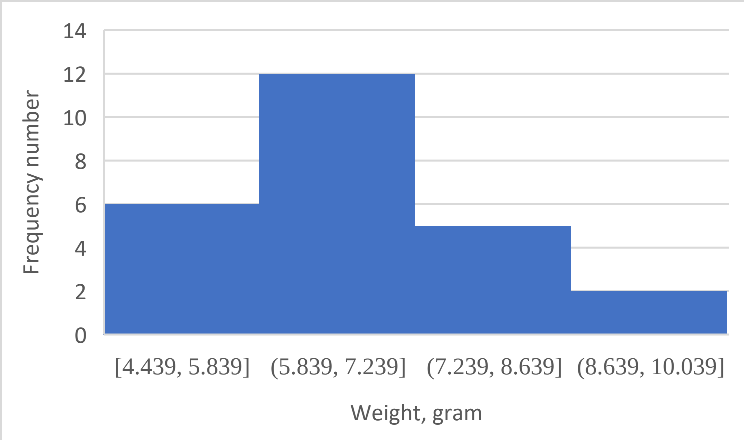


Figure 4.14 The dry matter weight distribution of 25 carrots

In Figure 4.14, the dry matter of carrots can be separated into four parts, and the distribution is close to the bell-shaped curve, which can be seen as a roughly normal distribution. The most common range of dry matter weight is between 5.839 and 7.239 g.

The dry matter may be related to the RR of carrots because previous studies reported that the soil respiration of other agricultural products like wheat and maize is curvilinear (Liu et al., 2013). Considering that the carrot root also grows underground, it may have a similar feature, so the linear regression of dry matter content and fresh weight is introduced. In the resulting regression the data points have significant spread on both sides of the fitted line, but the S remains at a small value in Figure 4.15. The dry matter content of carrots illustrates a linear relationship with RR, with the R^2 of the regression line over 0.5. This suggests that the dry matter content of carrots can influence the RR of carrots.

The linear regression of fresh weight and RR is shown in Figure 4.16. The fresh weight and mean of RR have a linear relationship but are not as strong as dry matter content. Besides, it shows a decreasing trend with fresh weight suggesting that carrot with less fresh weight tends to have a higher RR value.

According to two previous studies, there are two pools for dry matter, e.g. storage or structural, and the loss of dry matter within is mainly from growth and maintenance respiration that occupies 46% of the roots (Hussain et al., 2008; Reid, 2019). The fresh weight of carrots and dry matter content both have a linear relationship with RR. The carrot with more dry matter content but less fresh weight at the end of the experiment tends to have a higher RR.

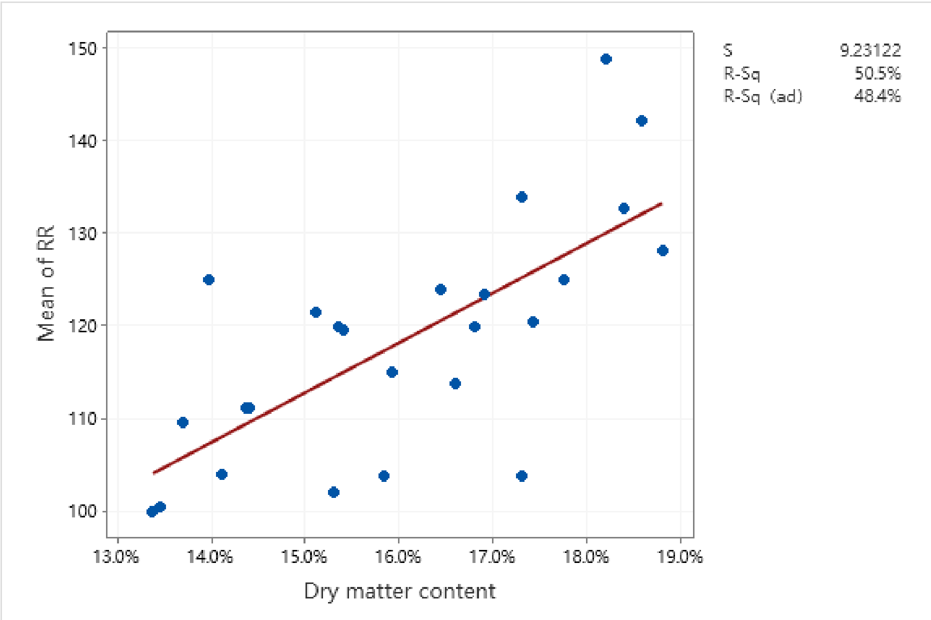


Figure 4.15 The linear regression analysis of dry matter content and mean RR of carrots of five temperature tiers (0, 4, 8, 12, 20°C).

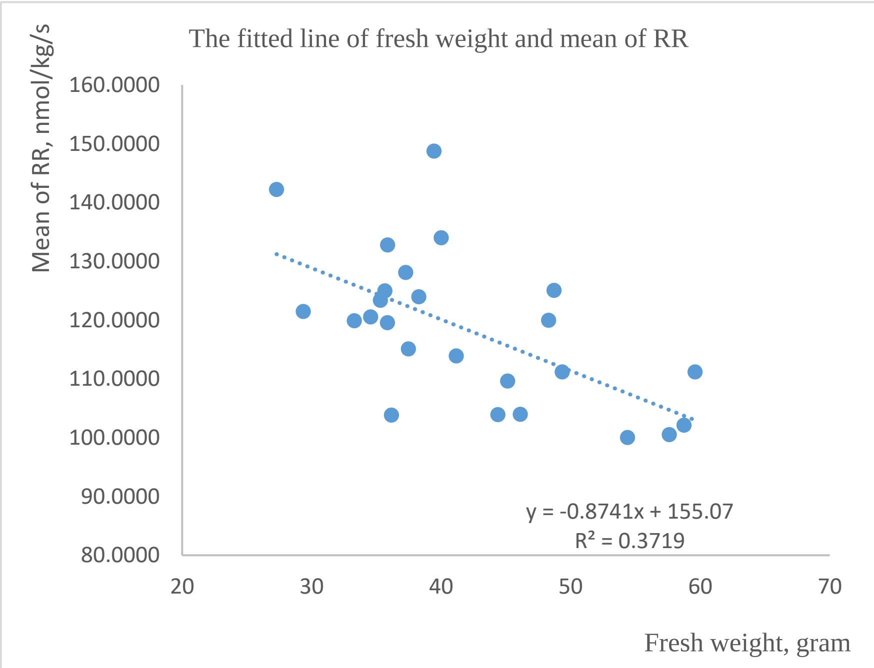


Figure 4.16 The linear regression analysis of carrot fresh weight and mean RR of carrots of five temperature tiers (0, 4, 8, 12, 20°C).

4.3.3.3. Carrot mass loss

The experiment lasted two weeks, and around 31% of the weight of carrots was lost. The average mass lost was 19.559 g, which is high. Hence, for the RR calculation of Exp 2 the initial weight of the carrots was used. Although the plastic package with holes is deemed an excellent method to restrict weight loss caused by transpiration during carrot storage (Correa et al., 2010), the weight loss was high. The plastic bag storage method should lead to higher RH within the package than the environment (Kader & Saltveit, 2002). The RH and temperature record of the environment inside the packs of carrots, collected with I-buttons are listed in Figure 4.17, and Figure 4.18, while the outside conditions are provided in Figure 4.19, and Figure 4.20.

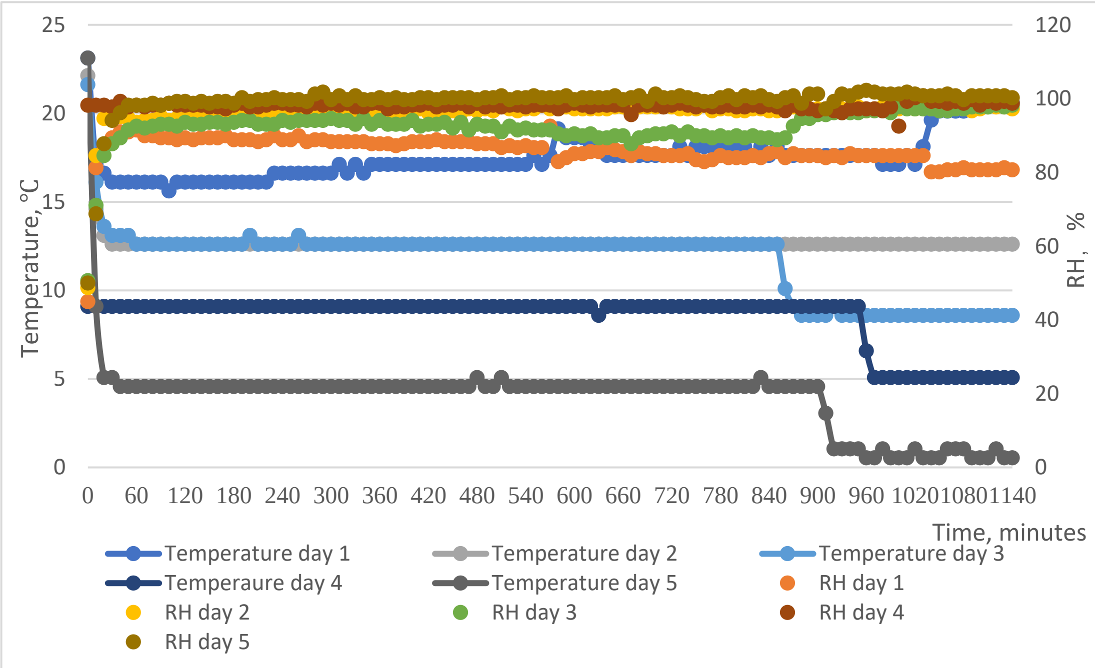


Figure 4.17 Temperature (°C) and Relative Humidity (RH, %) of I-button sensor G2 were measured over five days, temperature data is represented by lines in shades of blue to grey for each day and RH is shown with following dots (yellow, orange, green, brown, and tan)

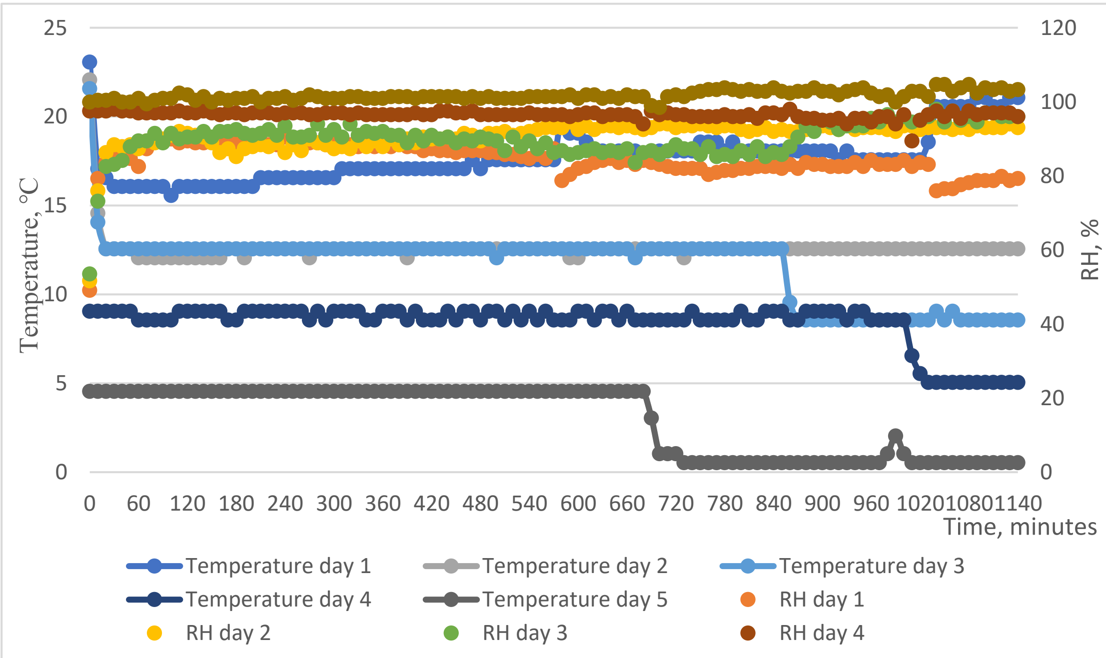


Figure 4.18 Temperature (°C) and Relative Humidity (RH, %) of I-button sensor G3 were measured over five days, temperature data is represented by lines in shades of blue to grey for each day and RH is shown with following dots (yellow, orange, green, brown, and tan)

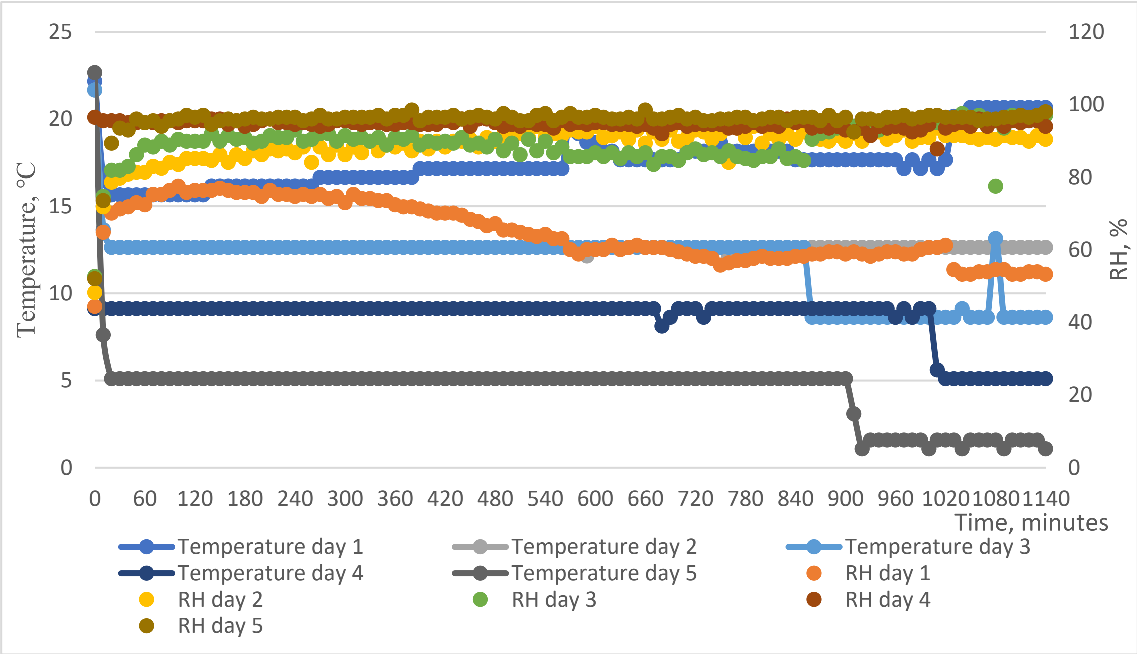


Figure 4.19 Temperature (°C) and Relative Humidity (RH, %) of I-button sensor G4 were measured over five days, temperature data is represented by lines in shades of blue to grey for each day and RH is shown with following dots (yellow, orange, green, brown, and tan)

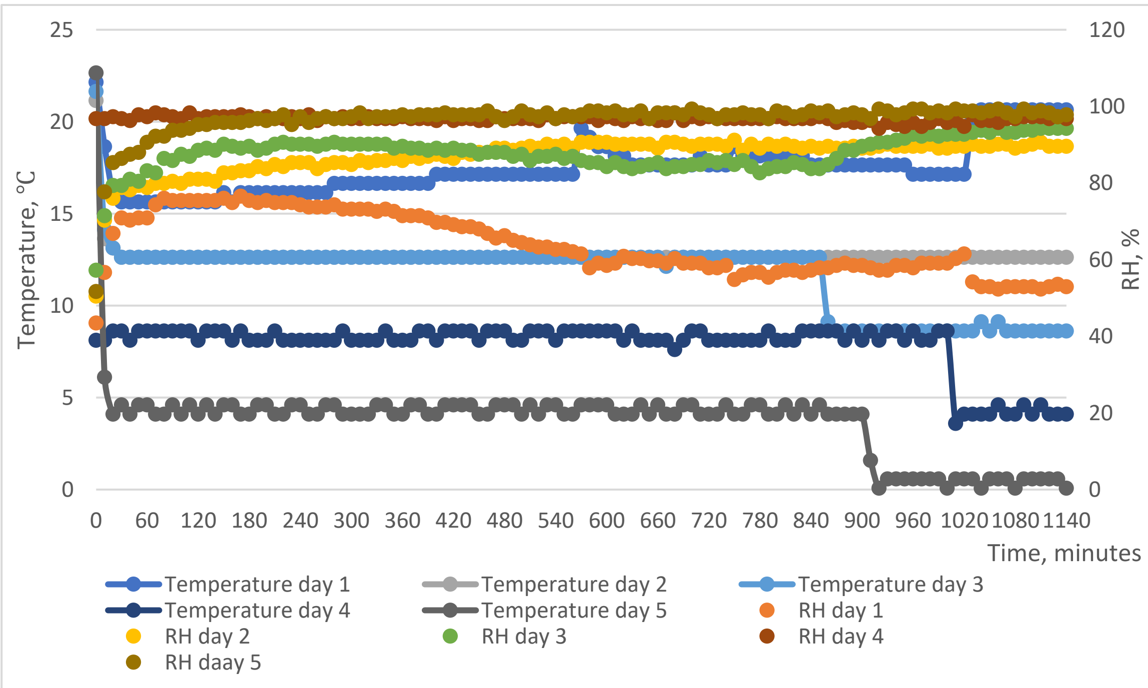


Figure 4.20 Temperature (°C) and Relative Humidity (RH, %) of I-button sensor G6 were measured over five days, temperature data is represented by lines in shades of blue to grey for each day and RH is shown with following dots (yellow, orange, green, brown, and tan)

The G2 and G3 are sealed in plastic bags with carrots, and the other two, G4 and G6, are outside. Hence, internal RH increases, and the temperature data decreases in experimental order. However, the RH increases, and all sensors recorded the RH of day 1 get the lowest RH level. Then, RR increases throughout the day and peaks on day 5. According to Figure 4.9, Figure 4.10, and Equation 4.6, the TR of carrots is related to the temperature and RH, so when the carrot is placed in an environment with low RH and high temperature, the carrot will lose more weight through transpiration. The temperature order of the experiment is from 20 to 0 °C, and assumes the carrots tend to have lower RR at the low temperature, so more waiting time will be arranged for groups under low temperatures to reduce weight loss, and the high-level weight loss during the experiment process may be caused by unstable RH and temperature change over 5 days storage. The high temperature and low RH accelerate carrot deterioration/browning, and high-level weight loss will cause smaller fresh weight values and hence also influence dry matter content measurements. This is a potentially major influence on the linear regression level of RR and fresh weight and the RR and dry matter content.

4.3.4. Conclusions from Experiment 2

The second experiment analysed the influence of temperature and dry matter on carrot respiration. Carrots stored under lower temperature had less RR, and those with higher dry matter content at the end of the experiment tend to have higher RR. The dry matter content is an inherent attribute of individual carrots, and the carrots are stored with the same conditions and period.

A temperature reduction for carrots interrupts the optimal condition of enzyme catalysis, so a decrease of RR from 4 to 8°C and 12 and 20 °C can be observed. A reaction speed change of two to three times for each 10°C change was observed. The introduction of polypropylene packaging can limit respiration and transpiration of carrots effectively, which helps to maintain the vitamin C content of carrots (Asgar, 2020). Based on the experiment results both temperature and dry matter content can influence carrot respiration. Carrots placed in a high-temperature environment and with high dry matter content may have the highest RR. Sometimes transpiration must also be considered, which can cause high-level weight loss under low RH and high-temperature environments.

4.4. Experiment 3 (Respiration of Carrots During Dynamic Temperature Change)

4.4.1. Introduction

The results of Exp 2 demonstrated that temperature is a significant factor affecting carrots RR. Low temperatures limit the respiration rate and transpiration rate of carrots. However, extremely low temperatures (< 0°C) will cause chilling damage to carrots (Den Outer, 1990). Also, the temperature of any storage facility may fluctuate within a limited range (Parsons & Day, 1970).

Homeostasis can be defined as an activity that helps maintain a process rate after an environmental stimulus, at or near the original rate seen before the stimulus (Kurimoto et al., 2004; Smith & Dukes, 2013). However, homeostasis is unnecessary for plants' normal physiological activities, such as plant carbon exchange responses to temperature and CO_2. Homeostasis launches when the carrots are exposed to an increase in temperature, and photosynthesis and respiration rates return to the rates seen before the temperature increase (Smith & Dukes, 2013). Besides, a previous study reported that when the plant homeostasis of R/P (photosynthetic CO_2 uptake and respiratory CO_2) is achieved across moderate growth temperatures, homeostasis is not maintained when plants are exposed to growth temperatures higher than usually experienced in the natural habitat (Atkin et al., 2007).

This experiment aims to explore the pattern of carrot RR under changing temperature conditions to explore carrot homeostasis. The temperature change was from 0 to 20 °C, representing the change occurring when carrots are taken out of a refrigerated environment.

4.4.2. Methods

Measuring RR and determining the temperature change of carrots is challenging. Hence, carrots were divided into two groups, one for respiration measurement and another for simultaneous temperature measurement. This assumes that the independent carrots being measured separately (respiration rate or temperature) are representative of each other. Ten (10) carrots were used for RR measurement, while three (3) carrots were used for temperature monitoring. Prior to the experiment, all 13 carrots and jars were placed in a 0 °C TCR overnight to adjust the temperature of all carrots, and the three carrots were placed together to get similar temperatures. Similarity checking was achieved by assuring similar carrot weight (67.938g – 78.038 g) as determined by electric balance (PG503-S, METTLER TOLEDO). A "Squirrel logger" was used in the experiment for temperature change recording. The logger requires connection to thermocouples and set time resolution (recording frequency) on a PC before the experiment starts. A recording setting of 1 minute was used. Six thermocouples were installed into six drilled holes on the surface and centre of three carrots. Carrots' natural shape is uneven, but they can be approximated as elongated cones. A ruler was used to determine the edge length. A mark was placed on the middle point on the surface, and a hole was drilled to the depth of the carrot radius.

The experiment design was to measure carrot RR under dynamic temperature from 0 to 20 °C. The logger was taken into TCR and connected with carrots via thermocouples. When the temperature logged data indicated the temperature was approximately 0 °C (± 3.7 °C), all carrots and the data logger were removed from the TCR room (0°C) and transferred to the laboratory (20°C). RR measurement occurred immediately. The frequency of RR measurement was 30 min during the experiment period, with the experiment running from 10 am to 5:30 pm. After the measurement, the carrots were returned to 0°C TCR overnight, with the RR measured at 0°C, the following day.

4.4.3. Results

The results of the surface and centre temperature of 3 carrots are provided below (Figure 4.21). The curve starts at a low temperature and increases gradually. The three curves of surface temperature rise more quickly than the centre temperatures, which indicates the time for heat to ingress into the internal location of the carrot. When carrots returned to 0 °C, the average respiration rate of the carrots averaged 23.1 nmol/kg/s. Figure 4.22 builds a relationship between respiratory rate (RR) and temperature over time.

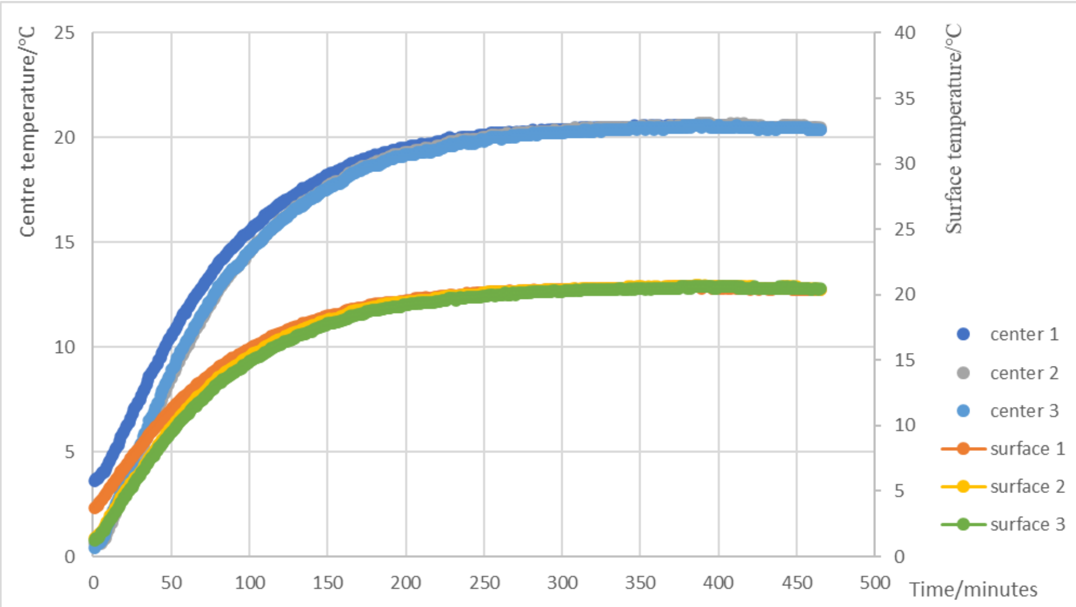


Figure 4.21 The temperature change of the surface and centre of the three carrots for temperature monitoring

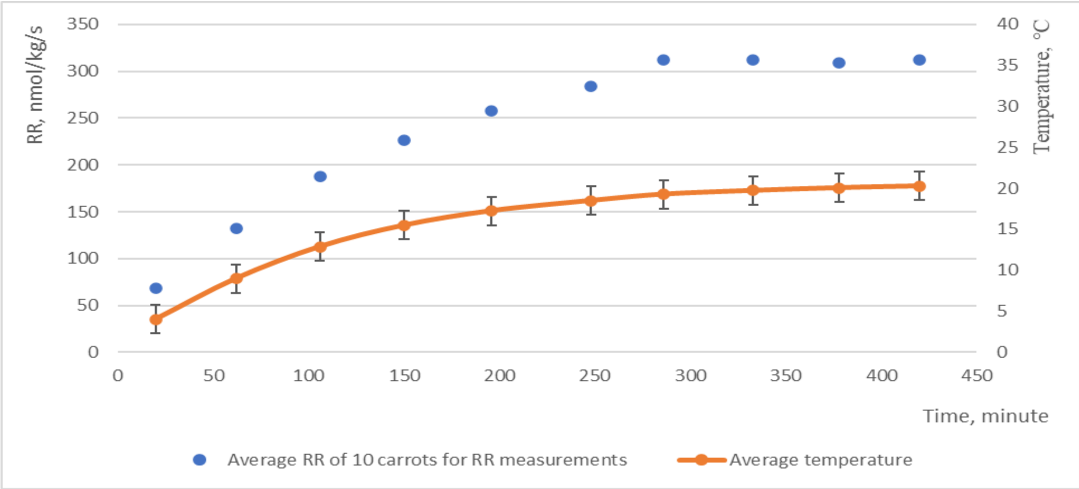


Figure 4.22 Relationship between average respiration rate (RR) of carrots at average temperature and time (The average temperature is the mean of carrot surface temperature and core temperature data was collected via thermocouple over 30 minutes, and the error bars of orange line represents the temperature variability ± 1.68 °C)

4.4.4. Discussion

Figure 4.22 demonstrates the average temperature increase of the carrots. For the period from the first measurement to the 196th minute, the rate of average temperature change is approximately 0.075°C per minute, while later that time point, the rate changes to 0.001 °C per minute until the end of the experiment. The RR data increases over time, but after the 196th minute, the RR's trend starts to differ from the average temperature. The temperature reaches relative equilibrium while the RR continues to increase until the 286th minute, then decreases slightly between the 286th minute and 378th minute, and then RR returns to an equilibrium.

According to Figure 4.22, when carrots are stored at low temperatures, the temperature sensitivity of RR stays high, and the same feature can be observed in Exp 2 (4.3). In Exp 2 (4.3), the RRs of carrots were measured under five tiers, but the increasing extent at the high-temperature range (12-20 °C) is much higher than in other groups.

However, there is the difference as well. The growth trend of RR under a dynamic temperature environment differs from the results of Experiment 2. After the 196th minute, the RR increase in extent is more significant than the temperature. When the carrot temperature reaches 20 °C, there is a slight fluctuation in the RR, and it seems the diversity between RR and temperature is caused by homeostasis. According to the reference, homeostasis can regulate CO_2 release, and the homeostasis extent is affected by the Q_{10} value. A higher Q_{10} value will lead to less CO_2 release of a plant (Kurimoto et al., 2004).

The degree of homeostasis (H) can be described by following equation (4.9)

$$H = 1- \frac{LTR_{10}-1}{Q_{10}-1} \quad (4.9)$$

In equation (4.9), LTR_{10} represents the long-term respiratory response to temperature, was calculated as the ratio of respiration rate, which can be seen as a constant in the experiment (Larigauderie & Körner, 1995). Based on the results of the last experiment, the Q10 value from 0 to 10 °C is 2.58, and the 10 to 20 °C is 3.17, which means the carrots at the range between 10 and 20 °C have more influence of homeostasis than carrots at 0 to 10°C.

But the equilibrium is vital for following experiments about physical damage on carrots because these experiments will be conducted in the lab where environmental temperature remains at 20 ± 1.7 °C. To ensure the carrots for damage-induced RR analysis reach homeostasis (after removal from cool-storage), the RR of intact carrots was recorded to compare with the control group. If the RR value is similar, it deems the carrot homeostasis has been reached.

4.5. Conclusion of Experiment 3

This third experiment was built upon Exp 2, and found that the RR of carrots follows a parallel relationship with temperature change. With the temperature rising and reaching equilibrium at the end, the same trend can be observed in the RR but with slight RR fluctuation, which is related to carrot homeostasis. Hence, the temperature influence on carrot RR is very significant. Meanwhile, while factors like the establishment of homeostasis can affect RR, they are generally not as influential as the temperature change. Hence temperature remains the most important parameter to be controlled in the following experiments.

In addition, this work found that the temperature change of carrots is not linear; the temperature rises fast when the temperature is much lower than the environment temperature (20°C), and the rising slope decreases when the carrot temperature is close to the environment temperature. This also means long-term storage is required to change the carrot temperature completely.

4.6. Summary of Chapter findings and conclusions

A few findings about the ordinary RR of carrots can be concluded from these three experiments. Firstly, the RR is an independent physiological process within carrots, so most of the carrot properties will not affect respiration. However dry matter content of carrots can influence RR. Secondly, temperature is a significant factor in controlling RR, but homeostasis can also affect RR. The temperature change of a carrot takes time, and hence the carrot requires a long period to change temperature completely. Thirdly, transpiration threatens carrot long-term storage weight loss, so plastic barriers that prevent moisture loss need to be applied to maintain the weight of carrots during long-term storage. In addition, some care in controlling RH and temperature of the storing environment should be done as both factors also influence TR. Hence, in the following experiments, temperature is an essential factor to be controlled, and multiple carrots are used as a controlled group to represent ordinary RR and to minimise the measurement error.

5. Respiratory Response of Carrots to Physical Damage Severity

5.1. Introduction

Mechanical stress enhances RR and ethylene production. Damage density, measured as the additional surface area created per gram of product, can describe the extent of damage. Hence, damage density can describe the respiratory response as a function of damage (Surjadinata & Cisneros-Zevallos, 2003). The objective of this work was to develop a description of the respiratory response of carrot to mechanical damage.

The RR change and data from other studies of the influence of physical damage on carrot RR are summarised in Figures 5.1 and 5.2. The rCO2w represents the wound-induced respiration rate, and the RR gap represents the gap between rCO2w and the initial non-wound respiration rate of carrots (rCO_{2i}) (Surjadinata & Cisneros-Zevallos, 2003). In these experiments, damage density (cm^2/g) was used to measure the physical damage level of the carrot. Figures 5.1 and 5.2 attempt to create a relationship between the RR gap of carrots (the control and cut groups) and damage density.

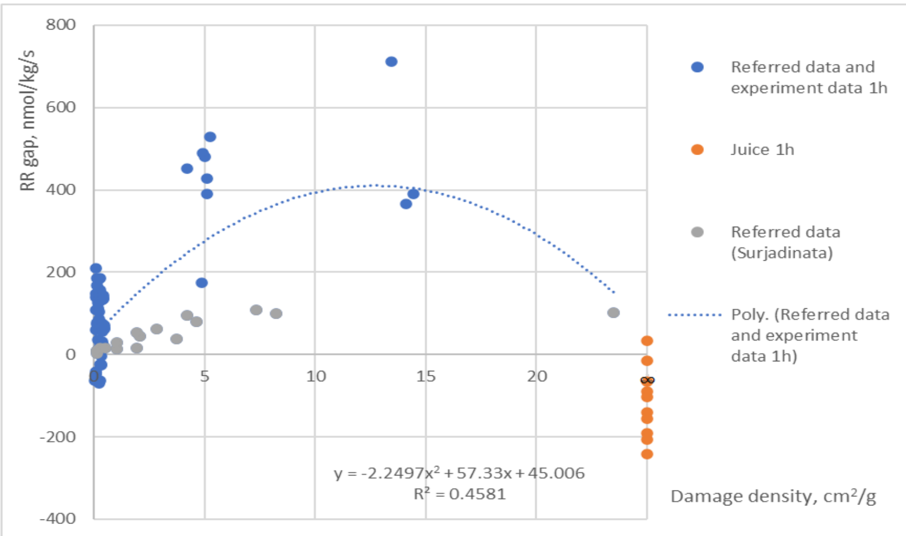


Figure 5.1 The relationship between damage density on carrot and 1h RR gap (Definition of RR gap is the gap between the measurement of the control group and 1h measurement on the cut group), the part of referred data for fitted line derives from work of Surjadinata (Surjadinata & Cisneros-Zevallos, 2003).

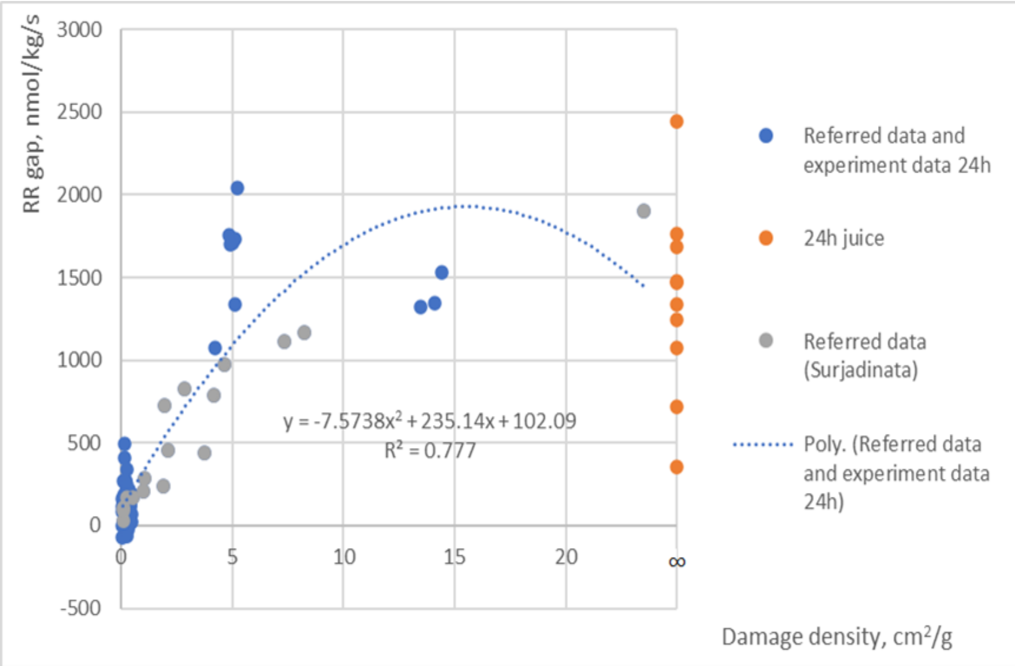


Figure 5.2 The relationship between damage density on carrot and 24h RR gap (Definition of RR gap is the gap between the measurement of the control group and 24h measurement on the cut group), the part of referred data for fitted line derives from work of Surjadinata (Surjadinata & Cisneros-Zevallos, 2003).

These experiments aim to establish a relationship between RR and physical damage. Figures 5.1 and 5.2 above demonstrate the relationship between damage density and enhanced RR based on experiment and reference data. The equations represent the relationship between damage density (x) and enhanced RR (y) after a 1h and 24h cut.

5.2. Methods

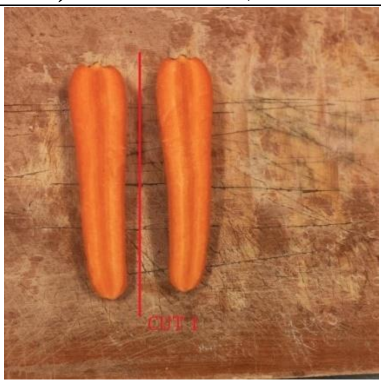
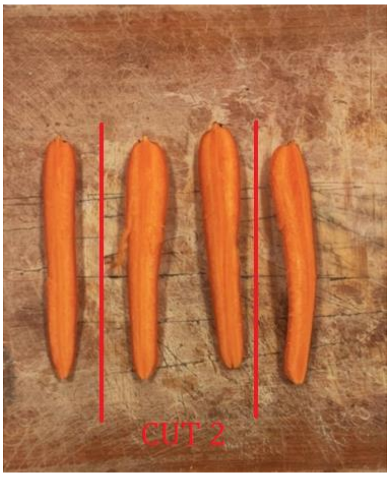
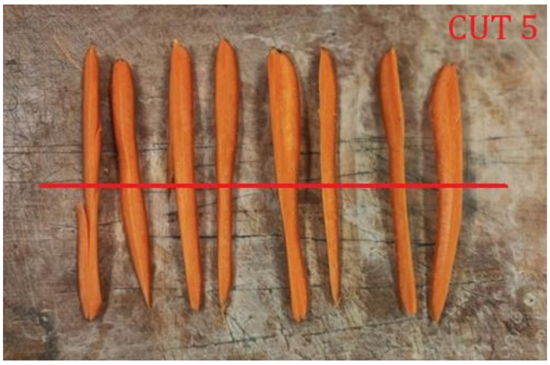
Due to the (time) effort required to conduct respiratory measurement, this work was done as a series of 5 experiments. The five experiments each aimed to analyse the physical damage effect on RR. In the first and second experiments (5.2.1, 5.2.2), carrot damage was achieved by manual cutting. In the third experiment (5.2.3), carrot shredding was investigated. The fourth experiment (5.2.4) returned to manual cuts (with a small additional of surface area increase). Carrots were manually cut into variable sections along the centre axis over the whole experiment time of 5 days, and the additional surface area caused by cuts was measured manually also. The fifth experiment (5.2.5) was designed to explore the RR under the extreme additional surface area, so carrots were juiced, and the additional surface area was recognised as infinite.

The RR measurement for the five experiments is different. The first experiment required sampling twice daily over the five days of the whole experiment period, and the time gap between two samples in one day was 5 hours. For the rest of the experiments, the sampling time point is similar; once the carrots were processed, the RR was assessed only 1 hour and 24 hours after the carrots were cut.

5.2.1. Experiment 1 (The RR difference between intact and cut carrots)

Exp 1 was the initial experiment exploring the influence of physical damage on carrot RR response. Ten (10) intact carrots were used as a control, while another 10 were cut. The cut carrots were cut into 16 sections (Table 5.1) by dividing into 8 sections with 4 cuts parallel to the centre axis, and then the 8 sections were divided into 16 parts with a 5th cut that divides these pieces into two even sticks. After cutting, the carrot's respiration rate was measured for 5 days. During this time, carrot weight loss was managed using plastic bags with punch holes, as was described in Exp 2 of Chapter 4.

Table 5.1 The cut methodology of carrot on day 1

1) The first cut, 2 sections	2) The second cut, 4 sections
	
3) The third cut, 8 sections	4) The fourth cut, 16 sections
	

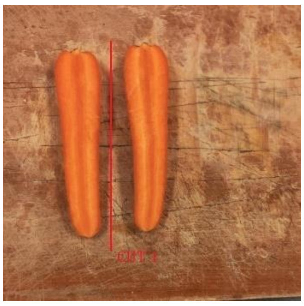
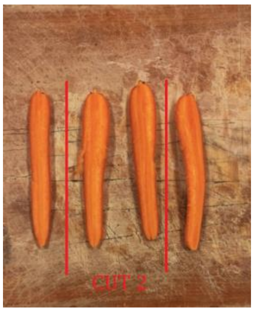
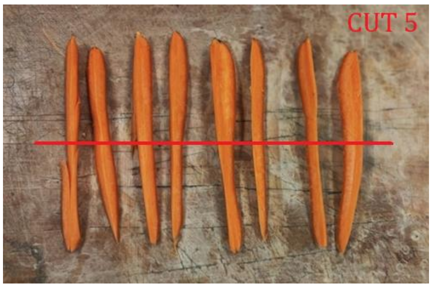
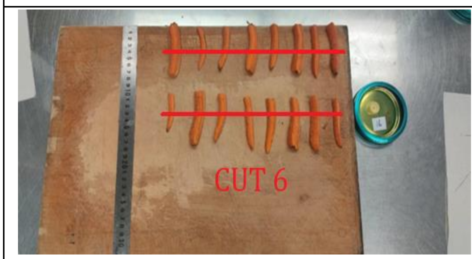
After the cutting treatment, RR measurement was conducted. Subsequent respiratory rate measurement frequency was twice per day over 5 days. An incubation time of 30 minutes was always used, while the time gap between two samples on the same day was 5 h. Between respiration rate measurements, carrots were stored in plastic bags at 20°C.

5.2.2. Experiment 2 (The RR change of carrot under dynamic damage)

According to the result of Experiment 1 (5.2.1), the RR of carrots rose significantly after cutting. An experiment was consequently established to study the RR change under various damage severities. The experiment was again run over 5 days, with the weight loss control remaining the same as Experiment 1.

Six (6) bags of carrots were sourced from a local supermarket. Fifty (50) carrots were chosen, consisting of 10 carrots as a control group (A) and 4 treatments of 10 carrots (a, b, c, f), with the treatments being different cut levels, as Table 5.2 illustrates. Halves, quarters and eights were created with cuts through the centre axis (following Exp 1) and 16th were made by halving the eights. An additional cut was used to develop 32nds using an additional cut through the middle of the sixteenth sections.

Table 5.2 The cutting method was used for four days during the 5-day experiment

(a) The first day (1 cut, 2 sections)	(b) The second day (2 cuts, four sections)
	
(c) The third day (4 cuts, 8 sections)	(d) The fourth day
	
(e) The fourth day (6 cuts)	(f) The fourth day (result, 32 sections)
	

The experiment was conducted over five days because there were two measurements on each treatment of 10 carrots per day, 1h and 24h after the cut. The cutting methodology of the first three days is a), b), and c) in Table 5.2, respectively, while the fourth day is (f). The experiment results are three RR measurements that occurred at different periods of the experiment. The first measurement happened before each measurement on intact carrots, and the other two measurements occurred at the 1h and 24h time points. During the experiment, the lab temperature remained stable at approximately 20 ℃. Thirty (30) minutes was used as a constant incubation period.

5.2.3. Experiment 3 (The influence of high-level additional surface area (shredding) on RR)

The results of experiments 1 and 2 implied that the RR of carrots will rise to a peak and then decrease later. Furthermore, the cut methodology on the carrots will affect the increasing rate of RR, so this experiment was conducted to analyse the RR change after the carrots got shredded.

The experiment is made of two parts: 1) Carrot shredding and 2) RR measurement. The RR measurements followed the previous design that divided the 20 chosen carrots as experimental materials into two groups; the control group and the shredding group, and 30 minutes was the incubation period for RR measurement.

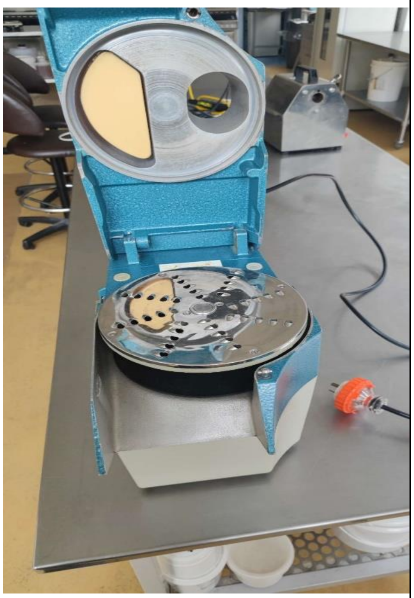
Carrot shredding was conducted by a rotating Grater-Shredding machine, which could shred the whole carrot into slices with similar shapes/damage density. The machine was introduced because the surface area calculation of shredded carrots is complex and cannot be achieved by conventional methods. In contrast, the machine can produce slices with consistent shapes quickly. The parameters like the weight of a single carrot slice (W_1), the weight for surface area estimation (W_2), the weight of an intact carrot (W_3), and the surface area of a single carrot piece (S_1) were recorded during the experiment period. The following equation calculates the estimated additional surface area (S) of each carrot:

$$S = S_1 \times (W_2/W_1) \quad (5.1)$$

W_2 divided by W_1 can estimate the number of slices produced by the machine, and S_1 is calculated based on the average surface area value of 100 carrot slices.

The operation had three measurements, e.g., the data came from intact carrots, 1 h after shredding, and 24 h after shredding. The surface area determination was conducted after the measurement was finished, and to prevent weight loss, the same management methodology as Experiment 2 of Chapter 4 was introduced. The graphs of the Grater and its driving machine are listed in Table 5.3 below.

Table 5.3 The graphs of the shredding machine

a) The Grater module and driving machine (The outer diameter of the Grater is 7mm, and the inner diameter is 5mm)	b) Assembled machine
	

The geometric shape of single carrot slices after shredding is made of two shapes: 1) uneven triangle pyramid and 2) flat pieces. The triangle pyramid slices have five faces, but the front and back faces of the original peels of carrots. So, a shredding machine produced the three faces in the middle part of the slices, and flat, ordinary slices have one/two faces that can be seen as flat squares and the geometry shape of the two sorts of slices are shown in Figure 5.3.

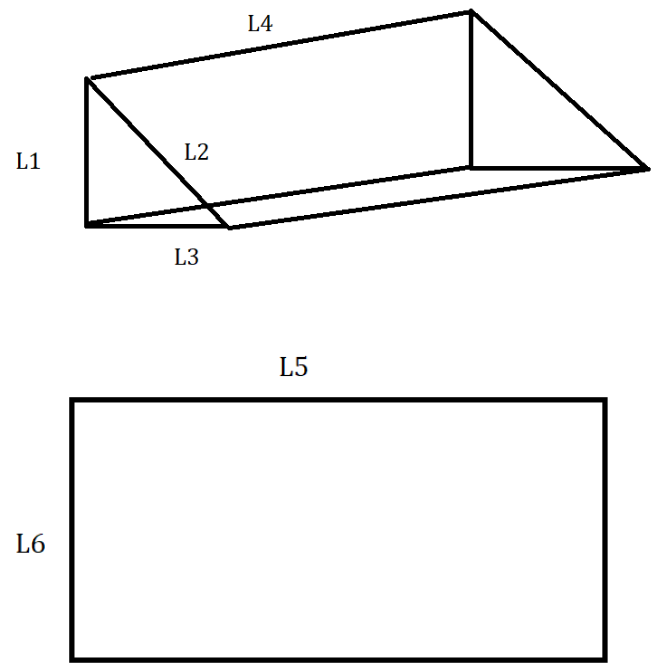


Figure 5.3 The geometric shape of slices with uneven triangle pyramid and flat square

Equations (5.2) and (5.3) are for additional surface area calculation of uneven triangle pyramids (S_2) and flat slices (S_3)

$$S_2 = (L_1 + L_2 + L_3) \times L_4 \quad (5.2)$$

$$S_3 = L_5 \times L_6 \times 2 \quad (5.3)$$

For the surface area determination, the length (L_4) and length of three edges (L_1, L_2, L_3) of triangle pyramid slices was collected to calculate the enhanced surface area of three faces. Additionally, the size (L_5) and width (L_6) of two face slices were determined. The 10 groups of shredded carrot slices were sealed within plastic bags with punch holes and labelled as Group 11 to Group 20

Figure 5.4 Stored carrot slices between two measurements

The assumption for the surface area estimation of the experiment is that the machine can produce slices with similar geometric shapes of carrots. In experiment operation, the slice shape of some groups was variable in comparison to other groups, in particular with respect to length. To assist with the estimate of the shredded carrot surface area of slices, slices were chosen from multiple carrots to assist with estimation accuracy.

5.2.4. Experiment 4 (The influence of low additional surface area (manual cuts) on the RR)

The introduction of shredding operation in Experiment 3 (5.2.3) explored the influence of high-level enhanced surface area that was caused by physical damage. From the data of Experiment 3, the RR fluctuates with the increased surface area of the carrots. As a result, an experiment was designed to determine the effect of manual cuts (low-level surface area) on the RR of carrot. The experiment lasted five days, and 50 carrots were chosen. Ten (10) carrots were treated as a controlled group (intact), and 4 groups of 10 carrots as four cut groups. The RR was measured in the control group and cut groups with different cut levels on each day. The 30 minutes incubation period was again used.

The measurement frequency was the same as in previous experiments, with measurement occurring at 1 h and 24 h after cutting.

The cutting methodology of the experiment was cutting one cut group (10 carrots) on each of the four days. There were four different cuts during the whole experiment, with the specific cutting method used on each day shown in Table 5.4.

The first cut was orientated to the centre axis and 1 cm away from the head. The geometric shape of a carrot can be deemed as a cone, but the shape of carrot slices is close to round or oval due to the uneven natural shape of carrots, and the geometric shapes of carrot cutting sections are shown in Figure 5.5.

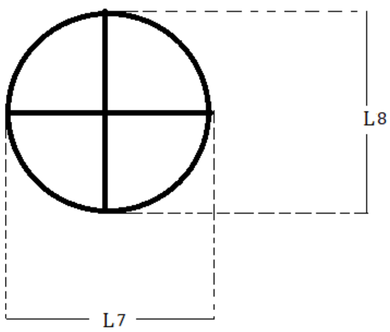


Figure 5.5 The geometric shape of cutting sections

Therefore, the additional surface area (S_4) can be calculated based on the following equations, which depend on the values of L_7 and L_8 (5.4, 5.5).

If $L_7 \approx L_8$, the cutting section is close to a round

$$\text{The additional surface area } (S_4) = \pi \left(\frac{L_7}{2}\right)^2 \quad (5.4)$$

If $L_7 \neq L_8$, the cutting section is close to an oval

$$\text{The additional surface area } (S_4) = \pi \left(\frac{L_7}{2}\right)\left(\frac{L_8}{2}\right) \quad (5.5)$$

Table 5.4 The specific cutting methodology on each day

a) The first day (The cut 1 cm away from the head, 2 sections)	b) The second day (The first cut on the 1 cm place, and the second cut on the 2 cm place, 3 sections)
	
c) The third day (The first cut on the 1 cm place, the second cut on the 2 cm place, and the third cut on the 3 cm place, 4 sections)	d) The fifth day (The first cut on the 1 cm place, the second cut on the 2 cm place, the third cut on the 3 cm place, and the fourth cut on the 4 cm place, 5 sections)
	

5.2.5. Experiment 5 (The influence of infinity additional surface area (juice) on RR, and RR determination and comparison with pomace)

Experiment 3 (5.2.3), and 4 (5.2.4) were designed to explore the RR change of carrots under high additional surface area (from 75 cm^2 to 701 cm^2) and low range (below 50 cm^2). To build the relationship between physical damage and RR, the experiment was established to explore the influence of extreme additional surface area on carrot respiration rate. Juicing was introduced to create an infinite additional surface area of the carrot in order to analyse the impact on respiration rate. In order to juice operations like crushing and yielding were required. The 20 carrots were used as experiment materials, and equipment such as a blender was introduced to crush the carrots into carrot mash (pomace with juice), and small plastic bags (130 × 200mm – 40 Micron) with three holes were used to separate the juice and pomace. Due to the application of the blender, the intact carrots were crushed into irregular particles and debris (mash). Then, carrot mash was yielded to derive juice and pomace, so the additional surface area value is difficult to determine. It was deemed that the extra surface area of carrot pomace was more than the shredded slices but less than the juice. Once the pomace and carrot juice were obtained, the weight of the juice and pomace were determined, respectively. However, equipment loss must be considered because not 100% of wet mash could be transferred from a blender container to the jars, and juice also needed to be transferred between two containers. Besides, the RR of both needed to be measured at the 1 h and 24 h time points as in previous experiments.

For the RR measurements, the pomace and juice were placed into 20 jars, respectively, and the CO_2 concentration was measured. As always, 30 minutes was used as the incubation period. Moreover, it was observed that the colour of fresh carrot juice turned from light orange to black after 23 h, which was probably caused by oxidation.

Figure 5.6 The blender and its driver

5.3. Results

5.3.1. Experiment 1

The Exp 1 was established to determine the effect of physical damage on the RR of carrots. Due to the RR diversity of individual carrots, data is reported as average RR. Moreover, the temperature is a significant factor affecting the RR of carrots. Unfortunately, during the experiment a temperature fluctuation occurred during the third measurement due to dysfunction of the room temperature control. To fix the error of temperature fluctuation, it was assumed the temperature and RR have a linear relationship, and based on the assumption, a temperature factor was introduced to modify RR to correct for the temperature differences.

The calculated RR, temperature, and their changes are listed in Figure 5.7 and Tables 5.5 and 5.6.

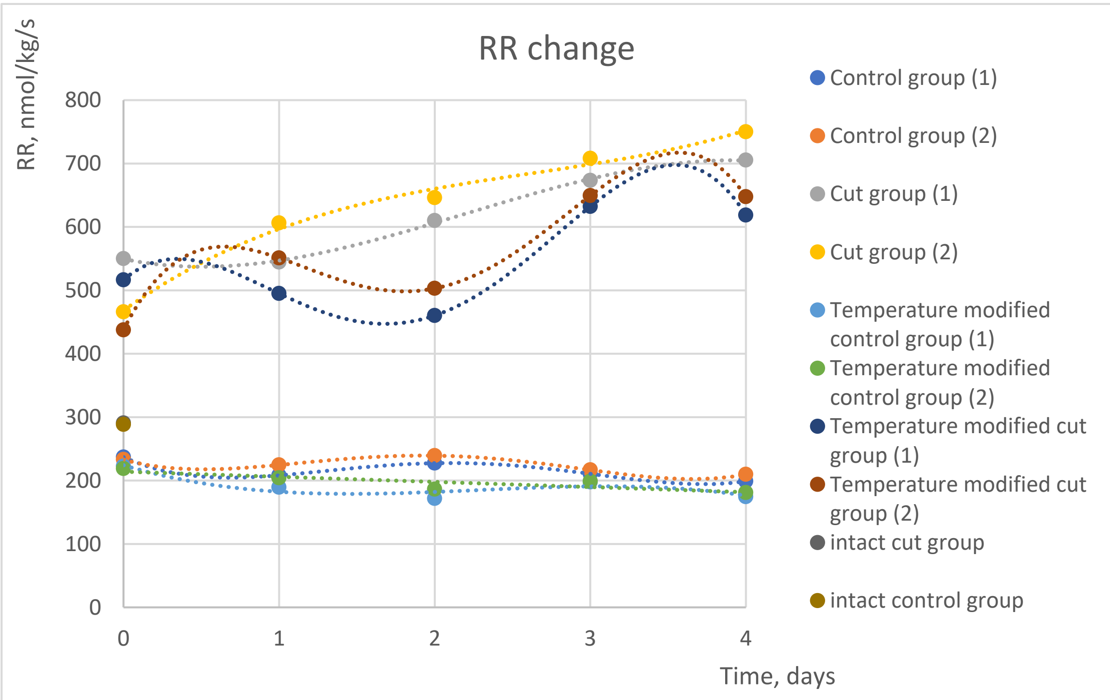


Figure 5.7 Average RR change (nmol/kg/s) over time (days) for different experimental groups, including control, cut, temperature-modified, and intact groups. (The temperature-modified group represents the estimated values calculated after accounting for laboratory temperature fluctuation errors, including control and cut group)

Table 5.5 The temperature change of the laboratory at ten sampling points for laboratory temperature fluctuation errors accounting

	First sampling	Second sampling
First measurement	21.3 ℃	21.3 ℃
Second measurement	22 ℃	22 ℃
Third measurement	26.5 ℃	25 ℃
Fourth measurement	21.3 ℃	22.3 ℃
Fifth measurement	22.8 ℃	23.5 ℃

Table 5.6 The original average RR values of two groups and temperature-modified

	RR (nmol/kg/s)				
Original control group	233.15	224.60	239.29	216.73	209.78
Modified	218.92	204.18	186.28	198.81	181.14
Original cut group	465.96	606.20	646.04	708.16	750.05
Modified	437.52	551.09	503.02	649.30	647.55

The 20 RR values were calculated based on the CO_2 concentration change, and the T-test was introduced to determine the significance of the difference between the RR values of the control and cut groups (Kim, 2015). The T-test results of ten groups that compare controlled and cut groups are contained in Table 5.7.

Table 5.7 The p-value results of T-test

Group	1	2	3	4	5
P value (two-tail)	1.56E-07	1.04E-05	8.01E-07	1.53E-07	1.86E-09
Group	6	7	8	9	10
P value (two-tail)	3.50E-08	2.47E-10	5.18E-11	2.43E-08	1.80E-07

5.3.2. Experiment 2

The carrots were measured three times in the experiment: intact, control and cut groups. The measurement of the intact group was the RR measurement after the carrots were prepared to determine the RR of all intact carrots immediately, and the measurement on the control group and cut group occurred after the cut was finished. Besides, the experiment data was from different time points, e.g. 1 h and 24 h after the cut operation.

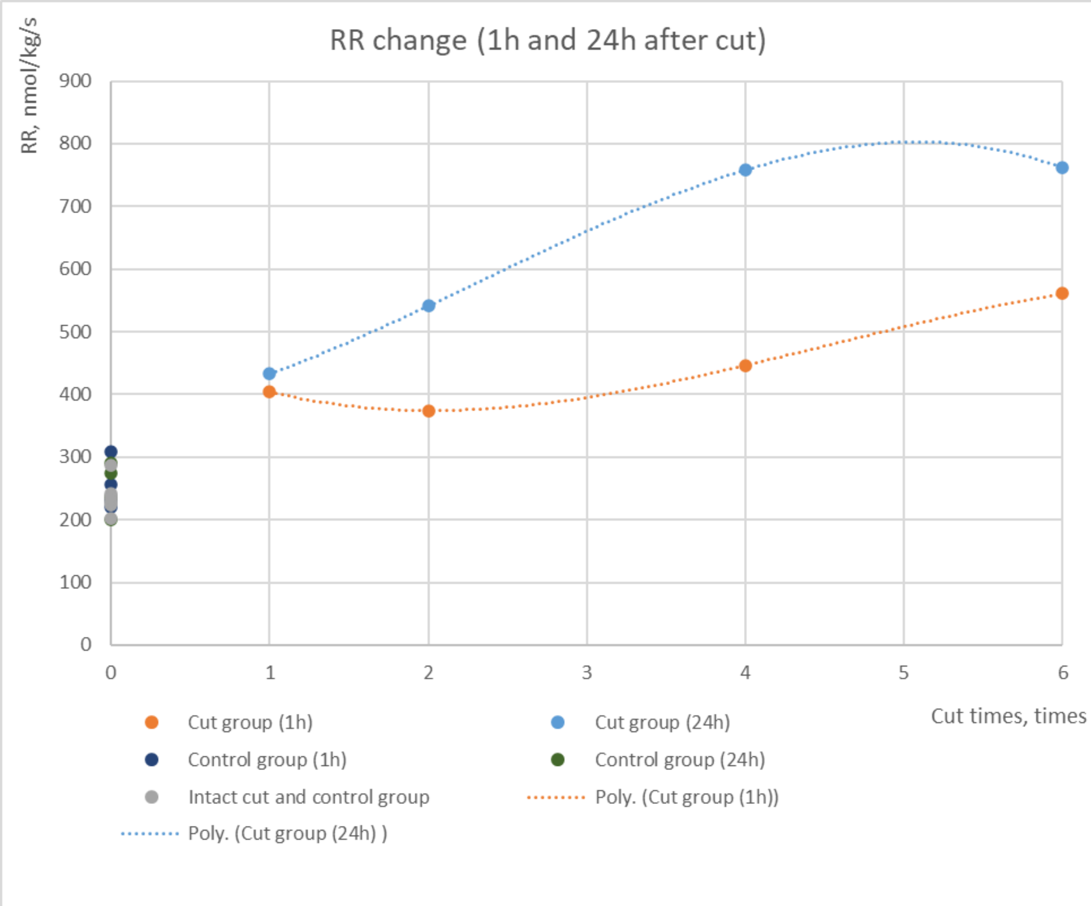


Figure 5.8 The RR change of carrot among different cut levels

Therefore, the results of Exp 5 are made by these three parts, as Figure 5.8 illustrates. The data representing the control and intact groups are on the left side of the graph, with the RR range being between 200 and 300 nmol/kg/s. The two lines, (blue and orange), represent the cut group RR measurement at 1 h and 24 h (respectively). Their trend indicates that the RR of the carrot will increase immediately after the physical damage occurs on the carrot (i.e. after 1 h). The trend shows that the RR of carrots continues to rise as the extent of physical damage on the carrots increases. The carrots with more physical damage have a higher RR. Furthermore, the blue line is always greater than the orange line. In comparison, only the RR gap of carrots with 1 cut at 1 h is not significantly different to the respiration rate at 24 h. Otherwise, the carrots tend to have higher RR after 24 h after the physical damage.

5.3.3. Experiment 3

Exp 3 aims to analyse the influence of high exposure surface area produced by shredding on carrot respiration rate (RR). The results of the experiment are made of two main parts:

5.3.3.1. Geometric parameters and weight of carrot slices

This content covers measuring and recording the geometric parameters of shredded slices, which is essential to estimate the surface area exposed due to shredding. The geometry parameters of the triangle pyramid and ordinary slices (two faces) are shown in a histogram in Figures 5.9 and 5.10.

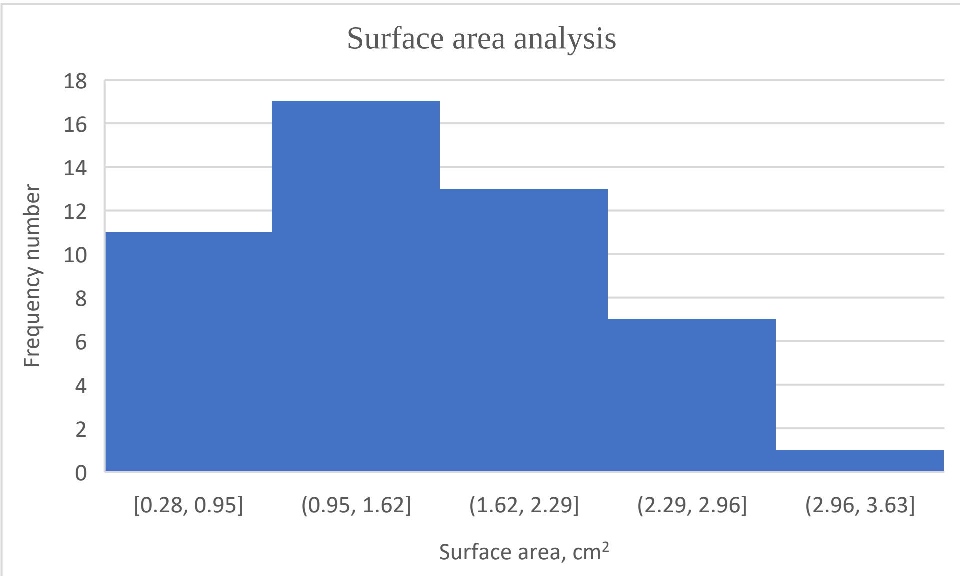


Figure 5.9 The additional surface area distribution of carrot slices of the G11 group in the shape of a triangle pyramid (Machine shredding)

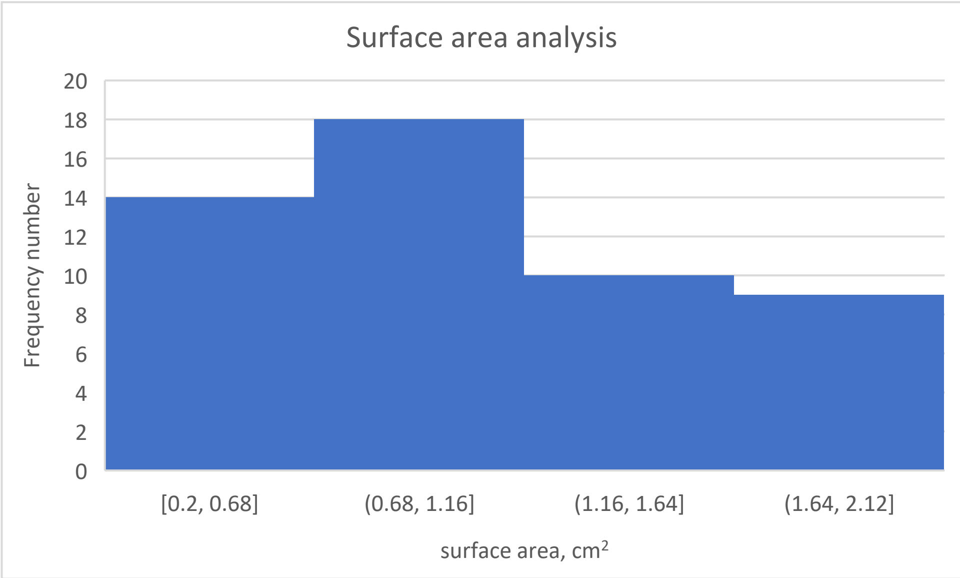


Figure 5.10 The additional surface area distribution of carrot slices of the G11 group in the shape of a square (Machine shredding)

Both histograms illustrate that most samples have relatively small to mid-range surface areas. The surface area values of triangle pyramid slices are closer to mid-range, spreading between 0.95 and 1.62 cm^2, while large surface areas are rare. The additional surface area distribution of square slices is balanced, and the value of additional surface area peaks at 0.68 to 1.16 cm^2.

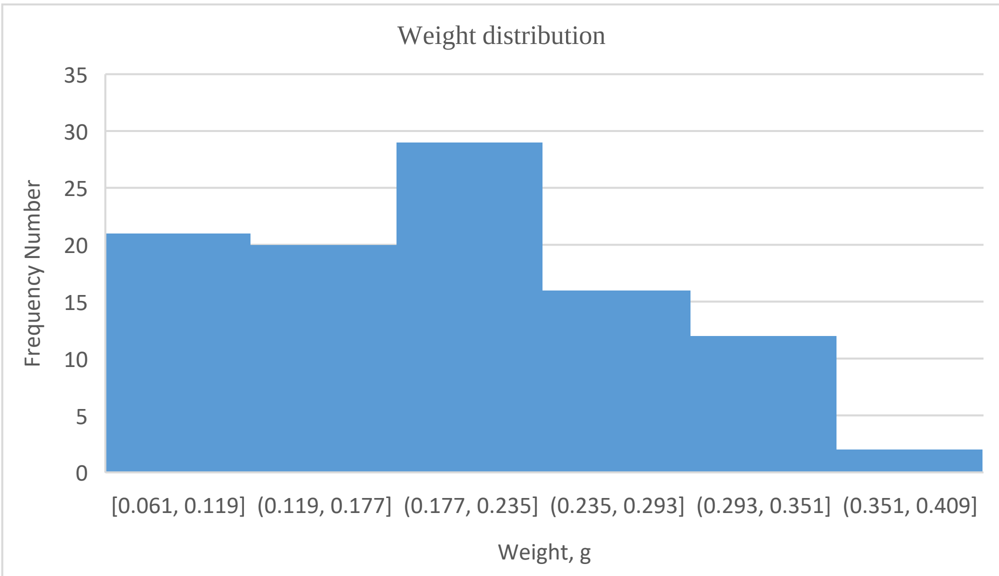


Figure 5.11 The weight distribution of 100 slices of Group 11

The weight distribution in Figure 5.11 indicates that the weight distribution of slices is balanced and close to normal, but the weight distribution slightly skews to the low range.

Due to the distribution analysis above, the averages of the measured values can reflect the data well. The total surface area of 100 slices is 128.893 cm^2, and the average value (S_1) is 1.29 cm^2. The average weight (W_1-1) of these slices is 0.199 g.

Figures 5.12, 5.13, and 5.14 demonstrate the surface area and weight distribution of carrot slices produced from other carrots.

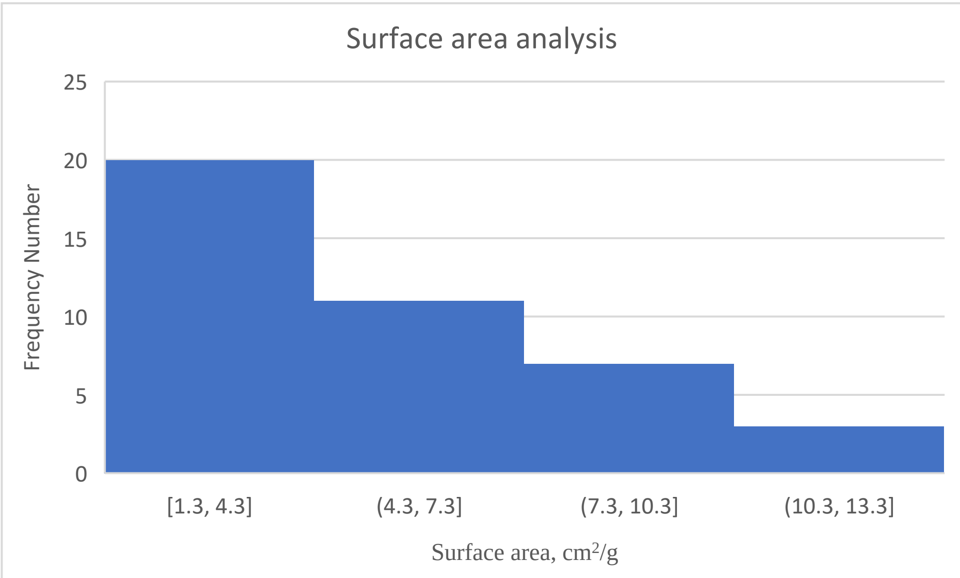


Figure 5.12 The additional surface area distribution of carrot slices of the G14 group in the shape of a triangle pyramid (Machine shredding)

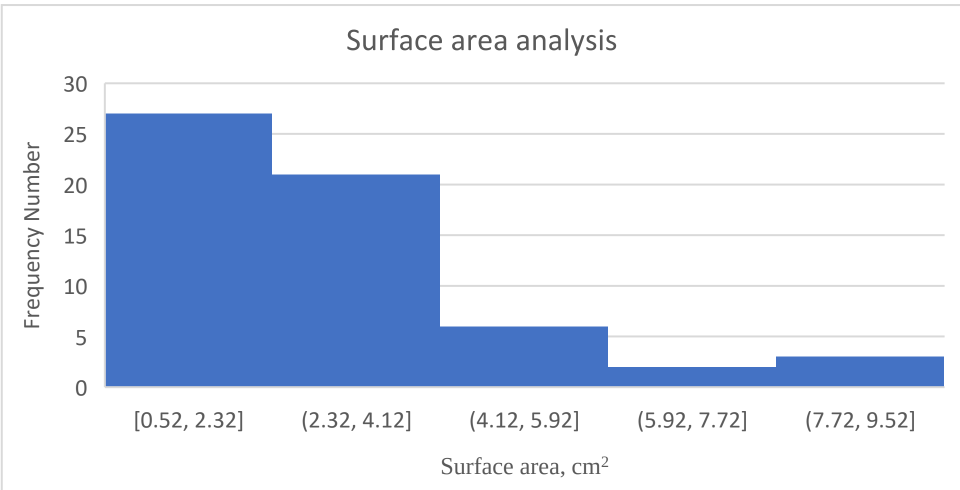


Figure 5.13 The additional surface area distribution of carrot slices of the G11 group in the shape of a square (Machine shredding)

According to the surface area analysis, both graphs skew to the low surface area range, and the number of square slices is greater than that of the triangle pyramid slices. The surface area of both slice peaks at the first bin in Figures 5.12 and 5.13.

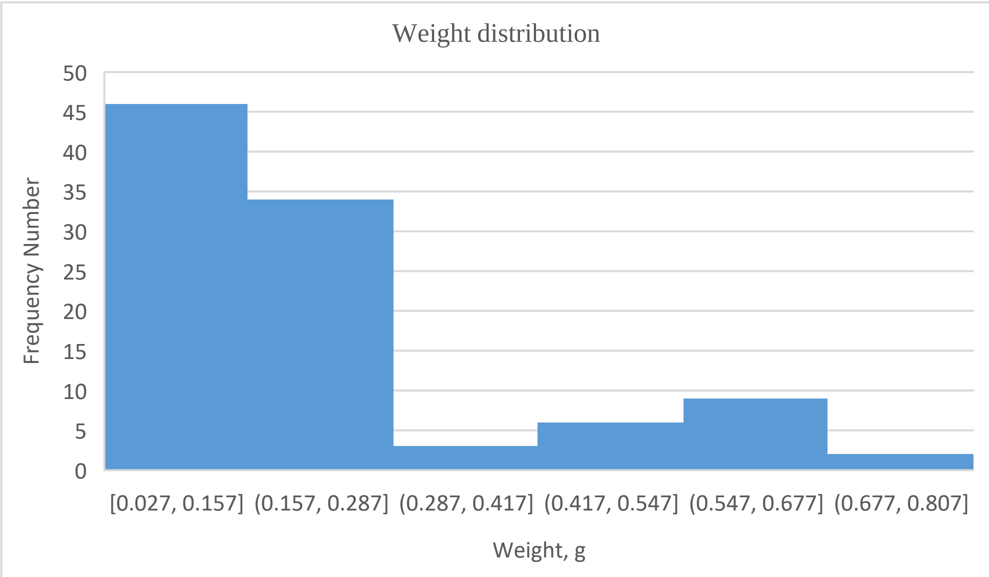


Figure 5.14 The weight distribution of 100 slices of Group 14

The weight distribution of slices in Figure 5.14 indicates that the weight of slices skews to the low range as well, and the large weight is rare.

The natural shape of the carrot probably causes a deviation in the surface area and weight. The carrot shape for some groups was more irregular, and hence the carrot could not be placed into the shredding machine directly, which led to longer carrot slices and more fragments (small slices with low mass and additional surface area).

The total enhanced surface area of 100 slices is 384.788 cm^2, and the average (S_1) is around 3.85 cm^2. The average weight (W_1-2) of these slices is 0.228g.

5.3.3.2. Surface area and damage density calculation and RR change analysis

The surface area results (S) of 10 shredding carrots were calculated via equation (5.1) and listed in Table 5.8 based on the surface area results of 200 slices. The damage density of carrots was determined by equation (5.6), and the RR change analysis based on damage density and RR gap indicates how shredding impacts the respiration rate of carrots.

$$\text{Damage Density} = \frac{S}{W_3} \quad (5.6)$$

Table 5.8 Surface area results of 10 shredded slices group

Weight (W_2), g	average weight (W_1-1/W_1-2), g	average surface area (S_1), cm^2	surface area (S), cm^2	Carrot weight (W_3), g	Damage density, cm^2/g
44.671	0.199	1.29	289.5758	68.862	4.21
22.324	0.199	1.29	144.7134	28.838	5.02
24.301	0.199	1.29	157.5291	30.786	5.12
22.173	0.228	3.85	374.4125	27.81	13.46
33.666	0.199	1.29	218.2369	44.748	4.88
38.603	0.228	3.85	651.8489	45.122	14.45
41.569	0.228	3.85	701.9327	49.746	14.11
18.374	0.199	1.29	119.1078	23.287	5.11
11.577	0.199	1.29	75.04688	15.316	4.90
21.564	0.199	1.29	139.7867	26.685	5.24

The RR as a function of surface area is shown in Figure 5.15. The RR of intact carrots (control) remained in a wide range, from 200 to 500 nmol/kg/s. The respiration measurement was at 1 h and 24 h after shredding. All RR values of 24 h were higher than the 1 h RR data, the same result as that observed in Experiment 2 (5.2.2). The RR values of the 1 h group fluctuate between 300 and 1000 nmol/kg/s. Most of the RR of the 1 h group is higher than that of the control group, and only two data points fall within the RR range of intact carrots. The RR increases after 24 h are much more apparent.

The two surface area values corresponding to the maximum RR of 1 h and 24 h groups are less than 400 cm^2. Overall, the RR of 1h fluctuates with the extra surface area changing, and the RR of 24 h illustrates a clearer trend, so the analysis of the relationship between RR change and additional surface area per gram (physical damage density) was introduced.

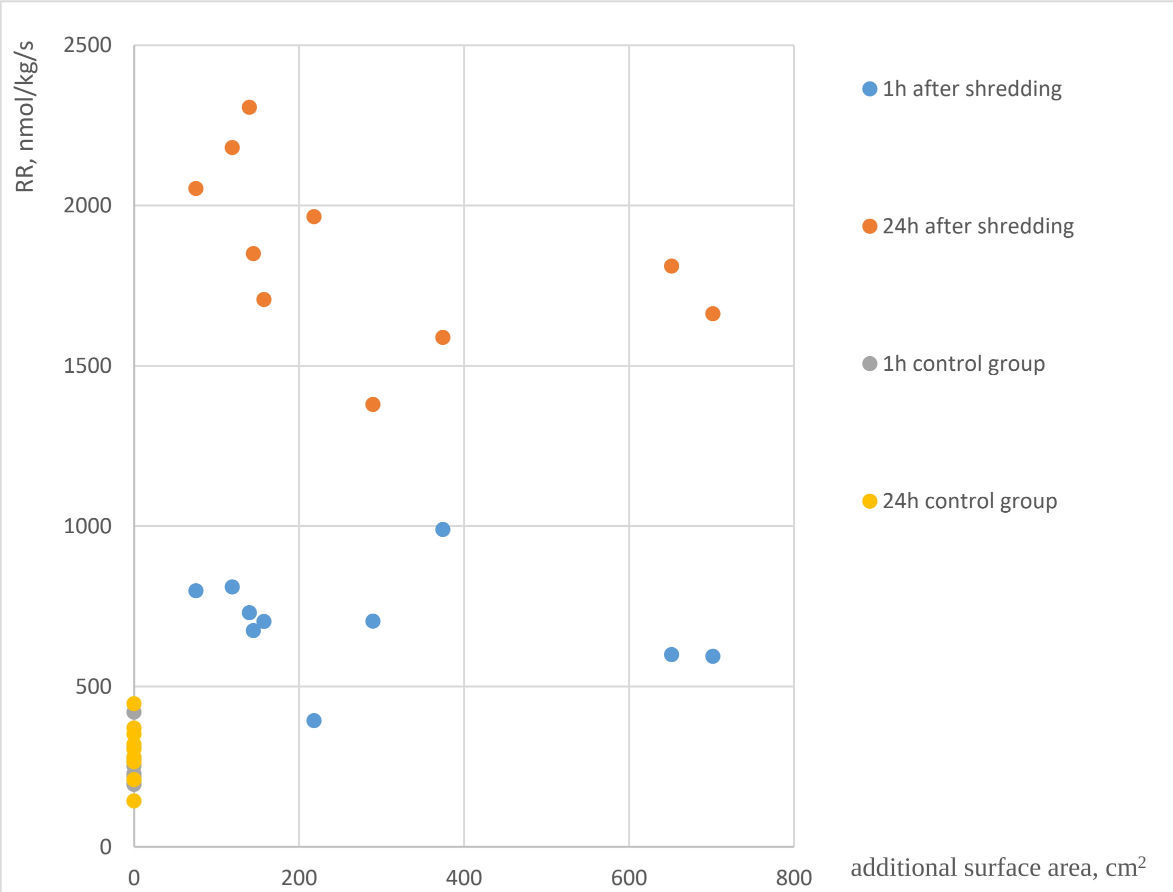


Figure 5.15 The RR change versus the additional surface area of shredding carrots

The damage density is used to reflect the physical damage on the carrot. The RR gap is the gap between the control group and the shredding group. The gap is enhanced by increasing damage density, but it is reduced when the damage density is at an extreme level of around 14 cm^2/g, as shown in Figure 5.16. Such a change can be observed in both time groups.

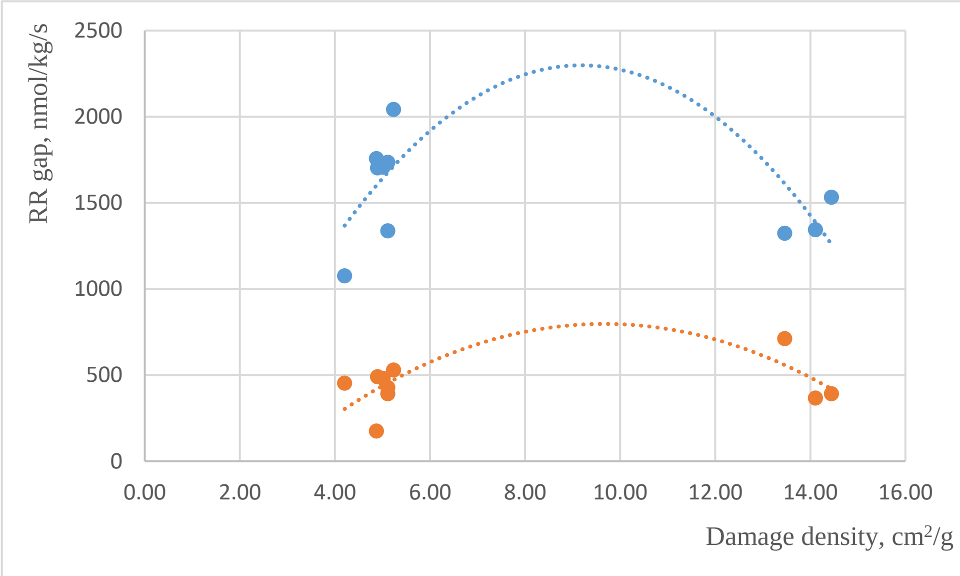


Figure 5.16 The RR gap of individual carrots versus damage density

5.3.4. Experiment 4

The experiment results are based on the surface area record and the RR change of two-time point groups based on the surface area value.

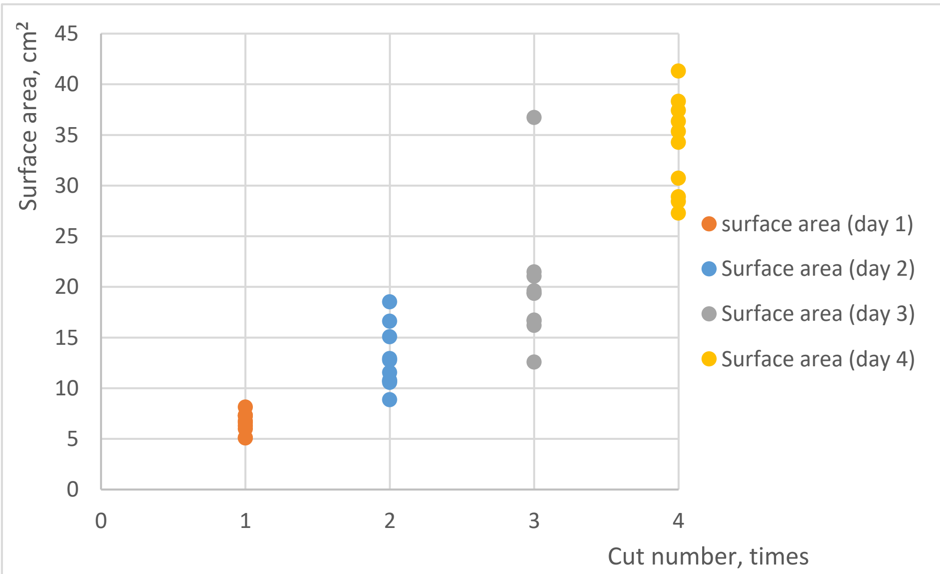


Figure 5.17 The surface area change of cut interface versus cut number on carrot

The surface area grows steadily with more cuts on the carrots, while the deviation level of the enhanced surface area also increases at 2 and 3 cuts and shrinks at 4 cuts.

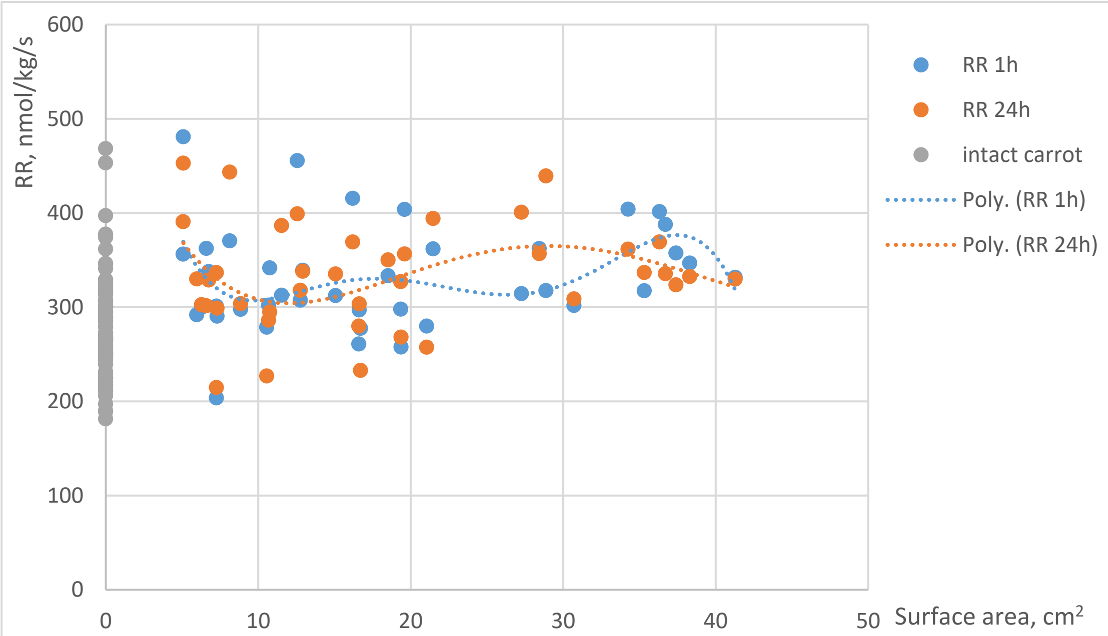
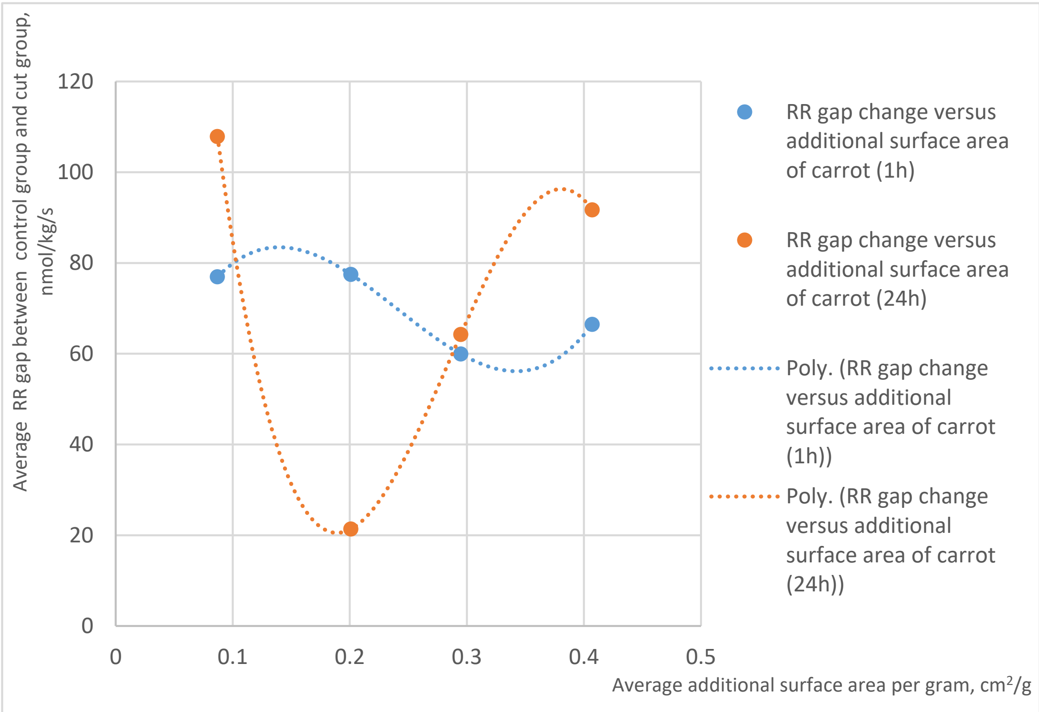


Figure 5.18 The RR change of the two-time group versus surface area

The carrot is cut manually, so the enhanced surface area is between 0 and 50 cm^2. Figure 5.18 illustrates that the RR of carrots with limited physical damage fluctuates wildly. Usually, the RR after 24 h would be expected to be greater than the 1 h group. However, in this set of data, the RR of the two groups fluctuate with additional surface area changes, and there is no trend evident. Furthermore, the RR of intact carrots also occupies a wide range from 100 to 500 nmol/kg/s, so the analysis of the RR gap between the cut group and intact group versus additional surface area per gram (physical damage density) of carrot was introduced.

The RR data in Figure 5.19 based on the gap between the control and cut groups is different from the RR versus surface area changing, which only shows the fluctuation. The two graphs of the RR gap indicate that the RR decreased when the carrot's damage density was extremely small, around 0.2 cm^2/g, while it turned to a rising trend after the carrot received more physical damage.

A)



B)

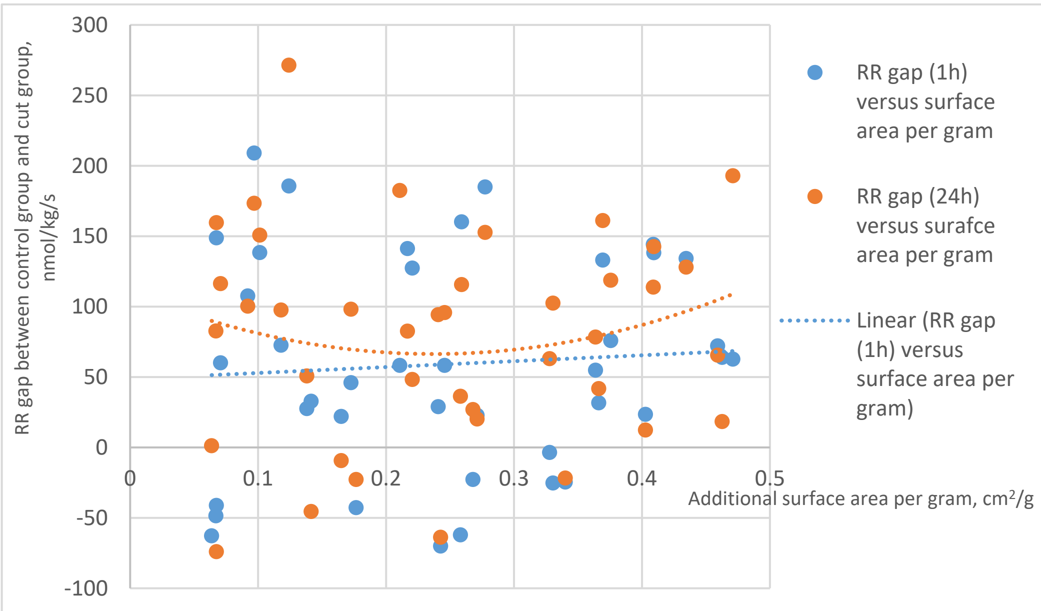


Figure 5.19 A) The average RR change versus damage density B) The individual RR change versus damage density

5.3.5. Experiment 5

The experiment's results include the yield rate from individual carrots, the RR result, and the change of juice and pomace. Table 5.9 contains the juice weight, pomace, yield, and equipment loss rate.

Table 5.9 The data of yield and its equipment loss

Juice	Intact	Pomace	Yield	Equipment loss
25.973	110.54	74.168	23.50%	9.41%
32.744	119.258	75.642	27.46%	9.12%
22.211	97.906	67.517	22.69%	8.35%
34.858	126.614	82.917	27.53%	6.98%
18.149	90.393	61.676	20.08%	11.69%
13.977	71.633	48.595	19.51%	12.65%
20.522	95.725	64.581	21.44%	11.10%
16.781	77.465	50.725	21.66%	12.86%
8.678	61.239	43.191	14.17%	15.30%
16.519	88.169	56.892	18.74%	16.74%

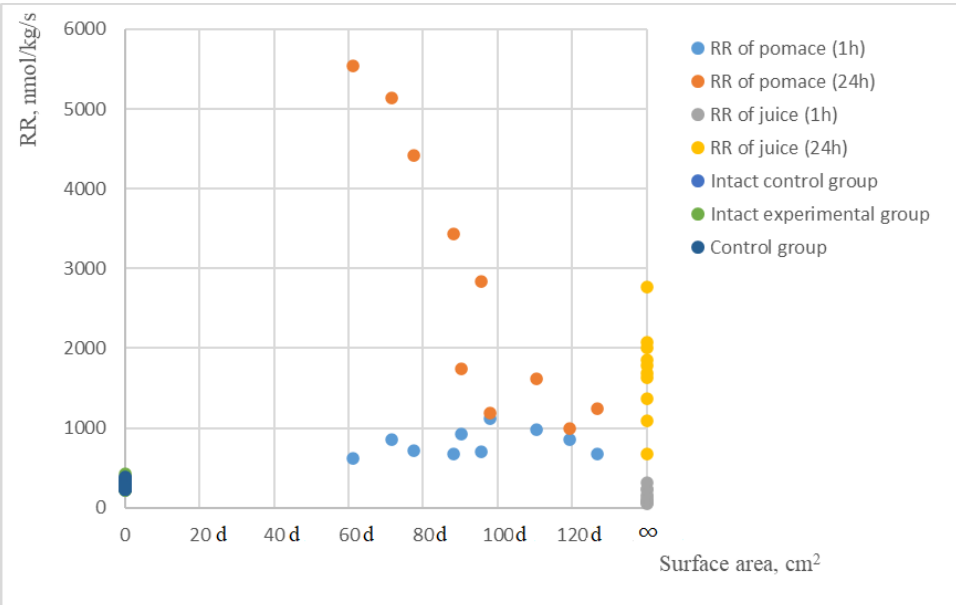


Figure 5.20 The RR results of juice, pomace, control group, intact ones, and their change of RR versus additional surface area (d is damage density)

When the blender finished the carrot crushing a moistened pomace was created. The pomace was made of carrot juice and irregular carrot fragments, (Figure 5.21). This outcome makes the specific values of the pomace difficult to estimate. Thus, the assumption about damage density gets introduced, which assumes the crushed carrots share similar damage density (d) due to the same crushing mode of the blender, so the surface area of pomace can be expressed as "weight × d" as Figure 5.20 illustrates. However, the specific value of the additional surface area is unknown, but it is deemed that the range is between shredding and juice, e.g., from 700 cm^2 to infinity. At the 1h measuring point, the RR of juice remains low but in a small range and is close to the RR range of the control and intact groups (regular group). The RR of pomace at 1 h is higher than that of the regular group carrots, but the extent is not great, and the RR of pomace fluctuates without a clear trend. After the 23 h period, both the RR of the pomace and juice groups rise significantly, which stays at a higher level than the regular group. Besides, the RR of pomace stays higher than that of juice but illustrates a falling trend with additional surface area enhancement, returning to the juice range when the additional rises to a certain level (between 80/d to 100/d cm^2).

A colour change of carrot juice was observed, (Figure 5.22). The jar stored carrot juice with a cap tends to be similar in colour to fresh carrot juice, but the carrot juice within the opening jar turns to a darker colour, nearly black.

Figure 5.21 The crushed carrot within the blender container

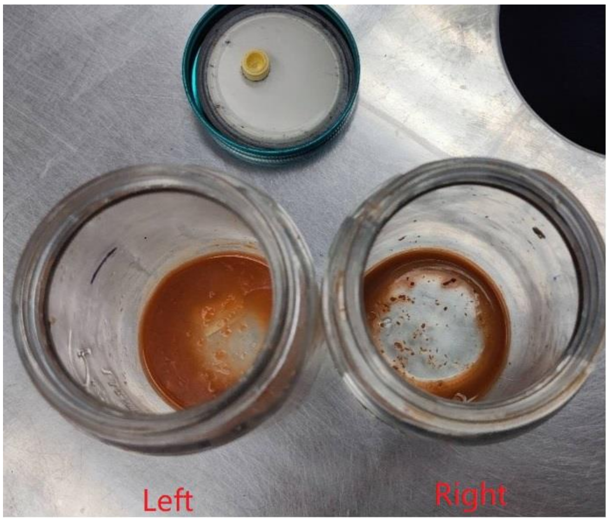


Figure 5.22 The left jar with cap for 24h; The right jar without cap for 24h

5.4 Discussion

The five experiments were created to explore the relationship between physical damage of carrots and the consequent RR response. The first and second experiments which demonstrated the influence of damage and dynamic damage on the RR, became the basis of the following experiments. In order to quantify the physical damage and build a relationship of RR to damage, surface area and damage density estimates were used. The surface area-related experiments processed the carrots by either shredding or manual cutting to create variable damage classes on the carrots.

Initially, experiment 1 aimed to determine the effect of physical damage on the RR of carrots. RRs were determined by CO_2 concentration change of controlled and cut groups during a 5 days experiment. The resulting p-values of the T-tests, (Table 5.7), were all less than 0.05 indicating significant differences, and means that causing damage to carrots results in an increased RR.

Figure 5.7 illustrates that the intact carrots have similar RR fluctuating around 200 nmol/kg/s. By contrast, RR of the cut group indicates a significant rising trend, after the carrots get physical damage.

In Experiment 1 (section 5.2.1), the carrots were cut into 16 sections, and both curves rose rapidly after the cut on day 1. The RR curve of the first measurement peaks on day 4 and the same trend also can be observed on the RR curve of the second measurement. In comparison with the referred data in Figure 5.24, the referred data and experiment data share similar features in the first three days of the experiment. However, the experiment RR increases and peaks on the fourth day, and then RR values decline slightly on the fifth day. The reference data was determined at 10 °C, while the RR data of the experiment is measured at 20 °C. There it is not surprising that the data collected in this experiment is higher. The reference RR data also illustrates that the RR of the cut carrots will return to a stable RR level that is close to the intact carrots in long-term storage (Surjadinata & Cisneros-Zevallos, 2003), although this was not observed in this experiment (Figure 5.24).

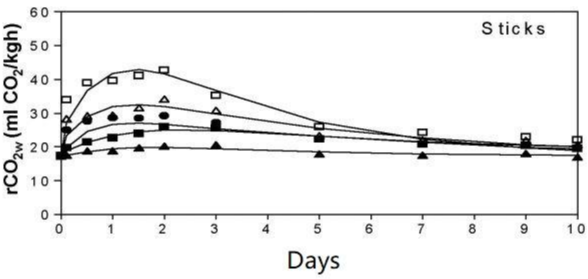


Figure 5.23 The RR change of carrot with physical damage (Surjadinata & Cisneros-Zevallos, 2003)

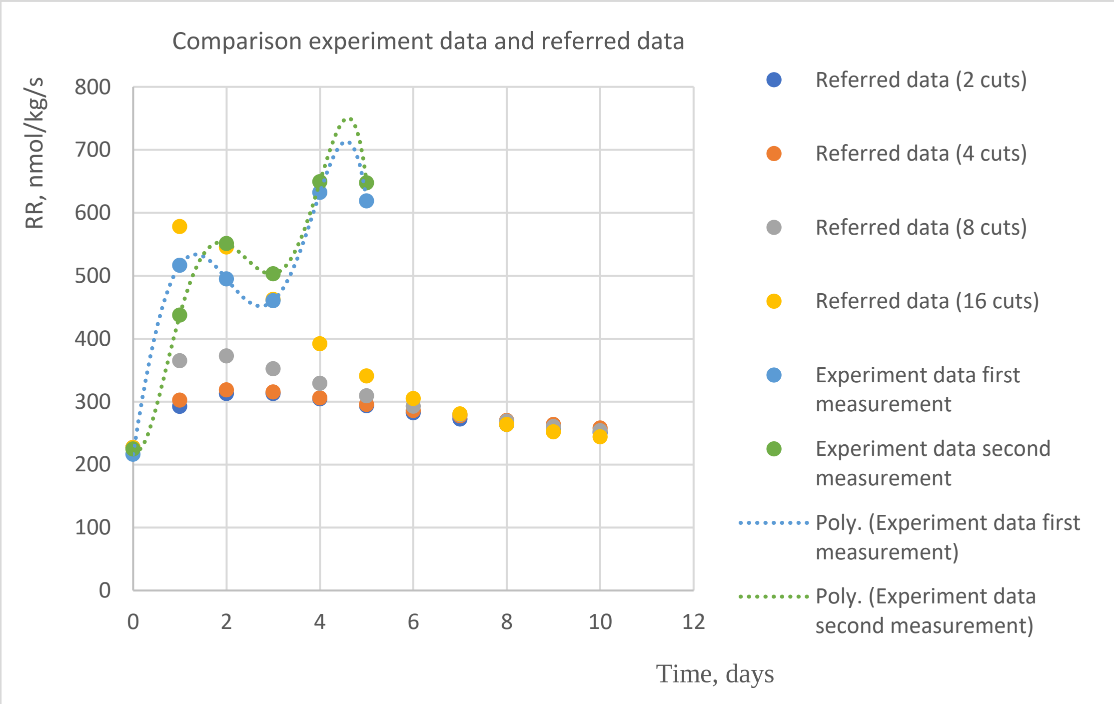


Figure 5.24 The comparison between RR data of Exp 1 and referred data

The factors that led to RR deviation across the experiment data include temperature, as the lab temperature was unexpectedly variable on occasions during the experiment. Still, the RRs of days 4 and 5 were higher than other days and that reported in the previous work. Possible reasons for this result may be: (1) carrot deterioration over the 5 days in the lab, at high temperature and (2) the damage density between the referenced data and carrots of the experiment may differ. A previous study reported that the increased RR is related to cell damage, so estimation of the damage density was introduced in the following experiments to quantify the damage level (Benoy, 1930; Papoutsis & Edelenbos, 2021).

Experiment 2 (section 5.2.2) chose cutting times on carrots as an assessment factor. The results indicate that carrots with more physical damage have higher RR, and the RR values of carrots at 24 h after damage are higher than 1h after damage. However, the difference in RR between the 1 h group and 24 h group in Figure 5.8 is not consistent. For instance, the RR gap between the 1 h group and the 24 h group before the fourth cut is enhanced with more cut times on the carrot, but such a trend is reversed at the sixth cut. For experiment 2 (section 5.2.2), the physical damage on the carrot of each measurement is different, which indicates variable cell damage level, so the RR increase extent of each cut group will be different (Papoutsis & Edelenbos, 2021). The cuts on the carrot will add to the surface area, representing additional physical damage. The following experiments, (sections 5.2.3 to 5.2.5), will quantify the damage extent, which helps establish the relationship between additional damage and RR.

The cut operation of experiment 2 (5.2.2) was completed manually, and the RR of carrots rises with more cuts on the carrots. However, manual cutting is limited and can only create a small additional surface area on a carrot. To get RR data under a higher additional surface area, the shredding process was applied in Experiment 3. According to the RR data in Figure 5.15, the respiration increased to a higher rate after carrot shredding and RR fluctuation was observed in measurement groups (1 h and 24 h measurements). The RR of both groups (1 h and 24 h measurements) peaked when the surface area was between 100 and 200 cm^2, but the peak of the 24 h group was more prominent, after this point the RR is more fluctuating as the surface area rises.

The analysis of additional surface area and RR doesn't indicate a clear trend, so the analysis of damage density and RR gap was applied. The result suggests that the RR curve tends to be a bell curve. The RR difference between the control and cut carrots remained low at low high damage densities. However, if the damage density value is > 5, but < 10 cm^2/g (mid-range), the RR difference tends to rise with more damage density.

Shredding was found to be one of the robust methodologies used to promote the RR of carrots, which was also previously observed. Shredding resulting in wounding of the cells and the exposed tissues causes damage-induced RR (Iqbal et al., 2008; Iqbal et al., 2009). However, the extreme damage density will limit the enzyme synthesis of carrots (Surjadinata & Cisneros-Zevallos, 2003)

For Experiment 4 (section 5.2.4), all of the cuts were completed manually, so the range of additional surface area was limited to 5 to 50 cm^2. The damage density of all groups was below 1 cm^2/g. The surface area gets larger with more cuts on the carrot, but a resulting change in RR is not obvious.

The analysis of RR based on the surface area doesn't show a clear trend. It finds that the RR fluctuates within a specific range close to the RR range of the controlled group. Therefore, the RR gap and damage density analysis are also included. Enzyme synthesis and decomposition can also explain the change in the RR difference. From Figure 5.25, the enzyme synthesis has a linear relationship with the damage density. In contrast, the trend of enzyme decomposition between 0 and 1 cm^2/g shows a sharp decrease, but the initial enzyme decomposition stays high. Thus, due to the high-level inactivation effect, the RR gap declines significantly when the damage density is lower than 1.2 cm^2/g. However, the synthesis of enzymes is positively related to the rising damage density, and the inactivation effect decreases as damage density becomes more significant. Hence, the RR gap illustrates a linear enhancing trend after 0.2 cm^2/g. The k_s will increase linearly with the growing exposure surface area, but the trending of k_d change is a single curve, as shown in Figure 5.25. The damage-induced RR can be determined by equation (5.7) below (Surjadinata & Cisneros-Zevallos, 2003).

$$rCO_{2w} = rCO_{2i} + rCO_{2s}^{max} (e^{-kd\,t} - e^{-ks\,t}) \quad (5.7)$$

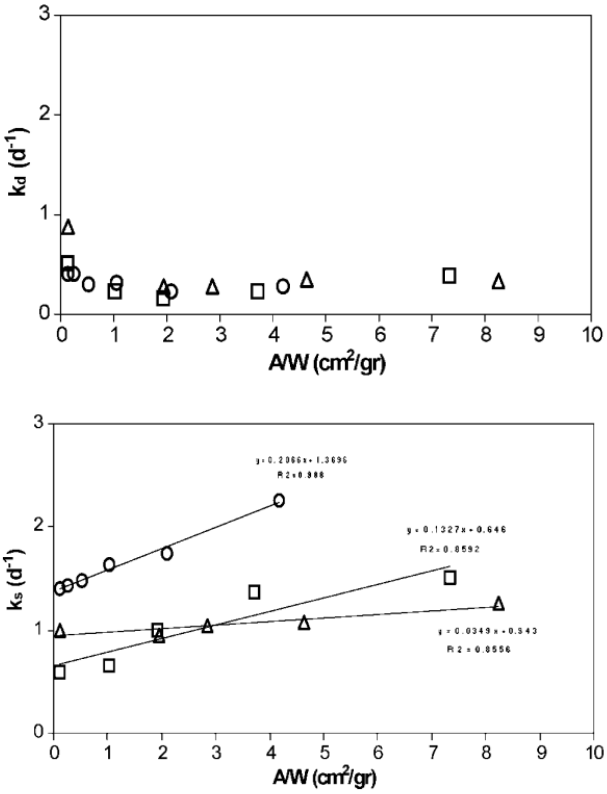


Figure 5.25 Enzyme synthesis kinetics constant (k_s) and inactivation system synthesis kinetics constant (k_d) compared with wounding intensity (A/W) for different types of cuts (Surjadinata & Cisneros-Zevallos, 2003).
○= carrot slices; □= carrot sticks; and △= combination of slices and sticks.

The Exp 5 (section 5.2.5) was established to find the RR of the carrot with an extremely exposed surface area, and the juice is recognised as the methodology to create an infinity surface area. The pomace is the byproduct of juice. Its specific surface area is hard to determine, but its range is from 700 cm^2 to infinite, and the surface area value of pomace is related to weight. The initial RR of pomace was found to be higher than that of regular carrots. Still, with an extended period passing, the RR of pomace fits a trend that the RR will decrease with more additional surface area on an experimental carrot. Besides, the RR of fresh carrot juice is close to the intact carrot and remains low. However, the RR of black carrot juice rises significantly.

The RR change of pomace and juice is attributed to the extreme surface area. Initially, the RR of carrots with high surface area is close to the intact groups, which can be observed when shredding carrots and pomace as well. The overall 24-h RR trend of shredded carrots and pomace follows a declining trend with the additional surface area increasing, which can be explained by the extreme physical damage on the carrots breaks the cell structure and restricts the enzyme synthesis that also limits carrot respiration.

The colour change of carrot juice is caused by the oxidation of pigments within the juice. Carrot is a nutritious food, so after it is converted into juice, the research reported a few nutritious compositions within carrot juice, such as carotenoids, α and β-carotene, minerals, water, and carbohydrates (Demir et al., 2007; Sharma et al., 2012). The existence of β-carotene within carrot juice is not only attributed to the nutrition but also a colouring agent, which is related to the colour stability of carrot juice, and the reference indicates the low colour quality of carrot juice is caused by low β-carotene (Sims et al., 1993).

In the experiment, the colour of carrot juice without a cap for 24 h turns dark, and the RR of the carrot rises significantly, which is probably affected by the oxidation of β-carotene and carotenoid within carrot juice (Burton et al., 2014). The molecular structure of carotene and carotenoid are made by many unsaturated double bonds, which makes the compound have antioxidation characteristics and can be a good protector from free radicals (Ozhogina & Kasaikina, 1995). Moreover, it has been reported that the formation of oxidized-beta carotene also needs to consume 1–2 moles of oxygen and release the same amount of carbon dioxide (Burton et al., 2014; Elefson et al., 2023). Thus, the carotenoid autoxidation and β-carotene oxidation of carrot juice within the jar will reduce the O_2 level but enhance the CO_2 concentration, which shares the same effect as respiration and hence has the potential to affect RR measurement by CO_2 production.

For the colour change aspect of carrot juice, fresh juice is dark orange and turns dark black after a prolonged storage period due to the appearance of these oxygen copolymers and cleavage compounds. The compounds with dark colours tend to have high levels of conjugation and polymerisation, which can offer good light-absorbing properties to the compounds (Li et al., 2021). Most of the products share a similar conjugation structure, which is made of a long alternating single bond and a double bond, so it seems most colours of canthaxanthin oxidation products may be dark. Furthermore, the oxidation profile is similar to the β-carotene (Esatbeyoglu & Rimbach, 2017). Moreover, previous study reported that when the β-carotene is oxidised by hydrogen peroxide within the chloroform-acetic acid solution, the colour of the solution turns immediately dark (Wendler et al., 1950).

5.5. Conclusion

Based on the results of the five experiments, it can be concluded that carrot RR will be influenced by physical damage. With more severe damage, the induced RR tends to be more significant. Damage density is a means to quantify damage level, regardless of size or mass, as it can reflect how the overall integrity of the material is compromised. The surface area analysis may assist in indicating the effect of physical damage on RR.

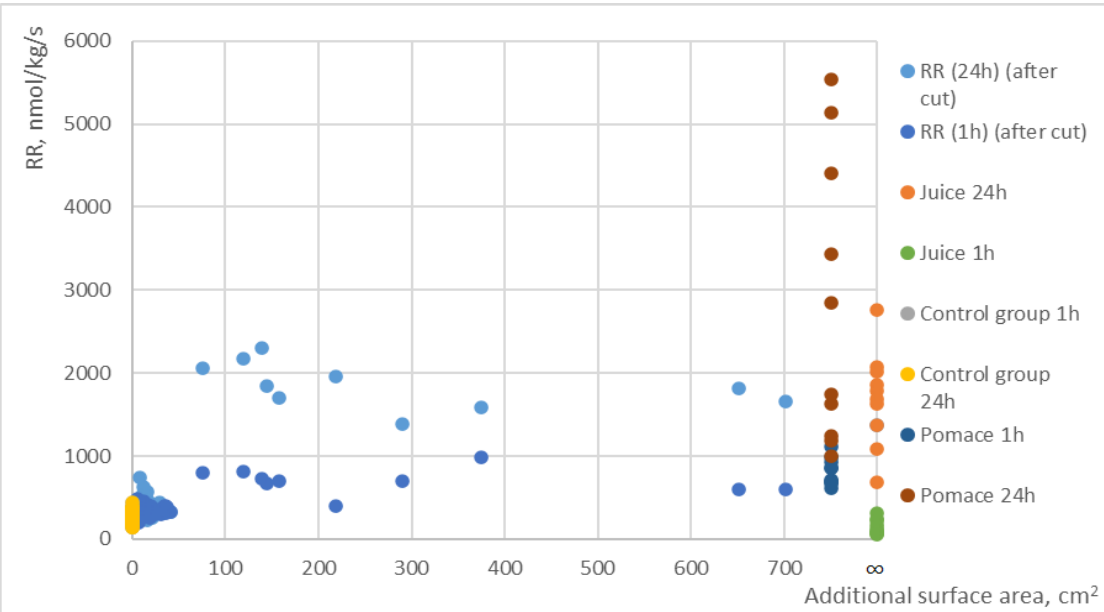


Figure 5.26 The change of 1h RR data versus addition surface area on carrot

Figure 5.26 shows the RR change of individual carrots after 1 h of cut and 24 h of cut as a function of additional surface area on carrots. The graph was created based on the results of Experiment 3 (section 5.3.3), Experiment 4 (section 5.3.4), and Experiment 5 (section 5.3.5).

The control at 1 h and 24 h are represented by pale dots and yellow dots, respectively, and these dots cluster at a low-level RR. The RR data of 1 h and 24 h both increase as the surface area increases. Carrots with an additional surface area below 100 cm^2 have a similar RR range as the intact and control groups. Except for the juice group, the carrots with more additional surface area tend to have a more significant RR value, with it peaking when carrots are converted into pomace. The specific additional surface area value of pomace is hard to determine due to the carrot crushing, while it deems the value should be over the maximum value of shredding but less than juice. The 24 h pomace group has the highest RR value, while the juice differs from other groups even if it has the most significant additional surface area. Hence, the additional surface area can enhance RR, but the extreme damage to the carrot will cause RR restriction.

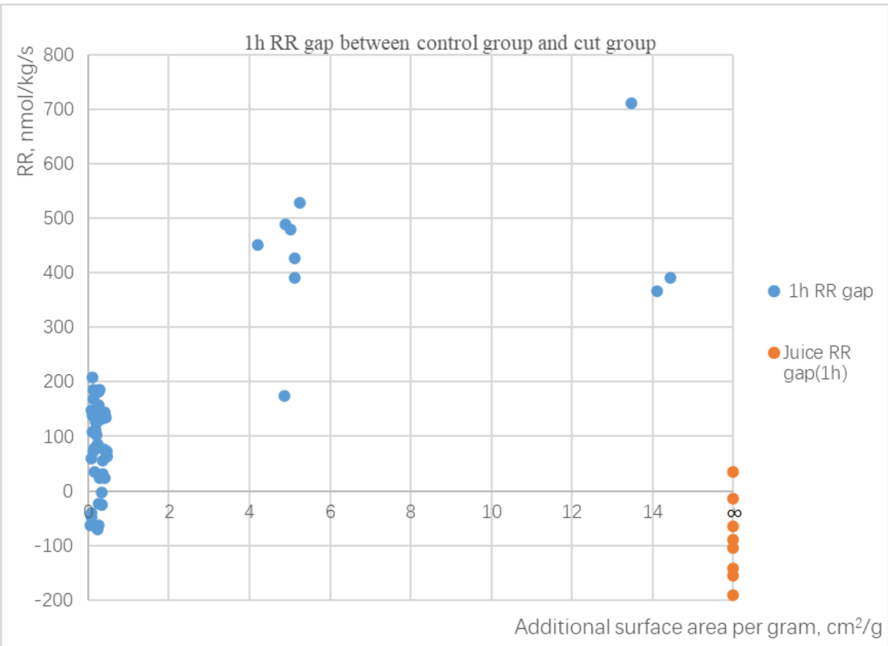


Figure 5.27 The 1h RR gap of individual carrots versus the change of additional surface area per gram on the cut group (pomace not included)

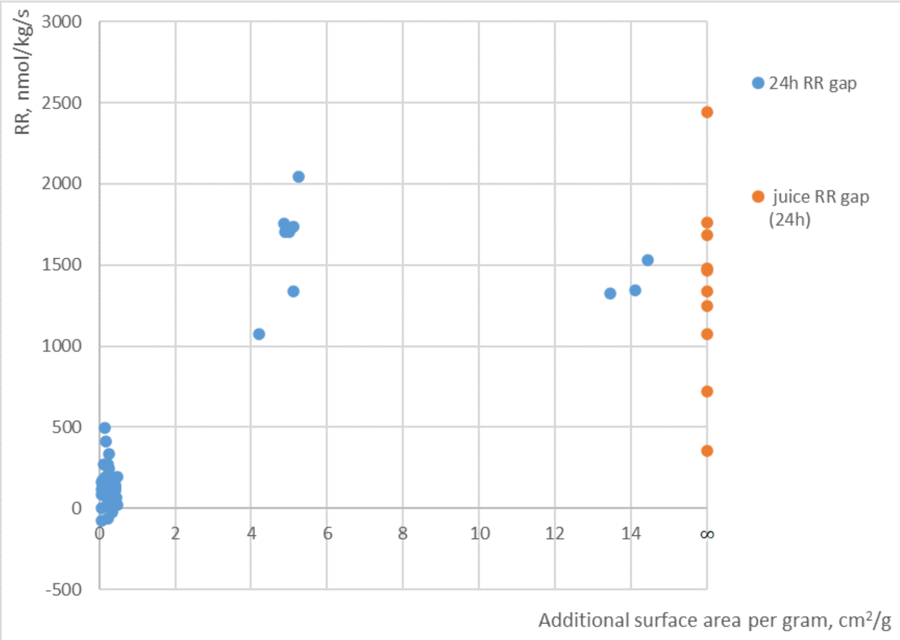


Figure 5.28 The 24h RR gap of individual carrots versus the change of additional surface area per gram on the cut group (pomace not included)

Figures 5.27 and 5.28 are created based on the results of Experiment 3 (section 5.3.3), Experiment 4 (section 5.3.4), and Experiment 5 (section 5.3.5). However, the pomace data is not included because the specific value of carrot pomace is hard to estimate to calculate carrot damage density. The RR gap, which is the difference between the RR values of the control group and the cut group, is used to measure the RR change.

The dots spread over the two graphs in Figures 5.27 and 5.28 show a similar pattern. The RR gap peaks at moderate damage density and then decreases at extreme damage density. However, the experiment lacks data on moderate damage density. The referred data is introduced to build a relationship between damage density and the RR gap. Their relationship can be summarised in Figures 5.29 and 5.30.

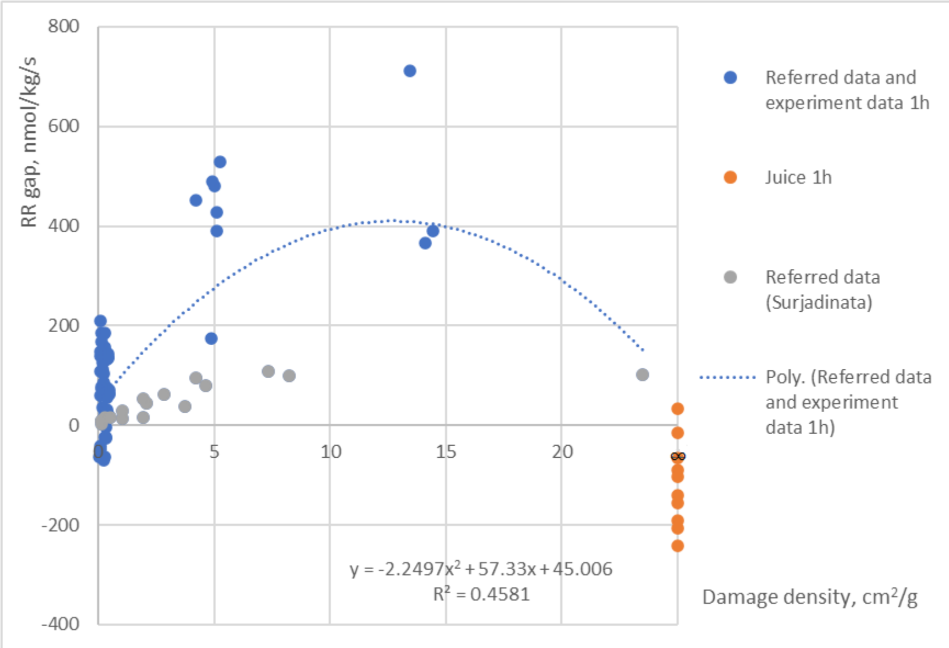


Figure 5.29 The fitted line of 1h RR gap and damage density, the part of referred data for the fitted line derives from the work of Surjadinata (Surjadinata & Cisneros-Zevallos, 2003)

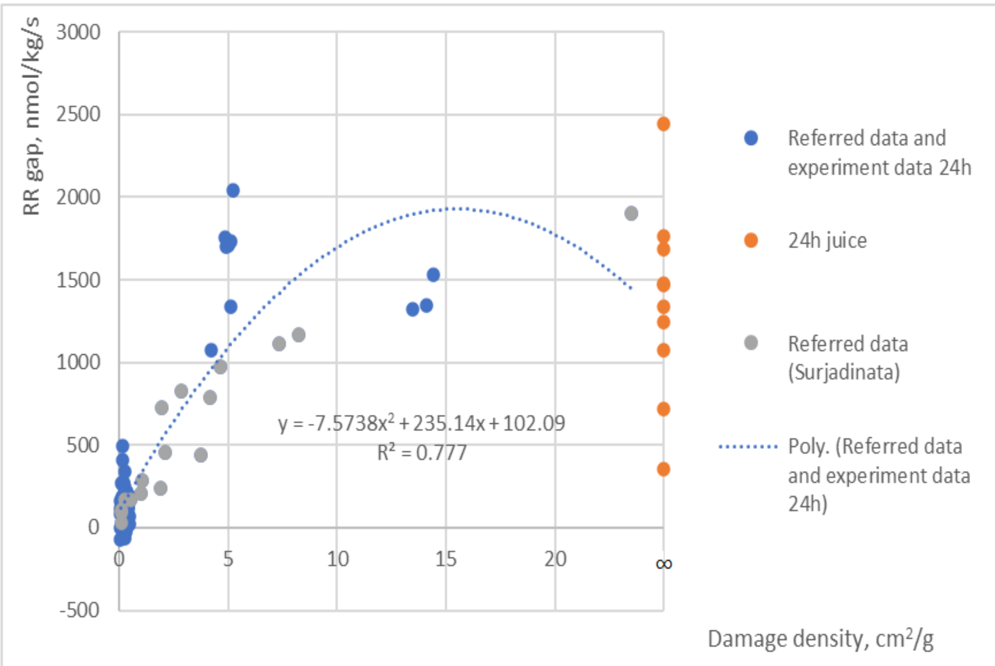


Figure 5.30 The fitted line of 24h RR gap and damage density, the part of referred data for the fitted line derives from the work of Surjadinata (Surjadinata & Cisneros-Zevallos, 2003)

The R^2 of both fitted lines is not strong because the referred data was derived under a 10 °C environment, and while the experimental work in this thesis was conducted at 20 °C, so the referred data needs to be estimated based on the Q10 value of Experiment 2. However, with the increased damage density, the RR will peak and then turn to a declining line, as the fitted line indicates.

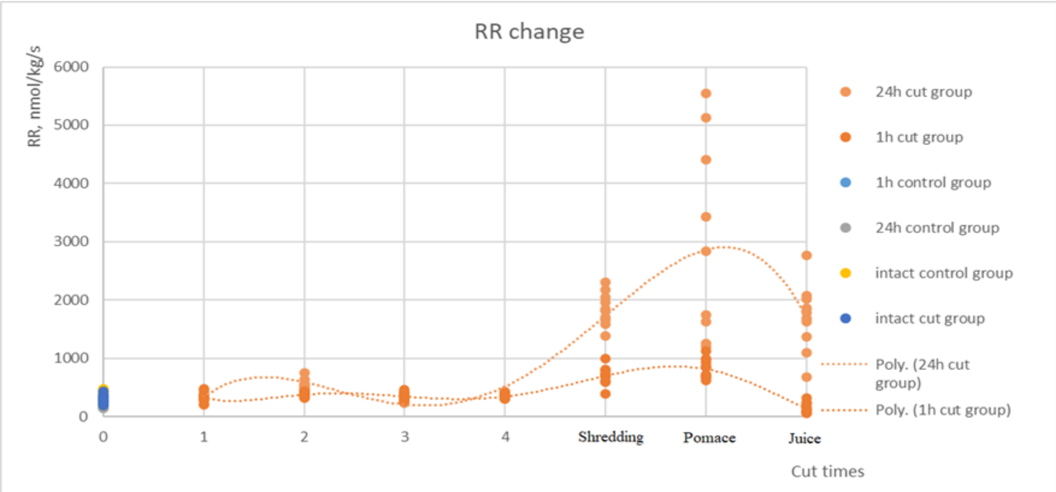


Figure 5.31 The RR change of carrots with different cut times

As the specific damage density is hard to determine, so the damage-induced RR over cut times on carrots are summarised. The control and intact groups overlap at the zero axis. Although the RRs of individual intact carrots are highly diverse, the range of intact carrots is close. All RR cut group data are shown in orange and share a similar trend. Still, the RR value of the 24 h group is higher than 1 h, especially on the shredding, pomace, and juice. The increase in damage-induced RR with small addition surface area is limited, with the RR values being close to the intact and control group. In comparison, he high additional surface area treatments, induced by shredding rises significantly, and the pomace and juice group are similar. However, it is noticeable that the variation of the pomace and juice group is much higher than other groups, which may have been affected by oxidation. After the carrot was processed, the contact surface area of pomace and juice was high, and the oxidation of carotenoids and β-carotene produced CO_2 which will affect the RR measurement. The oxidation extent of each unit within the pomace and juice group is different, leading to a high-level deviation of data.

6. Final Conclusions and Recommendations

Carrot respiration rate represents the metabolism that provides the necessary energy for the carrot to maintain regular physiological activity. The RR of carrots does not remain stable. Several factors affect the RR of carrots, such as temperature, dry matter content, physical damage, and storage time.

RR measurement can be conducted based on CO_2 production. The RR results are determined using data of measured CO_2 concentration. When conducting a sensitivity analysis of the parameters used to calculate respiration rate, most parameters were found to not substantially affect the RR accuracy. An exception to this was the calibration of the CO_2 analyser, which could result in a significant error.

The experiments conducted in this thesis can be divided into two types. In Chapter 4, experiments 1, 2, and 3 (4.2, 4.3, 4.4) were established to determine factors that affect whole carrot RR. Alternatively, Chapter 5 explores the influence of physical damage on the carrot respiration rate.

Chapter 4 provides the basis of the research and indicates factors that influence carrot respiration rates. The experiments checked basic factors like carrot density, equipment (CO_2 analyser and syringes) operation practice, and carrot weight and how these influenced RR. The results of these experiments indicate the diversity of individual carrots. For example, the density of individual carrots is different, as is the density. The RR of carrots also demonstrated individual diversity, while the weight of the carrot did not significantly affect the RR. At the same time, temperature and dry matter content were found to be important factors that influence RR. Carrots with high temperature and dry matter content will have higher RR, but the influence of temperature is more significant than that of dry matter. Although the carrots stored under high-temperature environments tend to have high RR, and the rate of change is uneven with temperature change from 0 to 20 °C. The RR was found to accelerate more when the temperature changes between 12 and 20 °C. Under dynamic temperature change conditions, the RR will change along with the temperature changes, and after the temperature reaches equilibrium, the RR of carrots will follow. Moreover, the RR is not the only factor that affects the quality of carrots during long-term storage; transpiration also contributes to the weight loss of carrots, and the application of plastic packaging is an effective methodology to control that.

Chapter 5 investigated the influence of physical damage on carrots. It was found that the RR will rise significantly when more damage is caused on the carrot within a low-level damage density. This level of damage stimulates the synthesis of the enzymes that assist in carrot recovery. When the damage density reaches an extreme level, carrot respiration will be limited by the physical damage. Juice could be considered as the most extreme form of damage creating infinite additional surface area but resulting in limited RR.

Overall, postharvest control of vegetables and fruits plays a vital role in the food supply chain, helping to control deterioration loss. The storage loss of carrots is affected by pre- and post-harvest treatments. The postharvest life of carrots is limited once harvested, but the change in storage environment can slow changes and extend storage life (Suojala, 2000). For example, β-carotene is an essential pigment within carrots that provides colour. Retail conditions at room temperature cause the highest reduction in β-carotene (−70%) and ascorbic acid (−70%). Hence best practice postharvest storage practices usually require low temperatures to maintain the quality of carrots (Seljåsen et al., 2013).

This thesis focused on the description of carrot respiration rate in response to minimal processing of carrot. This information assists in establishing carrot storage strategies for different damage levels. For instance, the carrot slices, sticks, and shreds have different RR based on the RR-damage density curve in Figures 5.29 and 5.30. Hence, any modified atmosphere packaging (MAP) solutions that may be applied to maintain carrot quality after minimal process may be required to be different. The application of MAP can reduce the RR by approximately 55% at 0 ℃, 65% at 5°C and about 75% at 10°C (Izumi et al., 1996). However, because of the diversity of damage-induced RR, the MAP strategy should be different. For instance, carrot sticks and shreds benefited from MAP atmospheres of 0.5% O_2 and 10% CO_2 when stored at 0 and 5°C but not at 10 °C (Izumi et al., 1996). However, the RR limitation of MAP was more significant at 5 and 10°C for slices and sticks but not for shreds (Izumi et al., 1996).

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8. Appendix

Figure 1 1L Jars

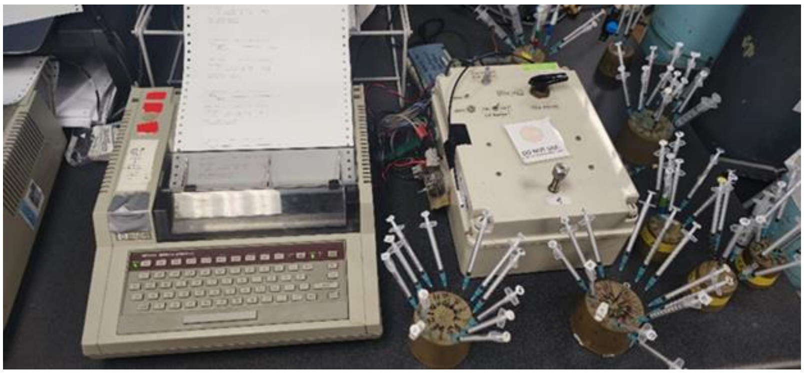

Figure 2 CO_2 analyser and syringes

Figure 3 Cylinder for drainage method (500ml, in unit of 50ml)