Diploma Thesis

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BUDAPEST

Effect of short-term high CO_2 treatment on post-harvest quality of fruits

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USED ABBREVIATION

Abbreviation	Meaning
CO_2	Carbon dioxide
MAP	Modified atmosphere packaging
MA	Modified atmosphere
CA	Controlled atmosphere
FAO	Food and Agriculture Organization
ppm	Parts per million
PFK	Phosphofructokinase
PK	Pyruvate kinase
ATPase	Adenosine triphosphatase
PDC	Pyruvate decarboxylase
NADP	Nicotinamide adenine dinucleotide phosphate
ADH	Alcohol dehydrogenase
SSL	Sensory Shelf Life
PPP	Pentose phosphate pathway
DNA	Deoxyribonucleic acid
TCA	Tricarboxylic acid cycle
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3
g	Gram
ANOVA	Analysis of variance
HSD	Honestly significant difference
USDA	United States Department of Agriculture

1. INTRODUCTION

Postharvest management is a critical aspect of ensuring the quality and shelf life of soft fruits (Chandra *et al.*, 2015a). Various strategies can be employed to effectively manage soft fruits after harvest including Modified atmosphere packaging (MAP), Modified atmosphere (MA) or Controlled atmosphere (CA), ethylene inhibitor etc. Among these factors, Carbon dioxide (CO_2) plays a significant role in fruit physiology and quality attributes. The short-term high CO_2 treatment had a significant impact on the post-harvest quality of fruits. The increase in levels resulted in delayed ripening, reduced weight loss, and decreased susceptibility to decay in various fruit samples. For instance, Rosaceae family includes a diverse range of economically important species, such as strawberry, raspberry, pear, apple, cherry, plum, peach, and so on.

Fragaria × *ananassa* (strawberries) and *Rubus idaeus* (raspberries) are two popular fruits worldwide. They have high commercial value in the international market. Both are non-climacteric fruits and highly perishable for soft texture and microbial infestation during post-harvest management (Chandra *et al.*, 2015b). *Fragaria* × *ananassa* (strawberries) and *Rubus idaeus* (raspberries) are very important fruits because of their flavor, taste, nutritional and phytochemical properties (Rao and Snyder, 2010). They are very susceptible to fungal diseases like botrytis fruit rot, the pathogen *Botrytis cinerea*, which causes grey mold during the growing period or later during post-harvest handling(Nakata and Izumi, 2020). To avoid decay, fungal attack, maintaining their texture, color and flavor those fruits have to keep within low temperature near 0°C during storage. But considering other factors like high respiration rate, high susceptibility to mechanical damage during handling and surrounding temperature at the end of the supply chain, maintaining low temperature isn't enough to extend the shelf life of those fruits to more than 14 days (González-Orozco *et al.*, 2020a). While low temperature storage increases the storage life of strawberries, it also results in a loss of cell structure (del Olmo *et al.*, 2022). For this reason, the grower, researcher and industry always searching for how to improve or increase the shelf life of agricultural commodities to avoid production loss. CO_2 is easily available and non-toxic that's why it's widely used to enhance as well as maintain fruit quality during post-harvest operations. Short-term treatment with high concentration of CO_2 has a good effect on keeping the quality of soft fruits and increasing their firmness which is previously studied (Goto *et al*., 1996a). Firmness can enhanced by treatments when these are kept in low temperatures. For strawberries, a short-term flush of 100% for 3h increases the firmness of the fruit (Goto *et al*., 1996b). CO_2 treatment extends the shelf life of soft fruits like *Fragaria* × *ananassa* (strawberries) and *Rubus idaeus* (raspberries) than air-stored fruit in case of firmness and lower the susceptibility to decay (Harker *et al.*, 2000). During the post-harvest treatment of fruits, it focuses on inhibiting ripening

processes regulated by ethylene activity or respiration (Krupa and Tomala, 2021). Short-term CO_2 treatment may delay the ripening of fruits under storage conditions (Li *et al.*, 2019a). Several experiments have been conducted on soft fruits to demonstrate their response during post-harvest management but still, many experiments are going on as it depends on various factors, such as fruit species, concentrations, maturity stage, temperature, light, and nutrient availability.

2. OBJECTIVES

Our aims with the current work were as follows:

1. To develop and test a simple method for short-term high CO_2 treatment for our lab conditions.
2. To assess the impact of short-term high CO_2 treatment of different concentrations on fruit quality, weight, brix level, and fruit firmness.

These objectives collectively might help to understand the impact of short-term high CO_2 treatment on the post-harvest quality of strawberries and raspberries, aiming to contribute valuable insights for the optimization of post-harvest handling practices and extending the shelf life along with other soft fruits. Also, the experimental method and results collected from this study might be useful for small farm owners with limited resources.

3. LITERATURE REVIEW

3.1. Importance of postharvest management

Post-harvest technology of fruits is a crucial aspect of ensuring the quality, safety, and marketability of fresh produce. Post-harvest treatments involve a series of activities aimed at preserving the quality, extending the shelf life, and adding value to the harvested produce. Effective post-harvest technology is crucial to reduce post-harvest losses and maintain the quality of harvested produce. One major cause of post-harvest losses is post-harvest diseases, which is responsible for 20-25% of fruit and vegetable losses (Taye, Saikia and Panging, 2023).

Fruit size and other morphological characteristics play an important role in the marketability, consumer acceptance, and overall economic value of fruit crops (Cirilli *et al*., 2021). Fruit quality is primarily based on firmness, color and chemical compositions (Harker *et al.*, 2000). Firmness can enhanced by treatments when these are kept in low temperatures. Fruit color is also an important factor in case of consumer's acceptability of fruit (Li *et al.*, 2019a).

One of the major challenges in this century is to provide high-quality products for its increasing population. The agricultural sector is facing serious issues like crop failure, crop damage, pollination problem, changes in chemical structure, post-harvest loss etc. due to environmental change and increase in temperature. A report prepared by Food and Agriculture Organization (FAO) mentioned that nearly 44% of total food losses by weight happened due to fruits and vegetables (Porat *et al.*, 2018). The fruits harvested and dispatched to retail outlets must maintain high-quality standards. According to FAO, a loss of up to 12% is acceptable due to the presence of low-quality fruits (Aglar *et al.*, 2017).

For this reason, the grower, researcher and industry always looking for how to improve or increase the shelf life of agricultural commodities to avoid production loss.

3.2. General physiological processes in post-harvest

Postharvest physiology of fruits, especially berries, is an important area of study. As those fruits have high nutritional value and bioactive compounds. Berries, such as blueberries, raspberries, and blackberries, are highly perishable and have a limited postharvest life. High respiration, softening rate, and susceptibility to mechanical damage and decay are responsible for the short storage life of berries (Horvitz, 2017). Berries are considered non-climacteric fruits which means they cannot ripen after harvest. Non-climacteric fruits, unlike climacteric fruits, do not experience a peak in ethylene production or respiration during the ripening process (Figure 1). However, due to their soft texture, some fruits have a short shelf life. Various techniques can be adopted to extend the shelf life of berries,

including harvesting at the right maturity stage, proper packaging, rapid cooling, refrigerated storage, and controlled atmospheres. These techniques help minimize undesirable changes in quality attributes during the postharvest period and reduce postharvest losses. Especially, Berries contain a significant amount of essential minerals, including phosphorus, potassium, and calcium, along with the presence of vitamins such as niacin (vitamin PP), retinol (vitamin A), thiamine (vitamin B1), and riboflavin (vitamin B2) (Krupa and Tomala, 2021). The compounds are mostly available in high-colored berries like strawberry, raspberry, blueberry and so on (Skrovankova *et al.*, 2015). Overall, postharvest physiology research plays a vital role in improving the shelf life and quality of fruits, including berries (Kumar *et al.*, 2018).

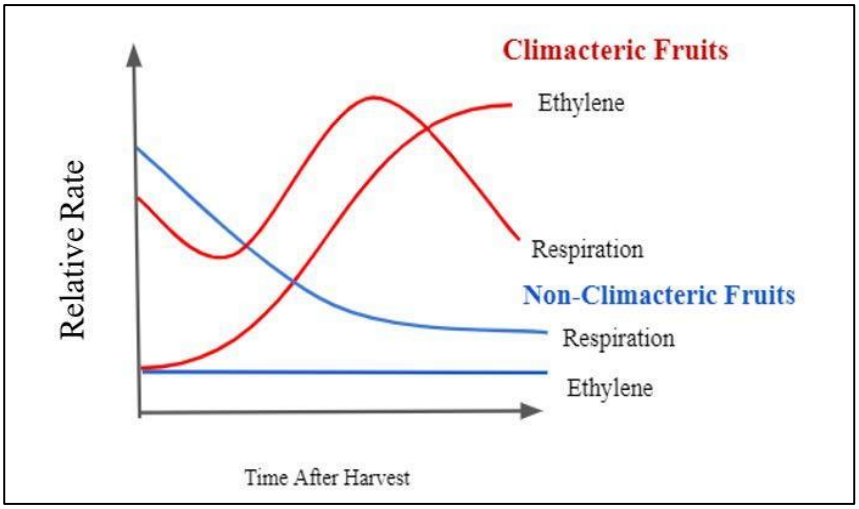

Figure 1. Fruit ripening patterns of climacteric vs non-climacteric (Source: University of California)

3.3. Post-harvest treatments

There are some special treatments such as physical, gaseous, heat, thermal, hydrocooling, irradiation which are involved in extending the shelf life of fruits by slowing down the ripening process, reducing the respiration rate, and inhibiting the action of ethylene. These are also important to preserve their freshness and quality before they reach the consumer's bowl. Nowadays, the consumer also gives importance to the fruit's appearance and overall fruit quality. Implementing proper handling practices like cleaning, sorting and grading, pre-cooling, packaging, storage, and transportation are essential in minimizing post-harvest losses (Shakeel *et al.*, 2022).

The physical treatments of fruits during post-harvest include heat, irradiation, and edible coatings. Edible coatings make a protective barrier that helps in reducing moisture loss, extending shelf life, preventing microbial growth, and maintaining fruit quality during storage and transportation. Irradiation exposes fruits to ionizing radiation to control pests, pathogens, and extend shelf life. Heat treatment helps to reduce microbial contamination, extending shelf life, and improving fruit quality. (Ramirez et al., 2019). Post-harvest heat treatments of fruit are also used to modify fruit responses to other stresses, for insect disinfestations and for disease control.

Gaseous treatments (ethylene, 1-MCP, MA or CA storage, and MAP) are used to extend the storage life of fruit by slowing down the ripening process (Giuggioli *et al.*, 2015). Modified atmosphere packaging (MAP), Controlled atmosphere (CA) involve packaging or storing of fruits in a modified atmosphere with reduced levels of oxygen and increased levels of CO_2 (Robertson, 2019).

These technologies also help in reducing postharvest losses, improving marketability, and ensuring a steady supply of fresh produce to consumers. By controlling factors such as temperature, humidity, and gas composition, postharvest technology can slow down the ripening process, inhibit microbial growth, and prevent physical damage to the products. Easily perishable fruits like strawberry, raspberry may provide significant advantages in storage by Modified atmosphere packaging (MAP), short term high CO_2 treatments.

3.4. Role of Carbon Dioxide in Post-Harvest Quality

It is widely recognized that CO_2 gas concentrations within the range of 15% to 20% exhibit inhibitory effects on fungal activity, respiratory processes, moisture loss, and textural deterioration in fruit berries, thereby prolonging their postharvest longevity (Romero *et al.*, 2022)

Generally, the typical ambient concentration of carbon dioxide in the atmosphere is 300 to 400 ppm (0.03% to 0.04%). Elevated levels, specifically at 0. 05% (500 ppm), have been observed to slow down the ripening process of certain products (Dhall, 2013). Short-term high CO_2 treatment or elevated treatment is widely practiced during post-harvest operations to control fungal decay as well as to increase shelf life. Several studies have reported that treatment increases the shelf life by delaying senescence and fungal decay which are mainly correlated with the reduction of respiration and ethylene production rates (El-Kazzaz, 1983). Throughout these experiments, CO_2 of different concentrations (5-30%) used for 5-20 days to see the outcome. In many experiments, is incorporated with different levels of oxygen to create a controlled atmosphere to identify the best possible result. In some experiments, the combination of and MA packaging resulted in higher overall scores, lower softening index, weight loss, and better freshness maintenance compared to treatment alone (Jin Choi *et al.*, 2016).

3.4.1. Previous Studies of CO_2 treatment on Strawberry and Raspberry

The application of high CO_2 concentrations during cold storage has been found to increase firmness, sugar content, and effectively preserve the color parameters of fruits, preventing undesirable color changes during storage. It also suppresses the spoilage caused by pathogens like *Botrytis cinerea* in strawberries. In a study, the researchers experimented with 'Minomusume' strawberries where stored them at 5ºC for 14 days in CAs containing 20%, 30% and 40% of CO_2. The results showed that the CA of 20% to 40% effectively delayed fungal growth and prevented external mold mycelia formation (Nakata and Izumi, 2020).

In an experiment, the post-harvest life of red raspberries was assessed through the application of two short-term CA treatments, mentioned as early (15% CO_2 , 10% O_2 for 3 days + air for 11 days) and intermediate (3 days in air + 3 days 15% CO_2 , 10% O_2 + 8 days in air), in comparison with continuous CA. The findings reveal that the early CA treatment exhibits prolonged Sensory Shelf Life (SSL) values and enhances color parameters consistently throughout storage as compared to the control group. Furthermore, the initial application of short-term CA treatments results in a reduction in weight loss during the first 6 days of storage (González-Orozco *et al.*, 2020a).

The 'Goha' summer strawberries were treated with different concentrations of and duration. Strawberries treated with 50% CO_2 for 1 or 3 hours had higher firmness compared to those treated with 15% and 30% CO_2 for 1 hour or the control group during 13 day cold storage period. The strawberries treated with 15% CO_2 for 3 hours exhibited improved quality, achieving higher scores in overall quality and visual texture while their softening scores remained lower for up to 9 days of storage compared with other different concentrations.

'Pájaro' strawberries were treated with different levels of (5-40% CO_2) for 0–3 days, and then stored at 0°C for a duration of up to three weeks. The application of treatments resulted in enhanced firmness of the strawberries, as well as 60% improvement in cell-to-cell adhesion (Harker *et al.*, 2000).

A popular cultivar in Korea 'Seolhyang' strawberries have a fragile outer layer and a short shelf-life, it is often treated with to extend their shelf life. However, the short-term high CO_2 treatment was investigated during a 9-day cold storage period which resulted in the decay rate, firmness, color, targeted metabolite profile, and antioxidant activity of Seolhyang strawberries (Ahn *et al.*, 2021).

Color is also an important indication of fruit's freshness and quality. Elevated (20% CO_2) delayed the changes in color values of the strawberry fruit. The degradation of chlorophyll in -treated fruit was achieved through the inhibition of chlorophyllase activities and the down-regulation of specific genes associated with chlorophyll metabolism(Li *et al.*, 2019b).

Similar experiments were conducted on other fruits to see the short term high CO_2 treatment. In an experiment with MiniKiwi (*Actinidia argute*), the findings suggest that the application of elevated concentrations (5-10% CO_2) successfully delays the ripening processes in fruit. After 12 weeks of storage, the fruit wasn't suitable for immediate consumption which indicates the extension of the storage period (Krupa and Tomala, 2021).

The study on the effects of high on the quality and antioxidant capacity of postharvest blueberries (*Vaccinium spp*.) showed that 25% CO_2 treatment significantly delays the respiration rate and preserves aroma compounds in blueberries. Additionally, the levels of aldehydes and limonene are enhanced, while esters and ethanol contents are decreased by 25% CO_2 treatment (Gao *et al.*, 2021).

3.4.2. Mechanism behind the extended life of fruits due to treatment

CO_2 treatment during post-harvest extends the life of fruits through several mechanisms. High CO_2 concentrations increase firmness and reduce decay incidence by regulating genes associated with cell wall degradation (Beiparysa et al., 2023). Moreover, CO_2 treatment stabilizes xyloglucans, preserves lamella integrity, and promotes the expression of genes responsible for maintaining cell turgor, thereby enhancing cell wall elasticity. Additionally, CO_2 treatment stimulates the production of oligogalacturonides, which serve as a defense mechanism against fungal pathogens such as Botrytis cinerea. Exposure to high CO_2 atmospheres inhibits the growth of decay-causing pathogens, such as Botrytis cinerea, reducing the incidence and severity of postharvest decay (Palou et al., 2016) (Wang et al., 2020). CO_2 treatment enhances the activities of enzymes involved in sugar metabolism, leading to the accumulation of soluble sugars and maintenance of fruit quality (Eum et al., 2021). Furthermore, CO_2 treatment reduces weight loss and maintains total soluble solids, fruit hardness, and chemical properties such as total phenolic compounds, total carotenoids, and vitamin C.

In an experiment with sweet cherries, it showed that high CO_2 treatment significantly improves the quality of sweet cherries, including respiration rate, weight loss, and firmness. The treatment enhances the pentose phosphate pathway (PPP) and decreases the glycolytic pathway (EMP) and tricarboxylic acid cycle (TCA) in aerobic respiration leading to improved respiratory metabolism. The treatment also increases the contents of NADP(H), source, and fructose, while reducing the levels of NAD(H), which contributes to better quality. High CO_2 treatment improves cell membrane integrity and enhances the activities of enzymes such as pyruvate kinase (PK), phosphofructokinase (PFK), and adenosine triphosphatase (ATPase), which are involved in metabolic processes. Additionally, high CO_2 treatment decreases anaerobic respiration by inhibiting the accumulation of pyruvic acid, ethanol, and acetaldehyde, as well as reducing the activities of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) (Gao et al., 2022).

The mechanism responsible for CO_2 induced firming of soft fruit tissue involves the modification of pectic polymers and the binding of calcium ions to the cell wall. High CO_2 pressure treatment stimulates an increase in firmness by inducing calcium efflux and enhancing calcium binding to wall polymers (Wang et al., 2014). This increase in firmness is primarily mediated by pectin polymerization, which is facilitated by the shift of water-soluble pectins to chelator-soluble pectins. The mechanism of firmness enhancement by CO_2 was possibly due to the change in intercellular pH and its solute composition (Siriphanich, 1998). Additionally, the formation of intramolecular bridges in pectin, mediated by calcium, is believed to play a role in the increase of firmness induced by CO_2 treatment (Goto et al., 1996c).

The duration of the treatment and the concentration of CO_2 used are important factors to consider as they can affect the response of the fruit. Off-flavors can develop when the concentration of CO_2 exceeds the tolerance threshold during a 3-day short-term gas treatment to maintain strawberry quality. For example, a 3-day treatment with 20% CO_2 is effective in maintaining strawberry quality, but higher concentrations (40% CO_2) applied for the same duration can lead to the accumulation of fermentative products, resulting in off-flavors. The initial optimization to enhance the storability of blueberries and raspberries should focus on refining both the duration of exposure and the dosage of CO_2 (Romero *et al.*, 2022).

3.5. Histological study of fruits

Fleshy fruit tissues are mainly formed by parenchyma cells with a thin primary wall. Strawberries are highly valuable but perishable fruits, prone to postharvest decay due to their soft texture. Analyzing the cell wall structures of soft fruits is crucial for understanding the mechanisms underlying fruit softening and ripening processes (Eum, Han and Lee, 2021). Understanding the mechanisms behind strawberry tissue degeneration is essential to understand to maintain the fruit quality and minimize bruising during postharvest management. A study found a wide range of cell sizes in strawberry tissue, ranging from 30 to 500 µm, with a peak frequency between 200 and 240 µm in ‘Flair’, ‘Malwina’ and ‘Sonata’ strawberries where ‘Flair’ and ‘Malwina’ strawberries had smaller cells than 'Sonata' (An *et al.*, 2023). Atomic force microscopy (AFM) mostly used in food science but nowadays it is used to study a complex biological process such as fruit ripening, focusing mainly on the role of pectin modifications during fruit softening. Pectin structure is preserved by postharvest treatments that improve fruit quality, indicating a direct correlation between pectin metabolism and softening which was found by using AFM (Posé *et al.*, 2019).

3.6. Visual analysis of fruits after postharvest treatment

Fruit quality is an important parameter to determine the acceptance rate of customers. Quality is mainly measured in two ways: external quality and internal quality. External qualities are typically evaluated based on size, color, shape, freshness, and the presence of surface flaws (Akter *et al.*, 2024). Furthermore, *Botrytis cinerea*, is considered as most common infection in strawberries which caused significant financial losses for the strawberry industry. *Botrytis cinerea* can damage vegetative cells as well as fruit and senescing organs, causing grey mold in fruit. The presence of any pathogenic symptoms or signs of microbial contamination on the surface of fruit can significantly degrade the value and appeal of the product to customers. Proper postharvest handling and storage practices are essential to maintain the competitiveness of food products in the market and ensure profitability for producers.

4. MATERIALS AND METHODS

4.1. Experimental design

In this study two separate experiments were conducted to test the effect of CO_2 treatment on berry fruits. In experiment 1, the primary aim was to develop a chamber in which CO_2 treatment can efficiently be tested on small quantities of fruits. This would allow more efficient research work, to assess the feasibility of the chamber and its practical applicability for small farm owners who do not have professional storage rooms or large quantities of fruits. For this purpose, we used 2 different species, and the most commonly suggested CO_2 concentration and treatment time.

In experiment 2, the method was further developed by small optimizations (adjusting fan position, reducing treatment time from 3 to 2 hours, etc.), and by increasing the number of chambers, The goal was to test the variance between different CO_2 concentrations which in one hand gives data on the efficiency of the chamber to answer research questions, as well as the direct impact of CO_2 concentration on treatment effectiveness. In this case, we only used one species but 4 treatments.

Both experiments involved analyzing in fruit texture, weight loss and sugar content (measured as Brix). In experiment 2 analytics were conducted in 0, 5, 8 days intervals and overall visual quality was also assessed after 22 days of storage period.

4.2. Fruit Source

In experiment 1, the strawberries and raspberries from unknown cultivars were obtained from a private gardener. On the day of the experiment, freshly harvested strawberries and raspberries were used. Approximately, 11 strawberries (112.8 grams) and 38 raspberries (128.6 grams) were divided into two plastic boxes without cover (Figure 2).

In experiment 2, 0.5 kilogram of ‘Senga sengana’ was bought from the market (Figure 2). Later 80 strawberries were divided into four plastic boxes (20 per box) and labelled as controlled and treated (10%, 20%, and 30%) of CO_2 . The weight was measured immediately. Fruits with relatively uniform color and size were selected and used in this study.

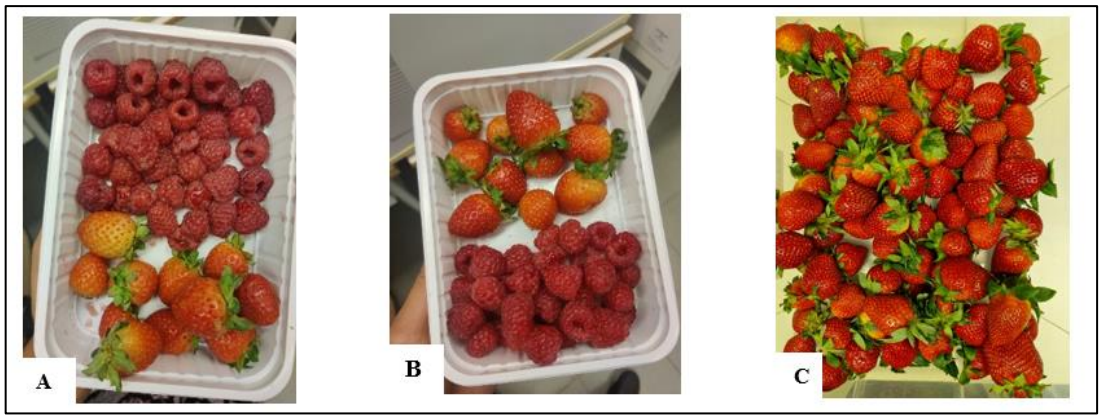

Figure 2. Strawberries and Raspberries before treatment A, B. Experiment 1 (unknown cultivars) C. Experiment 2 ('Senga sengana')

4.3. Gas treatment

A 30L airtight plastic container was used to conduct this experiment. Two holes were made in the cover to let the gas in and out as well as sealed it properly to avoid contamination. As CO_2 is heavier than air it has chance to settle down the container as a result the fruits might not get gas treatment uniformly. To avoid this issue a fan was put inside to supply air (Figure 3). After the experiment 1, more improved and accurate equipment is used to develop the experimental procedure. For example, transformer, ventilator, plastic fan, taps to control flow were used later on (Figure 4).

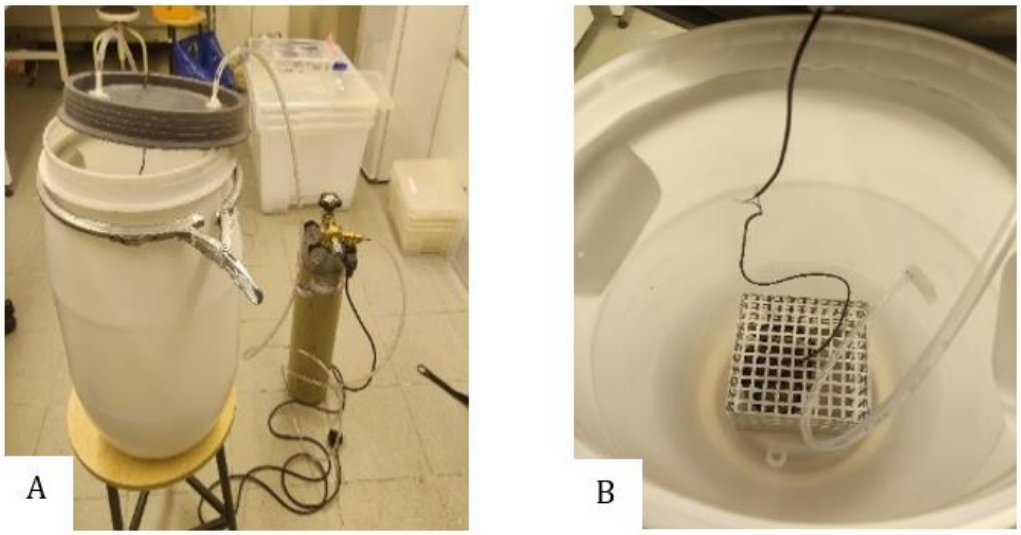

Figure 3. A. Compressed gas cylinder along with 30L container B. Position of fan for air circulation

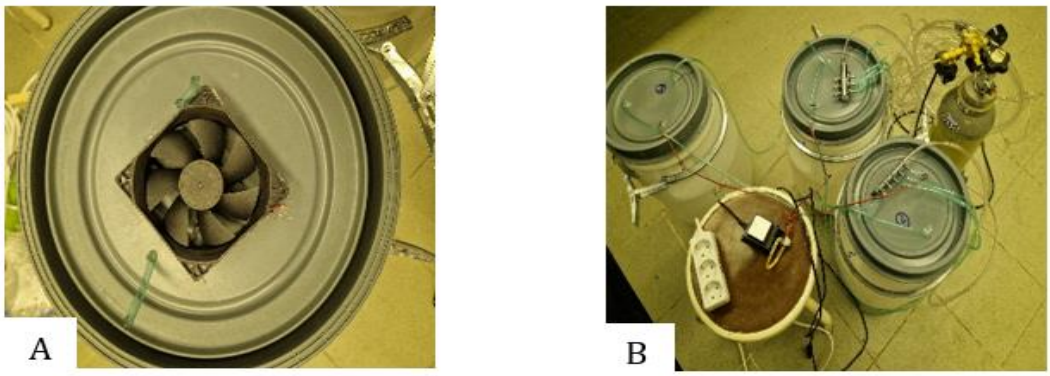

Figure 4. A. Adjusted plastic fan under the cover in experiment 2 B. Three containers for different concentration of CO_2 with taps, transformer in experiment 2

Two plastic boxes containing Strawberries and Raspberries were used for controlled (Air) and gas treatment. The box was placed inside the airtight plastic container and the lid was closed tightly. Around 20% CO_2 gas was passed through the tube into the container for 45 seconds. After that, the tube was sealed immediately. Later, the switch of the fan was turned on to supply air for the homogenous mixture of gas inside the container. The fruits were treated with gas for 3 hr. After the treatment, the controlled (Air) and treated samples were stored at 4º C in the cold storage chamber. The raspberries were kept for 10 days and the strawberries for 11 days in cold chamber.

Following the protocol mentioned above, in case of repetition, the treatments were conducted at room temperature with three containers. Different concentrations (10%, 20%, 30%) of CO_2 were used to treat strawberries for 22, 44 and 66 seconds. When concentration reached at expected level in different treatments, the containers were completely closed and air was supplied for 2 hr. After the fruits were treated with gas for 2 hr they were kept in the cold chamber (4º C) for 22 days.

To assess the impact of short term high CO_2 treatment the following experiments were carried out at the laboratory: 1) measurement of brix using a digital refractometer, 2) texture analysis using Brookfield texture analyzer, 3) measurement of weight, 4) Microscopic viewing of cells

4.4. Fruit quality analysis

Brix measurement

Individual brix measurements were taken for all the fruits sampled (n=8) using an digital refractometer (Figure 5). Brix was measured from both controlled and treated samples obtaining the

juice by squeezing individual fruit. In case experiment 1, the brix was measured in controlled and CO_2 treated sample after a duration of 10 days. For the second setup, brix was measured from control samples initially (0 days), followed by measurements in both controlled and treated samples at an interval of 5, 8 days beginning from the storage period.

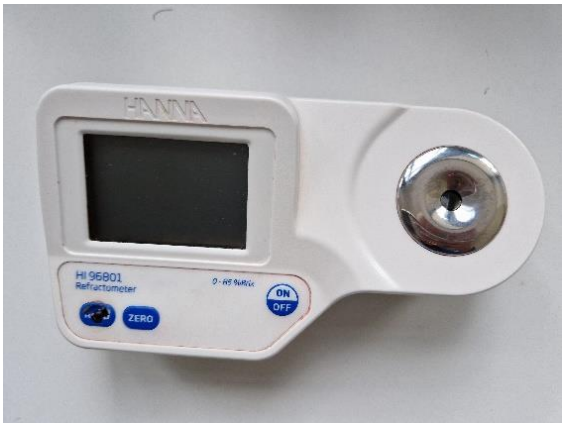

Figure 5. A Digital Refractometer

Fruit firmness Test

Penetrometric and puncture test methods belong to the simplest methods and are widely used in practice. Mostly the maximal force needed for the penetration of the deforming body to a given depth is measured. The hardiness of Strawberries and Raspberries was measured using a penetration test by Brookfield texture analyzer (Figure 6). For this assay, the samples both controlled and treated were obtained after 10 days of cold storage. The experiment was performed on the whole fruit using TA39 probe which is commonly used for soft fruits (Figure 6). The speed of penetration was set at 5 mm s^{-1}; the test was stopped after a maximum penetration depth of 5 mm. Using this approach, fruit hardiness was immediately measured in the controlled sample. Later, strawberries that were both controlled and treated were measured at an interval of 5, 8 days for experiment 2. Eight strawberry fruits from each replicate were randomly selected and a total of 32 data from four replicates were averaged from each treatment. All measurements were taken at room temperature.

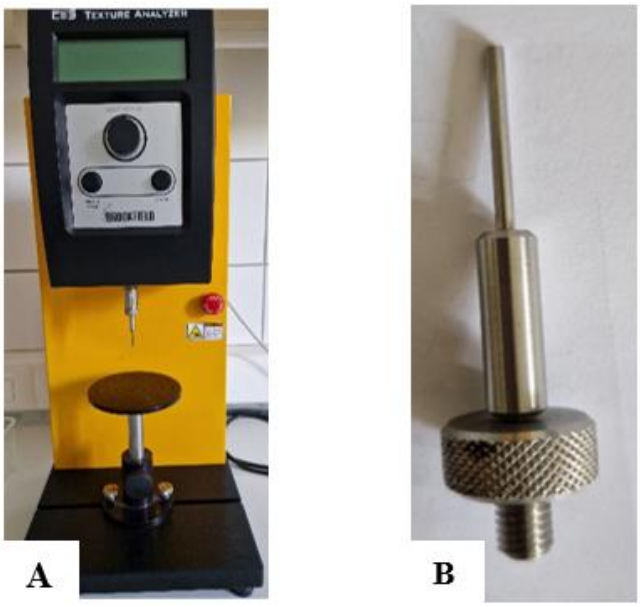

Figure 6. A. Brookfield texture analyzer B. TA39 probe

Additionally, the samples were weighed with a digital balance before and after the treatment as required.

4.5. Microscopic analysis

4.5.1. Sample collection and preparation

For the evaluation of the tissue samples, a small portion (around 15 mm) were cut from treated and untreated fruit samples. The samples were collected on 8th day of storage at 4^{o}C. Each tissue samples were placed into fixative solution containing 70% ethyl alcohol and 30% acetic acid. They were kept in the fixative solution for at least a week to dissolve tissue materials and become transparent. Then they were examined for their cell structure and thickness. The preparation of fruit tissue samples followed the protocols below.

The fixed fruit tissue pieces were placed in sealable cassettes. These cassettes were then placed in the Microm STP 120 Spin Tissue Processor machine, which has 12 vessels. The samples were treated in each compartment according to the specified protocol for a certain duration:

Table 1. Protocol for fruit tissue sample preparation

Container	Solution	Treatment time
1	75% ethanol	2 hours
2	80% ethanol	1.5 hours
3	90% ethanol	1.5 hours
4	96% ethanol	1 hour
5	96% ethanol	1 hour
6	99% ethanol	1 hour
7	Empty	no treatment
8	Roticlear	1.5 hours
9	Roticlear	1.5 hours
10	Paraffin	2 hours
11	Paraffin	2 hours
12	Empty	no treatment

After this, the samples were embedded in paraffin. Once fully solidified and cooled, sections of 15 micrometers thickness were cut using a microtome and placed in a warm distilled water bath. Then these sections were placed onto glass slides coated with Mayer's egg albumin to adhere the sections to them, and then transferred to a drying oven, where they spent approximately 2 hours at 30-40°C.

After drying, staining was performed in 9 tanks according to the following steps:

Table 2. Protocol for staining of fruit tissue

Solution	Treatment time	Purpose of treatment
Roticlear	5 minutes	Paraffin release
96% ethanol	5 minutes	Hydration
70% ethanol	5 minutes	
50% ethanol	5 minutes	
0.01% toluidine blue solution	approximately 45 seconds	Painting
Distilled water	3.5 minutes	Removing excess paint from slide
50% ethanol	3.5 minutes	Dehydration
70% ethanol	3.5 minutes	
90% ethanol	3.5 minutes	

After staining, the slides were let to dry, and then Euparal was dripped onto them to mount cover slips on the sections.

4.5.2. Microscopy

For cell size measurements, the fixed and transparent tissue sample pieces were placed onto microscope slides with their abaxial sides facing upward, and their epidermis was examined under a light microscope at a magnification of 200X, and three images of the cell were captured from each sample.

All microscopic images were made by a Zeiss Axio Imager A2 (Carl Zeiss Microscopy, Munich, Germany) compound microscope. For making images, we used a Zeiss AxioCam HRc camera (Carl Zeiss Microscopy, Munich, Germany).

4.6. Statistical Methods

Welch ANOVA model was applied to assess the differences of brix and hardiness between controlled and treated samples. The test was performed for each treatment. Welch ANOVA model had the assumptions of normally distributed residuals and non-homogeneity of variances, besides independent samples. Normality was graphically represented by bar plot. To compare all possible pairs Tukey HSD test was done to see whether the samples were significantly different from each other or not. One-way ANOVA tells us whether the means of the groups are significantly different or

not, but we still need to do a post hoc multiple comparison test to dig further. One of the widely used methods is the Tukey HSD test.

An unpaired t-test was applied to identify the relationship of weight between treated and untreated samples. Independent t-test or (unpaired t-test**)** is used to compare the means of two unrelated groups of samples. Before the experiment 2, experiment 1 was conducted with two fruits (strawberry, raspberry) by one replication that resulted in some similar attributes. All used statistical tests were two-sided, and the significance level was set at $\alpha=0.05$.

Statistical analyses were performed by using the statistical software package R (Version 4.4.1. R Foundation for Statistical Computing, Vienna, Austria).

5. RESULTS AND DISCUSSION

In order to evaluate the short-term high CO_2 treatment the following experiments were carried out at the laboratory: weight, brix and hardiness measurement.

5.1. Effect of CO_2 treatment on brix level

Experiment 1

Firstly, brix was measured in experiment 1 where CO_2 treated strawberry samples had higher brix levels than untreated ones (Figure 7). But it wasn't statistically significant by Welch ANOVA model, might be due to the low sample size and lack of repetition. Overall, treated strawberries showed higher brix compared to untreated control samples. However, CO_2 treated raspberries showed lower brix levels than the control berries at the end of storage. At the end of storage the weight loss of untreated raspberries was (92.8 g) whereas the treated raspberries was (106.2 g). Therefore, the low values of brix could be explained by cultivar and harvest season influence, for example, weight loss (such as water loss) could influence brix measurements (González-Orozco *et al.*, 2020a). In a study also showed that short-term CA exposure (15% CO_2, 10% O_2) significantly reduced the weight loss of raspberries during the first 6 days of storage (González-Orozco *et al.*, 2020b).

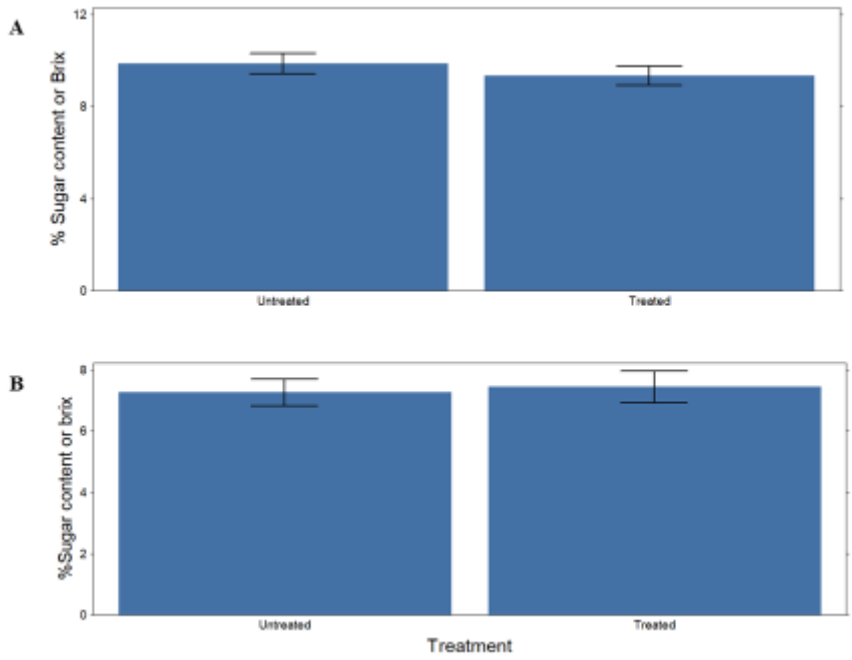

Figure 7. % Sugar content or Brix in experiment 1, A. Raspberry after 10 days of storage B. Strawberry after 11 days of storage at 4ºC where samples were treated for 3hour with 20% CO_2.

Experiment 2

Later on, the repetition with the strawberry samples in experiment 2 showed a significant difference in brix level.. Overall, like the experiment 1, CO_2 treated samples showed higher brix compared to untreated control samples. Among the treated samples, sugar content of 20%, 30% of CO_2 for 2 hour treatment showed insignificant differences with untreated control samples, whereas samples treated with 10% CO_2 for 2 hours showed significant differences ($p<0.05$) with untreated control samples (Figure 8). Additionally, the Tukey HSD test showed slightly significant differences in 10% and 20% of CO_2 concentrations ($p=0.04$).

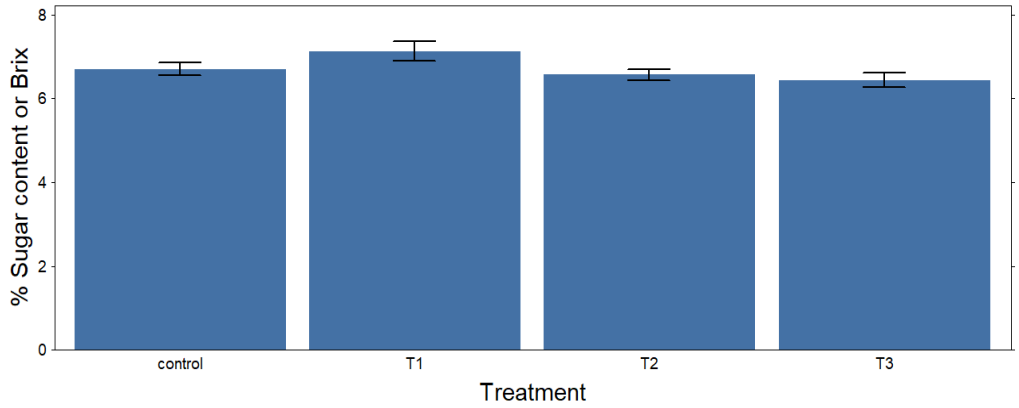

Figure 8. % Sugar content or Brix of Strawberry in experiment 2 after 8 days of storage at 4ºC where (T1-10%, T2-20%, T3-30% of CO_2) treated for 2 hour.

In 'Goha' strawberry brix levels slightly increased or maintained during storage in all treated (15%, 30%, 50% CO_2) samples except control (Chandra *et al.*, 2015a). (Goto et al., 1995) identified there was little effect of treatment on total sugar content in the 'Hoko Wase' variety when they were treated with 30%, 70%, 100% of CO_2.

Brix level based on storage days in experiment 2

Brix was measured from untreated (control) samples initially in experiment 2 and then in both controlled and treated samples at an interval of 5 and 8 days to see is there any variation observed throughout these days. For graphical representation, some variable data were filtered out from all treatments (control and treated) to maintain the quality of the data.

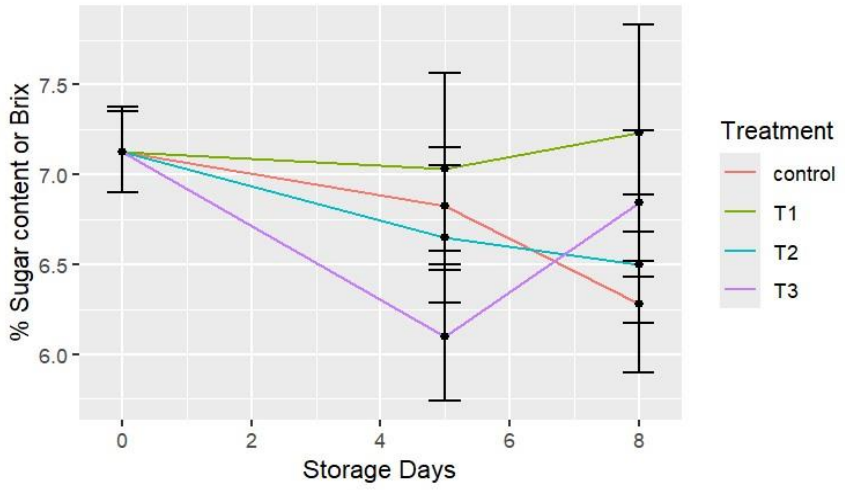

Figure 9. Changes in Brix level of Strawberry in experiment 2 during 8 days of storage at 4ºC among different concentrations (T1-10%, T2-20%, T3-30%) of CO_2 and untreated (control) samples.

It was observed (Figure 9) that the brix level was high in control samples initially and the control sample's sugar content steadily decreased over time. This decline suggests that natural ripening processes may lead to a reduction in sugar levels. In case of CO_2 treated samples (T1 and T3) the sugar content increased as the storage days progressed. However, T2 and T3 had lower brix levels than control samples on the 5th day. On the 8th day all treated samples (T1, T2, and T3) showed higher brix levels compared with control samples. As well, T2 samples showed a similar pattern like controlled samples where sugar content decreased over time. This unexpected rise in T1 and T3 samples could be due to the treatment affecting sugar synthesis during ripening (Durán-Soria *et al.*, 2020). Moreover, T3 samples lost less weight (99.4g) throughout the storage periods.

(Chandra *et al.*, 2015a) also found the strawberry samples treated with for a longer duration (3hour) showed a sudden decline on 3 days of storage and the brix was recovered thereafter. In their experiment, among all treatments, 15% CO_2 for 3hour increased insignificantly until the end of storage (13 day). (*Fragaria* × *ananassa* cv. Selva) showed no differences in brix levels among treatments (10%, 20%, or 40% CO_2 at 5 °C for 10 days) (Gil, Holcroft and Kader, 1997).

5.2. Changes in fruit firmness

Experiment 1

Firmness is a major index for evaluating the quality of fruits. The firmness of the strawberry fruits exposed to short-term high- treatment was slightly higher than that of control fruits which were observed in different experiments. In experiment 1 and 2, Welch ANOVA model was applied to identify differences among samples.

In experiment 1, no significant changes in raspberry firmness were observed during storage with treated samples compared with the untreated samples (Figure 10). In a similar study, there were no significant changes in 'Adelita' raspberry firmness between treated and untreated samples although the continuous CA treatment (5% CO_2 + 10% O_2 for 14 days) showed significant increase compared with the other treatments from day 2 onwards (González-Orozco *et al.*, 2020b). The firmness of the decayed fruits was also investigated during storage. However, decayed fruits had a soft texture when touched so it might have influenced the overall results.

The firmness of strawberries was higher than untreated samples (Figure 10) though there was no significant difference most probably due to the low sample size and lack of repetition.

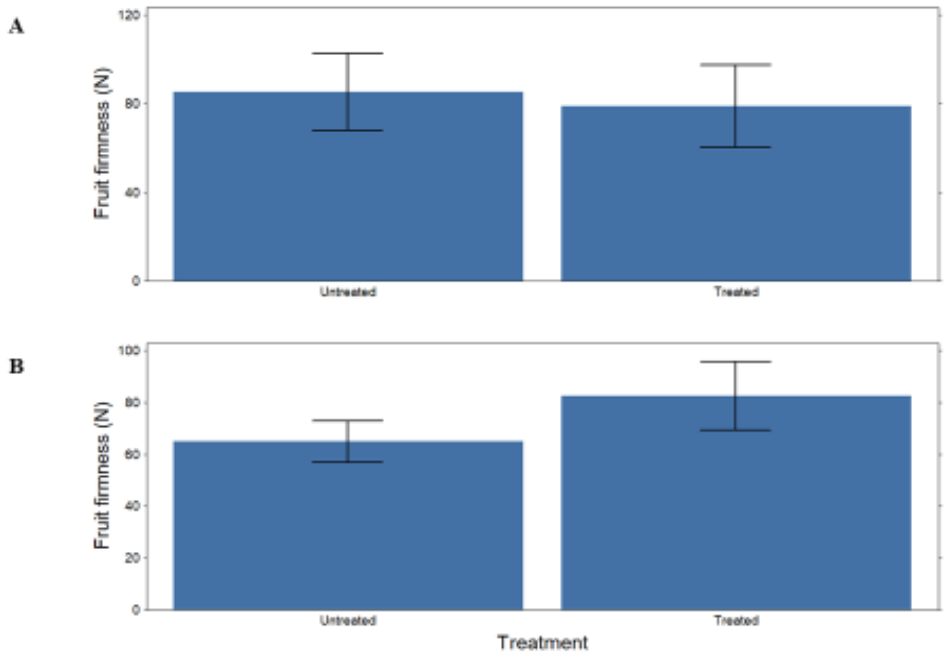

Figure 10. Changes in fruit firmness (N) in experiment 1, A. Raspberry after 10 days of storage B. Strawberry after 11 days of storage at 4ºC where samples were treated for 3hour with 20% CO_2.

Experiment 2

In experiment 2, treated samples (10%, 20%, 30%) of CO_2 showed significant differences ($p<0.05$) with untreated (control) samples. Among all T1 showed highest difference ($p<0.005$) compared with control (Figure 11). From Tukey HSD test, significant difference was also found ($p<0.05$) between 10% and 20% of CO_2.

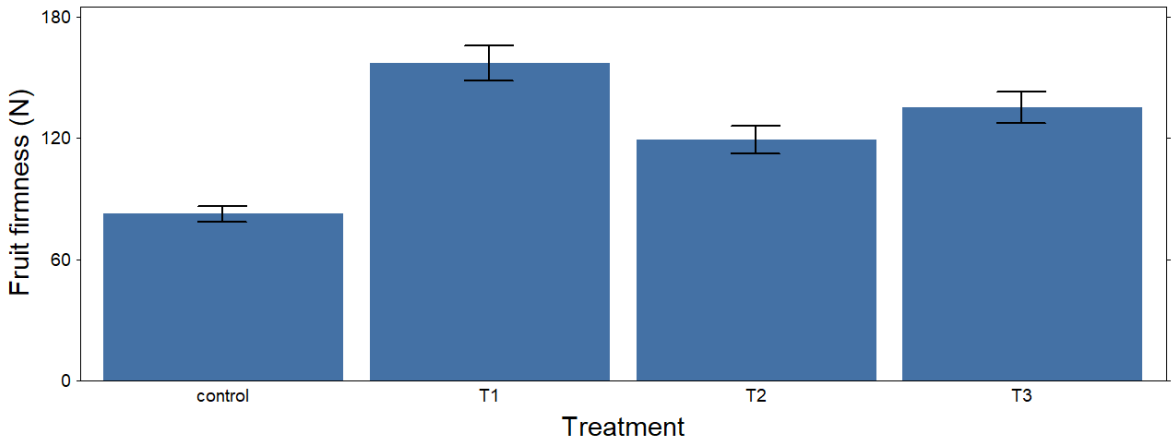

Figure 11. Changes in fruit firmness (N) of Strawberry in experiment 2 after 8 days of storage at 4ºC where (T1-10%, T2-20%, T3-30% of CO_2) treated for 2hour.

Although increases in fruit firmness were reported in several studies by the application of CO_2 but the time of treatment and concentration are also affect the firmness of strawberry (Chandra et al., 2015a). (Eum, Han and Lee, 2021) found 30% CO_2 for 3hour increased the fruit firmness in (*Fragaria* × *ananassa* Duch. 'Seolhyang') variety. The rise in fruit firmness observed at the end of storage may be attributed to the loss of water, which results in a firmer texture.

The variations of the increase in fruit firmness also depend on different cultivars based on pectin solubility in water and alkali (Matsumoto et al., 2010). The firmness also increased in response to 100 kPa CO_2 for 24 in 'Maehyang' strawberry in response to fruit cooling, and differences for fruit with and without epidermis (Hwang *et al.*, 2012). In an experiment with three cultivars of strawberry where flesh firmness of 'Aromas' was not affected by storage time or CO_2, but that of 'Diamante' and 'Selva' increased over storage time and in response to high CO_2 levels (air+20 kPa CO_2) (Pelayo, Ebeler and Kader, 2003). It's also an evident that changes of fruit firmness after postharvest treatment also depend on cultivar.

Fruit firmness based on storage days in experiment 2

In experiment 2 fruit firmness (N) was measured from untreated (control) samples initially and then in both controlled and treated samples at an interval of 5 and 8 days to see if there was any variation observed throughout these days. For graphical representation, some variable data were filtered out from different treatments to maintain the quality of the data.

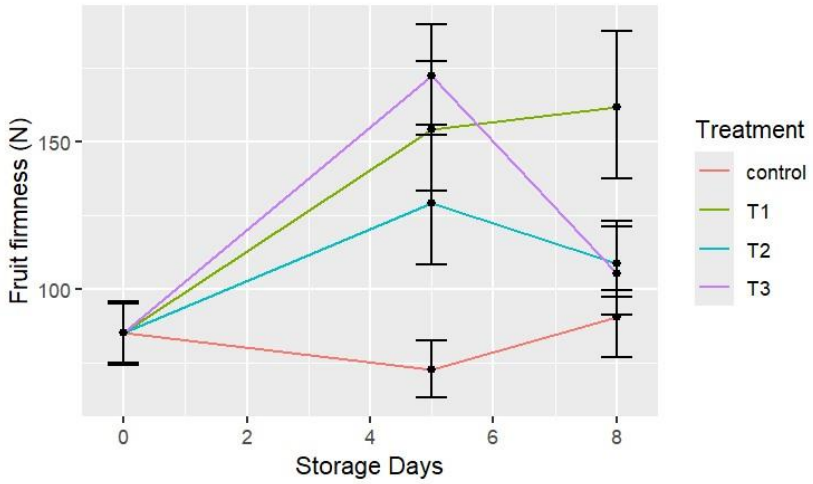

Figure 12. Changes in fruit firmness (N) of Strawberry in experiment 2 during 8 days of storage at 4ºC among different concentrations (T1-10%, T2-20%, T3-30%) of CO_2 and untreated (control) samples.

The control group exhibited a gradual increase in fruit firmness over the 8 days (Figure 12). The natural ripening process might be the possible reason behind it. However, on the 5th day CO_2 treated samples (10%, 20%, and 30% CO_2) showed higher firmness compared with control. Initially, T1 (10% CO_2) treatment results in a sharp increase in firmness on days 5 to 8 (Figure 12). Similarly, T2 treatment showed an initial increase in firmness on day 5 but lower than T1 and T3 and by day 8, the firmness decreased (Figure 12)**.** T3 treatment showed a drastic drop in fruit firmness right from the beginning which represented rapid softening of strawberries.

Short-term high CO_2 treatment has been shown to have both positive and negative effects on the post-harvest quality of fruits. On one hand, high CO_2 treatment can delay ripening, reduce respiration rate, and maintain fruit firmness, thus extending shelf life. On the other hand, it can also lead to the

development of off-flavors, excessive softening, and internal browning in certain fruits(Li *et al.*, 2022a) (Eum, Han and Lee, 2021). Therefore, it is crucial to optimize concentrations and storage conditions to maximize the benefits of high CO_2 treatment on fruit shelf life and quality.

5.3. Weight loss

Water loss from lower relative humidity and microbial activity may account for the weight loss experienced during postharvest. Over time in storage, a gradual loss in fruit weight could be observed. The weight was measured initially from each box before gas treatment and the final weight was measured at the end of storage period for both control and treated samples in case of experiments 1. Untreated control samples of strawberries and raspberries showed weight reductions of 1 g/fruit (11 g for 11 fruits) and 0.36 g/fruit (13.4 g for 38 fruits), respectively, compared with the treated samples. CO_2 treated samples showed less weight loss at the end of storage.

In experiment 2, the strawberry weight profiles for control and CO_2 treated fruit were different throughout the storage period as weight was measured at 0, 5, and 8 days before and after the gas treatment. However, there was no significant difference in mean strawberry weight between the treatments as measured using a two-tailed unpaired t-test. The mean weight of 10%, 20%, 30% CO_2 treated strawberries were respectively, (116.7 g, 117.4g, 99.4 g) higher compared with the control (91.3 g) at the end of storage time, but this difference was not significant as determined by the two-tailed unpaired t-test (Figure 13).

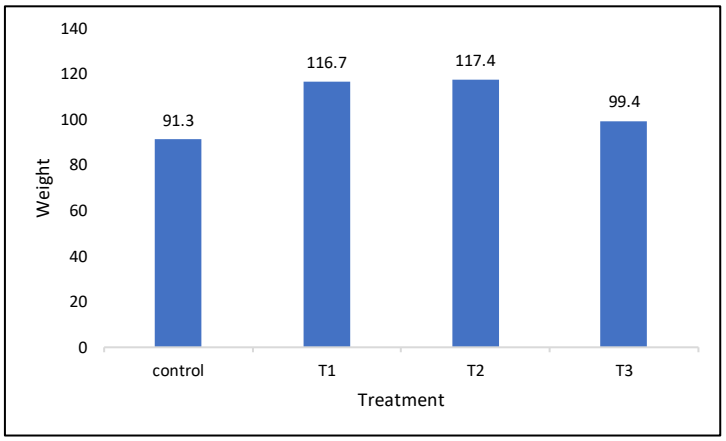

Figure 13. Weight loss of Strawberry in experiment 2. Weight was measured on 0,5 and 8th days of storage at 4^{o}C among different concentrations (T1-10%, T2-20%, T3-30%) of CO_2 and untreated (control) samples

5.4. Visual quality of strawberry

Cold storage slows down the senescence process of the fruit by decreasing its metabolic rate and preventing the growth and enzymatic function of pathogens (Mohammadi *et al.*, 2021). In experiment 2, after 22 days of storage at 4 C the decay was observed in control samples result in soft and deformed texture. Also, the fruit skin color was changed in control. Lower firmness, natural ripening and higher weight loss might be the possible reasons behind this degradation. All treated samples (10%, 20%, and 30% of CO_2) exhibited overall better quality than control samples (Figure 14).

In a similar research 'San Andreas', 'Albion' and 'Murano' cultivars were stored in 15°C retained high quality and shelf life after 7-day storage. The quality of strawberries stored in a conventional air storage was slightly lower compared to the fruit stored in a controlled and modified atmosphere (Błaszczyk *et al.*, 2022). Fruit color was not affected by treatments in 'Aromas', 'Diamante' and 'Selva' cultivars which were kept at 5 °C in air or air +20 kPa CO_2 for up to 15 days (Pelayo, Ebeler and Kader, 2003).

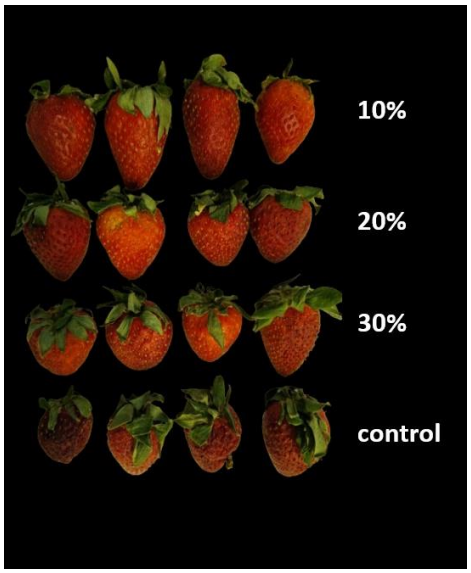

Figure 14. Visual quality of strawberry in experiment 2 among different concentrations (T1-10%, T2-20%, T3-30%) of CO_2 and untreated (control) samples after 22 days of storage at 4ºC.

Among all the samples with varying concentrations, specifically at 10%, 20%, and 30% of CO_2, only one fruit treated with 20% CO_2 concentration exhibited early signs of fungal infection caused by

Botrytis cinerea while two fruits (out of 4) from control samples showed spoilage due to the same pathogen (Figure 15).

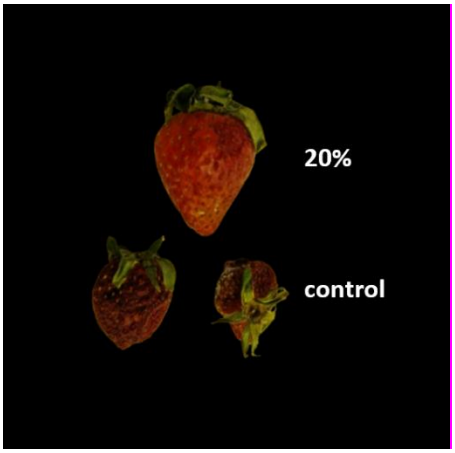

Figure 15. Sign of infection in strawberry after 22 days of storage at 4ºC between 20% CO_2 treated and control samples.

The utilization of high CO_2 levels, specifically 20 and 30 kPa CO_2, in combination with 10 kPa oxygen (O_2), has been found to significantly reduce strawberry fruit spoilage caused by *Botrytis cinerea*. This is attributed to lower respiration rates and higher pH levels in the fruit (Li *et al.*, 2022b). High-induced the expression of oligogalacturonides, thereby conferring defense against *Botrytis cinerea* in strawberry fruits, and lowering the decay incidence at seven days after its inoculation which was studied in experiment with (*Fragaria ananassa* Duch. 'Seolhyang') varieties after 3 h of exposure with 30% CO_2 (Eum, Han and Lee, 2021).

5.5. Microscopic images of strawberry cell

Untreated and treated tissue samples are observed under microscope to see the difference in cell structure and thickness. Untreated sample exhibited loose cell structure where treated one defined more cell integrity. Also, the untreated one was also irregular in shape while treated one showing more or less regular cell formation. The average cell wall thickness measured in untreated sample was (7.184 µm) and in treated sample (5.858 µm) (Figure 16). The cell wall thickness was also higher in treated samples visibly. Though it needed more repetition and further analysis to make any decision about the impact of CO_2 in this experiment. As the structures, functions, and properties of cell walls show wide and complex biological variations. A previous study found that exposing strawberries to

30% CO_2 for a short period of time delayed the degradation of the middle lamella, which is associated with cell wall degradation during ripening (Bang *et al.*, 2019).

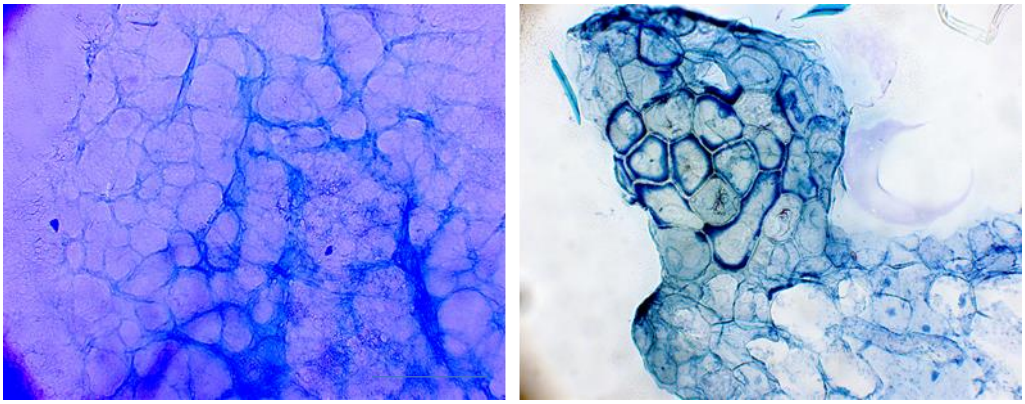

Figure 16. Microscopic view of untreated (left) and CO_2 treated (right) sample. Average cell wall thickness of untreated and treated sample under 200x magnitude and see scale bar.

6. CONCLUSION

- The short-term high CO_2 treatment had a significant impact on the post-harvest quality of fruits. Primarily. A set up was developed in lab. In experiment 1, the strawberry displayed higher weight, brix level and fruit firmness compared to untreated samples after 11 days of storage period in 4ºC**.** While, raspberry exhibited lower or similar brix level and fruit firmness compared to untreated samples, though at the end of storage period (10^{th} day) it showed lower weight loss. Although the effect of treatment was visually confirmed, due to the low sample size no statistically significant differences were found in experiment 1.
- After implementing minor adjustments such as increasing chamber number, reducing treatment time, relocate fan and installing taps, Experiment 2 was conducted using new strawberry samples. All treated samples at 10%, 20%, and 30% of CO_2 exhibited significant differences in fruit firmness compared to the control samples. Specifically, the 10% CO_2 treatment showed a significant difference in fruit firmness compared to the control. Additionally, both the 10% and 20% CO_2 treated samples demonstrated significant differences in brix level and fruit firmness. Overall, the 10% CO_2 treatment displayed significant differences from the control samples in both brix level and fruit firmness after 8^{th} day of storage.
- Based on timescale data, it was examined how treatment affects sugar content and brix levels of fruit during storage. Control samples showed decreased sugar content and increased firmness over time, while treated samples, especially at 10% and 30% CO_2, had increased sugar content, possibly due to enhanced sugar synthesis during ripening. Specifically, 10% CO_2 treatment led to a sharp increase in firmness from days 5 to 8, while 20% CO_2 treatment initially increased firmness but declined later. However, 30% CO_2 treatment led to rapid softening of strawberries, with a drastic drop in firmness observed. Overall, treated samples showed higher brix levels and firmness by day 8 compared to controls. Different studies on CO_2 treatment during post harvest suggests that variations in brix levels and firmness during storage depend on factors like variety, concentrations, storage conditions, and initial fruit quality.
- In Experiment 2, after 22 days at 4°C, control samples decayed, resulting in soft texture and color change. CO_2 treated samples (10%, 20%, and 30%) showed better quality overall. Only one fruit at 20% CO_2 showed early fungal signs, while two control fruits spoiled.
- Overall in our experiment CO_2 treatment showed positive effect on post harvest quality of fruits though it needs further experiments and more repetition especially in case of microscopic analysis. Moreover, we have to filter out some biased data and lack of information about the fruit source and absence of freshly harvested fruits might have an impact on result In several

experiments, different cultivars exhibited variations in their results. The result from our experiment might be beneficial for the small scale farmers to improve the shelf life of soft fruits after harvest.

7. SUMMARY

Postharvest management of fruits is crucial in ensuring food security and reducing wastage. It involves various practices from harvesting to storage and transportation. Postharvest treatments play a crucial role in post-harvest management by enhancing the quality, safety, and marketability of fruits, ultimately reducing post-harvest losses and ensuring that fresh produce reaches consumers in optimal condition. Various strategies are used to effectively manage soft fruits after harvest including MAP, MA or CA, ethylene inhibitor etc. As these fruits are highly perishable in nature. Controlling the levels in the storage atmosphere can also impact fruit respiration and ripening, which can affect fruit quality.

The current research aimed to understand the short term high CO_2 treatment on postharvest management of strawberry and raspberry. The short-term high CO_2 treatment had a significant impact on the post-harvest quality of fruits. The increase in levels resulted in delayed ripening, reduced weight loss, and decreased susceptibility to decay in various fruit samples. From the experiment 1 and 2, the difference in weight loss, brix level and fruit firmness were measured between treated and untreated samples. Additionally, the visual appearance also represented after the cold storage of 22 days at 4^{o}C.

In experiment 1, treated strawberry (20% CO_2 treated) showed less weight loss, higher fruit firmness and brix level compared with untreated samples though it wasn't significant ($p>.05$) due to low sample size and lack of repetition. Raspberry had lower or similar brix level and fruit firmness with untreated samples but it showed less weight loss (106.2 g) at the end of the storage. Though there was no significant differences between samples.

In experiment 2, short term high CO_2 treatment was done under different concentration (10%, 20%, and 30% of CO_2) on strawberry. After treatment, 10% CO_2 treatment had significant difference in brix level with untreated samples ($p<.05$). Therefore, all CO_2 samples showed significant differences (10%-$p<.005$, 20% and 30% - $p<.05$) in fruit firmness with control samples. However, 20% CO_2 exhibited significant difference with 10% CO_2 ($p<.05$) both in brix level and fruit firmness.

Furthermore, it was also presented the impact of treatment on brix levels and fruit firmness during storage. Control samples exhibited declining in sugar content and firmness over time. 20% and 30% CO_2 had lower brix level than control on 5th day of storage, though on 8th day CO_2 treated samples (10%, 20%, and 30%) had higher brix level than control samples. Fruit firmness was higher in CO_2 treated samples (10%, 20%, and 30%) at the end of storage period (8th day) comparing control one. In addition, the 20% CO_2 initially increased firmness but declined thereafter. Conversely, the 30% CO_2 had rapid softening of strawberries on 8th day. Only, 10% CO_2 had higher firmness overtime.

Overall, CO_2 treated samples (10%, 20%, and 30%) exhibited higher brix levels and retained firmer texture by day 8 compared to controls. Though we observed some notable increased or decreased of brix and fruit firmness among CO_2 concentrations on different day of storage which also support the studies where CO_2 concentrations showed variation on overall fruit quality depending on various factors, such as fruit species, maturity stage, temperature, light, and nutrient availability. It can significantly affect fruit physiology and quality attributes, but the effects can be complex and has been found to impact gene expression related to cell wall degradation, leading to improved fruit quality and extended shelf life. In our experiment untreated sample had loose cell structure and thickness than treated one though it needs further repetition.

CO_2 treated samples (10%, 20%, and 30%) exhibiting superior quality after 22 days at 4°C, as evidenced by reduced decay and maintenance of texture and color. One fruit at 20% had early sign of fungal infection whereas control samples experienced higher spoilage rates. This final result also proved the efficiency of short term high CO_2 treatment during postharvest management

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Figure

Table

10. ATTACHMENTS

DECLARATION

on authenticity and public assess of final essay/thesis/mater's thesis/portfolio

Student's name: Sadia Tabssum Tanni

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Title of the document: Effect of short-term high CO_2 treatment on post-harvest quality of fruits

Year of publication: April, 2024.

Department: Fruit growing

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STATEMENT ON CONSULTATION PRACTICES

As a supervisor of Sadia Tabassum Tanni (Neptune code: DNA5HU) I here declare that the final essay/thesis/master's thesis/portfolio has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules.

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