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Effect of packaging materials and storage conditions on development on tip rot in carrot

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Plant science

Acknowledgements

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Samandrag

Tuppråte er eit aukande problem ved lagring av norske gulrøter, og fører til mykje svinn og tap av god

Gulrot er ein av dei viktigaste grønsakene dyrka i Noreg. I 2020 vart det produsert 54 000 tonn gulrot som utgjer ein handelsverdi på 465 000 norske kroner. Også dei siste 10 åra har produksjonen av gulrot vore aukande. Ei stor menge gulrøter som vert produsert med tanke på matforbruk går derimot tapt under lagring. I samband med lagringa, er svinnet av gulrøter estimerte til å vere om lag 25%. I pakkeria vert om lag 10 000 tonn sortert vekk årleg, og lagringssjukdommar fører til halvparten av denne utsorteringa. Tuppråte har vorte eit aukande problem gjennom det siste tiåret. Auka kunnskap om korleis lagringsforhalda påverkar utviklinga av tuppråte vil vere viktige for å redusere svinn av gulrot gjennom distribusjonskjeda og bidra til ein meir berekraftig produksjon.

Tuppråte skuldast ikkje berre eit enkelt patogen åleine, men av fleire ulike patogen som enten opptrer åleine eller saman som eit kompleks. Soppane *Mycocentrospora acerina* og *Cylindrocarpon destructans* synes å vere sentrale i sjukdommen.

I denne oppgåva har effekten av korleis emballasjen og lagringstilhøva påverkar utviklinga av tuppråte vorte studert. Dei fire gulrotsortane 'Brillyance', 'Dailyane', 'Namdal' og 'Romance' vart enten lagra som friske kontrollar eller smitta av tuppråte og inokulert med enten *Mycocentrospora acerina* eller *Cylindrocarpon destructans*, og lagra i 3 ulike emballasjevariantar. Emballasjane hadde enten høg perforering som gav høg CO2 – og låg O2-konsentrasjon eller ei låg perforering som gav låg O2 og høg CO2-konsentrasjon. Pakkane vart lagra i ein periode på 21 dagar for å simulere lagring i distribusjonskjeda. Gjennom denne perioda, vart pakkane anten lagra ved 4°C i 3 dagar og deretter i 18 dagar ved 6°C, eller ved 4°C i 3 dagar, 20° i 3 dagar og deretter 6°C i 15 dagar. Dette simulerer lagring i daglegvarebutikk der gulrøtene ofte vert lagra i romtemperatur.

Gulrøter som vart lagra i kjølege omgjevnader gjennom heile lagringsperioden synte redusert roteutvikling samanlikna med gulrøter som vart lagra 3 dagar ved romtemperatur. Pakkane med låg perforering fekk raskare høg CO2 konsentrasjon ved romtemperatur og den auka CO2 reduserte roteutviklinga. Patogenet M.acerina gav djupare og meir alvorleg tupprote symptom enn C.destructans. Patogenet C vart observert å auke CO2 konsentrasjonen i pakken. I gulrot infisert med patogen fann ein at TPS innhaldet vart høgare.

Abstract

Carrot is one of the most important vegetables grown in Norway. In 2020, 54 000 tonnes were produced with a retail value for 465 000 NOK. The carrot production has also been increasing over the last decade.

A lot of the carrots produced for food consumption is however, lost during storage. Carrot wastes in storage are estimated to have a mean of about 25%. In packing houses 10 000 tonnes are graded out annually, and half of these are due to postharvest diseases. During the last decade tip rot disease has become an increasing problem. Getting a better understanding of how tip rot development is influenced by storage conditions will be an important step in reducing future carrot wastes in the distribution chain and contributing to a more sustainable production.

Tip rot is not caused by a single pathogen, and several possible agents may cause the rot in the tip, either on their own or in a complex of several pathogens. The pathogens *Mycocentrospora acerina* and *Cylindrocarpon destructans* have been found to be important agents in the disease.

In this thesis, the effect of packaging material and storage conditions on the development of tip rot in carrots has been studied. The four carrots cultivars 'Brillyance', 'Dailyance', 'Namdal', 'Romance' were either non-inoculated control carrots or inoculated by either *Mycocentrospora acerina* or *Cylindrocarpon destructans* and packaged in three different package treatments. The package types either had a high perforation giving high CO_2 - and low O_2-concentrations or a low perforation giving low O_2 and high CO_2-concentrations. The packages were stored for a 21-day period, simulating the storage in the distribution chain. During this storage period, packages were either stored at 4°C for 3 days and then 18 days at 6°C, or they were stored at 4°C for 3 days, followed by 20°C for 3 days, and then the last 15 days at 6°C. This simulates the storage in grocery stores, where carrots often are stored in room temperature.

Carrots stored in chill conditions through the whole storage period showed decreased rot development compared with carrots that had 3 days of storage at room temperature (20°C). Packages with low perforation quickly got high CO_2-concentration during storage at room temperature and the elevated CO_2 reduced the rot development. The pathogen *M.acerina* gave deeper and more severe tip rot symptoms then *C. destructans*. The pathogen C. destructans was observed to increase the CO_2-concentration in the package headspace. In carrots infected with pathogens, the polyphenol content (TPC) was found to be higher.

1. Introduction----------

1.1 The carrot

Cultivated carrot (*Daucus carota* subsp*.sativus*) is the most widely grown crop in the Apiaceae (syn. Umbelliferae family (Iorizzo et al., 2013). Carrot is a cool season biennial (Que et al., 2019). During its first growing year it stores carbohydrates in its root, (*figure 1*), and then it flowers in the second year (Stolarczyk & Janick, 2011). The roots have a high content of carotenoids. The type of carotenoid and the content depends on the colour and cultivar of the carrots (Simon et al., 2019). Orange carrots have a high content of α- and β-carotenes, making carrots a good dietary source for provitamin A (Simon et al., 2019; Stolarczyk & Janick, 2011). Polyacetylenes are compounds present in carrots, and human consumption can have both beneficial and negative effects. The main polyacetylenes found in carrots are: falcarinol, falcarindiol and falcarindiol-3-acetate. The concentration is highest in the periderm (Baranska & Schulz, 2005). Falcarindiol contributes to bitter off-taste in carrots and has been reported to be toxic to fungi (Kjellenberg et al., 2012).

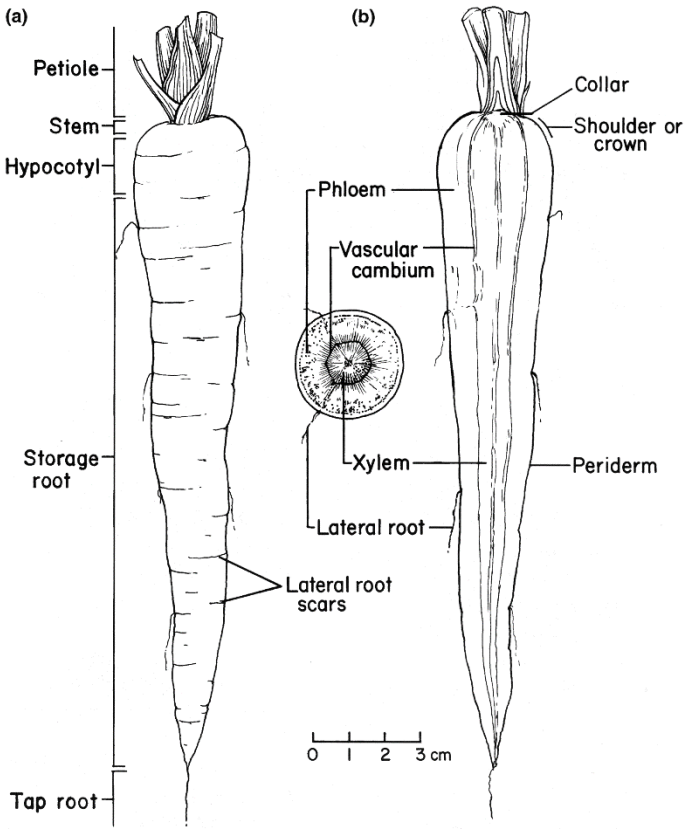

Figure 1. Carrot storage root anatomy: (a) longitudinal and (b) cross-section. Available at:

https://vcru.wisc.edu/simonlab/sdata/pimages/vegetableumbelliferae/index.html

1.2 Economic importance

The world production of vegetables was 1.15 billion tonnes in 2020 (FAO, 2020). Of this, 3.5% or 41 million tonnes was carrots and turnips. Asia contributed 63.8%, Europe 20.1% and America 9.4% of the production. China is the country with the largest production.

In Norway, 6440 hectares was used for vegetable production in 2020 (FAO, 2020; NIBIO, 2020; SSB, 2020), (*figure 2A*). Of this, 25% was used for carrot production. Over the recent decade, there has been a more or less steady increase in the carrot production, and in 2020 it reached approx. 54 000 tonnes, which is about 28% of the vegetable production, (*figure 2B*). The retail value for carrots in Norway in 2020 was 465 000 NOK (NIBIO, 2020).

Carrot wastes in storage in Norway has been estimated to range from 10 to 40% with a mean of 25% (Franke et al., 2013). In packing houses 10 000 tonnes of carrots are out-graded annually, and half of the out-graded carrot losses are due to postharvest diseases (Bond, 2016).

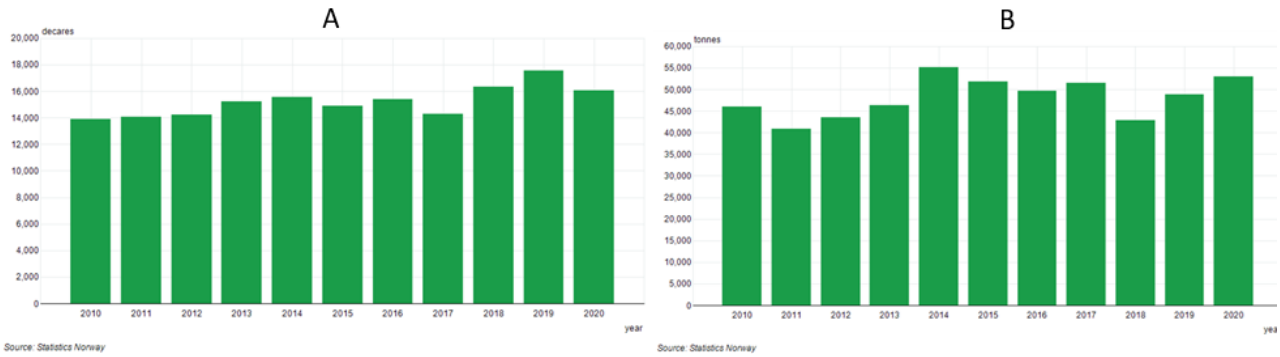

Figure 2. A) Decares used for carrot production in Norway annually from 2010 to 2020. B) Tonnes of carrots produced in Norway annually from 2010 to 2020. (SSB, 2020)

Available at: https://www.ssb.no/en/statbank/table/10507/chartViewColumn/

1.3 Tip rot

Tip rot has been observed with an increasing frequency over the recent decade and was found at 92% of the carrot storages in a survey conducted as part of the Optirot project in 2017 (Asalf et al., 2018). Tip rot has been found in carrots grown under different growing conditions (Nærstad, 2015). Tip rot is not caused by a single pathogen, and several possible agents may cause the rot in the tip, either on their own or in a complex of several pathogens. Pathogens found in relation to tip rot are liquorice rot (*Mycocentrospora acerina*), *Fusarium avernaceum*, *Cylindrocarpon destructans*, (Asalf et al., 2021; Nærstad, 2015), grey mould (*Botrytis cinerea*) and *Neonectria ramulariae* (Nærstad, 2015). The ones found to be most important for tip rot were *M.acerina* and *C. destructans* (Mohamad, 2021).

1.3.1 Mycocentrospora acerina (liquorice rot)

M*ycocentrospora acerina* is a necrotrophic pathogen and the causal agent for liquorice rot in carrots (Le Cam et al., 1994; Wall & Lewis, 1980). Liquorice rot is the most important post-harvest disease occurring in long-term storage of carrots in Norway (Hermansen et al., 2008).

1.3.1.1 Symptoms:

M*ycocentrospora acerina* causes a black, porous and juicy rot, (*figure 3*), that goes deep into the root, (*figure 3A*), and usually has a lighter brown zone next to healthy tissue (Hermansen et al., 2008; Hermansen, 2011). Infection can occur anywhere in the root, but the most common site is from the tip of the root (Hermansen et al., 1999). Symptoms are normally first seen after 2-3 months in storage (Hermansen et al., 2008). The foliage can also be attacked during the growing season and infection causes irregular brown to black spots, often along the edge of the leaves (Hermansen et al., 2008; Hermansen, 2011).

Figure 3. Mycocentrospora acerina *symptoms in carrot: (A) Internal rot going deep into the carrot. Photo: (Belachew, Asalf Tadesse); (B) Soft rot symptom. Photo: (Erling, Fløystad); (C) Dry rot symptom. Photo: (Arne, Hermansen). A and B Available at:*

https://www.plantevernleksikonet.no/l/oppslag/1270/

1.3.1.2 Survival

Mycocentrospora acerina is a deuteromycete with no known sexual stage (teleomorph). It survives in soil with chlamydospores (resting spores) (Hermansen et al., 2008). The fungus has a large range of host plants (Wall & Lewis, 1980), including vegetables (like parsley, celery, parsnips and lettuce), and ornamentals (*Viola tricolor)* and weeds (*Viola arvensis) (Hermansen, 1992; Hermansen et al., 2008; Hermansen, 2011)*. In a field experiment in Norway, carrots were still infected by *M. acerina* after an eight year crop rotation without cultivated host plants (Hermansen et al., 2008).

1.3.1.3 Epidemiology

M*ycocentrospora acerina* is a wound pathogen and rarely causes infection in healthy roots with an intact periderm (Davies & Lewis, 1981; Louarn et al., 2012). Mechanical injuries often occur during harvest, and infection in storage may originate from chlamydospores (resting spores) or mycelium in soil adhering to the roots (Hermansen et al., 2012; Louarn et al., 2012). Optimal mycelial growth occurs at 18°C, but the pathogen can grow at temperatures as low as -3°C (Hermansen et al., 2008; Hermansen, 2011). Another infection pathway is through the crown (Hermansen et al., 1999). The foliage can become infected through water splashing by conidia (asexual spores) produced from germinating chlamydospores (Hermansen et al., 2008).

1.3.2 Cylindrocarpon destructans

C*ylindrocarpon destructans* is a soilborne fungus and a common cause of root rot in several crops, e.g., in ginseng and seedlings of conifers and fruit trees (Buscot et al., 1992; Seifert & Axelrood, 1998; Seifert et al., 2003; Song et al., 2014).

1.3.2.1 Symptoms:

In carrots the rot has been described as either dry or wet, and from brown to darker brown or black, (*figure 4*), with a darker colour seen at higher temperature or after longer storage time (Mohamad, 2021).

Figure 4. Tip rot symptoms from Cylindrocarpon spp. on split carrots. Photo: (Rizan, Mohamad)

1.3.2.2 Survival:

The teleomorph (sexual stage) of *C. destructans* is the ascomycete *Ilyonectria radicola* (Farh et al., 2018). The fruiting bodies (perithecia) are generally not found in culture and are known primarily in vivo (Seifert & Axelrood, 1998). The fungus has a diverse range of hosts (Seifert & Axelrood, 1998; Seifert et al., 2003). Several species produce resting structures called chlamydospores that allow the fungus to remain viable in soil for prolonged periods without susceptible host plants (Dumroese & James, 2005). In gingseng production, *C. destructans* has been found to survive in soil after 10 years in rotation with non-host crops (Song et al., 2014).

1.3.2.3 Epidemiology:

In the fields, the fungus spreads by transportation of contaminated soil or via conidia (asexual spores) distributed either by irrigation or rain water (Dumroese & James, 2005). The optimal temperature for mycelial growth

is 18°C (Rahman & Punja, 2005). In ginseng roots, wounding of the roots was a prerequisite for infection by weakly virulent strains, and enhanced disease severity by more virulent strains (Rahman & Punja, 2005). Chlamydospores in the soil adhering to the carrots, and wounds is likely an important entryway during storage (Richard et al., 1998).

1.4 Factors affecting quality during storage

Carrots are classified based on root size, shape, uniformity, surface smoothness, surface and flesh colour, firmness, sweet taste, the characteristic odour and flavour of terpenes, and lack of sprouts, cracks, rot, and bitterness (Edelenbos et al., 2020).

Carrots go through several steps in the distribution chain before reaching the consumer. During the period between harvest and consumption, the quality can be affected by several factors, including mechanical damage, temperature, relative humidity and package atmosphere (Seljåsen et al., 2013).

The optimum storage temperature for carrots is 0-1°C and a relative humidity at 98-100%, and carrots can be stored for 6-8 months under these conditions (Edelenbos et al., 2020). At 0-5°C and 90-95% RH, carrots can be stored for 5-6 months (Edelenbos et al., 2020).

1.4.1 Temperature

High temperature can have negative effect on shelf life and quality of carrots in itself, but may also affect the RH (Shibairo et al., 1997) and the gas concentration in the packages, which are important factors that affect the quality during storage (Edelenbos et al., 2020; Seljåsen et al., 2013).

Higher temperatures lead to increased rates of respiration in carrots (Iqbal et al., 2009), a process that provides energy for plant biochemical processes, through the breakdown of organic substrates. During aerobic respiration,

O_2 is consumed, and CO_2 produced. Respiration can be expressed as CO_2-production (RCO_2) or O_2-consumption (RO_2) (Iqbal et al., 2009). The ratio of CO_2 produced to O_2 consumed is the respiratory quotient (RQ) with a normal range of 0.7-1.3. Too low O_2 or too high CO_2 results in anaerobic respiration, which reduces the quality (Iqbal et al., 2009).

The respiration rate is minimal in long-term cold storage of carrots at 0°C (Iqbal et al., 2009). Increased temperatures enhance the concentration of ethanol, CO_2 and ethylene and decrease the concentration of O_2, as well as the sucrose and total sugar content (Seljåsen et al., 2004). High temperatures (20°C vs 2°C) increased the intensity of the negative characteristics ethanol flavour and odour, aftertaste, earthly flavour, terpene flavour and bitter taste (Seljåsen et al., 2004). However, the temperature had inconsistent effects on the β-carotene content during storage (Seljåsen et al., 2013).

In carrots, low temperatures restrict fungal growth, but bacterial growth is not inhibited (Edelenbos et al., 2020; Seljåsen et al., 2013). In packinghouses, disease incidence of carrots was positively correlated with storage degree hours with a base temperature of 0°C (Asalf et al., 2018). Degree hours is a measure of how much (in degrees) and for how long (in hours) the air temperature increase above the base temperature.

1.4.2 Air humidity

Transpiration has various adverse effects on postharvest quality and shelf-life of fresh fruits and vegetables. The water release can lead to direct weight loss and moisture condensation inside packages (Bovi et al., 2016). Generally, the rate of moisture loss is proportional to surface area of the carrot root and the water vapour pressure deficit (WVPD) which is determined by temperature and RH of the surrounding air (Shibairo et al., 1997). This means there is a higher transpiration rate and water loss for larger than smaller carrots, and in storage conditions with a low RH.

The direct effect of water loss is a decrease in weight, which can in prolonged storage at low RH lead to loss of one-fourth of the original weight (Edelenbos et al., 2020), and lower weight also means a reduced market price. The loss in weight can cause carrots to shrivel and become susceptible to disease (Shibairo et al., 1997), as well as causing a reduction in β-carotene, fructose and glucose (Soria et al., 2009). In order to preserve the content of health-related compounds, the sensory quality and increase shelf life, a high RH at 98-100% is required (Seljåsen et al., 2013).

Condensation on the inside of packaging materials can have a negative effect on postharvest quality and shelf life, as free water facilitates pathogen infection and growth (Bovi et al., 2016). The temperature where this occurs is known as the dew point (Holcroft, 2015). Condensation occurs on any product that is below the dew point temperature of the surrounding air (Holcroft, 2015). Condensation inside packaged fresh produce occurs when evaporated water stays within the package instead of transmitting through the packaging film (Bovi et al., 2016). Temperature fluctuations, breaks in the cold chain, and insufficient ventilation promote condensation (Holcroft, 2015).

Humid saturation is achieved using a perforated plastic lining on the boxes in bulk storage (Seljåsen et al., 2013).In the distribution chain in Norway today, the most common packaging for carrots are pouches made of laser-perforated 40 µm biaxially oriented polypropylene (BOPP) film (ScanStore Packaging AS, Middelfart, Denmark) or carrots on trays placed in pouches of laser-perforated 25 µm BOPP-film. (Larsen & Wold, 2016).

1.4.3 Package atmosphere

Storage in controlled atmosphere (CA) or modified atmosphere (MA) is a method used to reduce respiration, and thus increase the shelf life of plant products (Seljåsen et al., 2013). Lower O_2-concentration and elevated CO_2-concentrations reduce the respiratory rate (Edelenbos et al., 2020).

Storage in an O_2 concentration at (7%) seems to have a negative impact on the sensory quality of carrots (Seljåsen et al., 2004). During anaerobic respiration in carrots, ethanolic fermentation is more important than lactic fermentation, and the ethanol content may increase and lead to a disgustingly sweet taste and ethanol flavour (Kato-Noguchi, 1998; Seljåsen et al., 2004). Low O_2 levels combined with high temperatures further increase the ethanol content (Kato-Noguchi, 1998). Storage in CA and MA should therefore be avoided (Seljåsen et al., 2013).

Packaging may create inappropriate MA through inadequate O_2 and CO_2 transmission through the film (Larsen & Wold, 2016; Seljåsen et al., 2004). As $O_2 \leq 7\%$ (Seljåsen et al., 2004) and CO_2 above 5% (Watkins, 2000) are injurious to carrots, MA- packaging (MAP) should be used with caution (Edelenbos et al., 2020). The O_2 and CO_2- permeability should therefore be high enough to avoid negative effects of low O_2- or high CO_2-concentrations. Elevated CO_2 (5-14%), does however decrease microbial rot, though low O_2 (3%) increases it.

1.4.4 Phenolic browning

Surface discoloration of carrot roots, commonly called browning or 'phenolic browning', is a serious problem in marketing and storage (Chubey & Nylund, 1969; Zhang et al., 2005) . Browning is initiated by physical damage to the carrot surface during harvest and postharvest brush polishing, thereby exposing the internal tissue to oxidation. It usually develops when carrots are on the market shelf after a period of cold storage. The brown colour originates from enzymatic degradation of phenolic compounds in the presence of O_2 at the periderm (Toivonen & Brummell, 2008). Total phenolic content (TPC) of carrots was found to be highest in tissue near the root surface and decreased with the depth of tissue (Chubey & Nylund, 1969).

1.5 Hypothesis

The main aim of this study was to determine the effect of the packaging material and storage conditions on the development of tip rot in carrots. Three different commercially used packaging materials with different gas and water properties were selected to study how the development of tip rot was influenced by the package microclimate. The quality and disease development in the four carrot cultivars 'Brillyance', 'Dailyance', 'Namdal' and 'Romance' were monitored during a storage period simulating the distribution chain. The quality and disease development of tip rot in healthy control carrots, and carrots tip inoculated by either *Mycocentrospora acerina* or *Cylindrocarpon destructans* were recorded in the study.

1. In Asalf et al. (2018) it was seen that disease incidence in carrots was positively correlated with storage degree hours, and storage pathogens have a higher growth rate at higher temperatures (Edelenbos et al., 2020). It is therefore hypothesized that tip rot development will be higher for carrots stored at room temperature in grocery stores than for carrots stored in cold store.

2. Based on findings in Larsen & Wold (2015 and 2016) the CO_2-levels can get very high in packages after only 3 days at room temperature. Too high CO_2-levels can have harmful effects on carrot quality. High CO_2-levels can also affect fungal growth negatively, and it is hypothesized that elevated CO_2-levels in the package will reduce the development of tip rot.

3. Loss of water due to low RH has negative effects on quality of carrots. In some cases, it may increase the susceptibility to pathogens, but the lack of free water also has negative effects on pathogen development. Packaging in a pulp tray, is hypothesized to give a lower RH in the package and reduced development of tip rot, though some loss of quality due to reduced weight may occur.

4. Carrot browning due to oxidation has become an increasing problem and is suspected to be related to changes in the metabolism in response to symptomless infections in early stages. It is therefore hypothesized that carrots infected with *Mycocentrospora acerina* or *Cylindrocarpon destructans* will have a higher content in polyphenols.

2 Material and Methods----------

2.1 Location of the experiments

The carrots and pathogens used in the experiment were provided from the earlier experiments at NIBIO, Ås. Healthy carrots and carrots infected at the root tip with *Mycocentrospora acerina* and *Cylindrocarpon destructans* were stored until tip rot symptoms developed at a cold store at Vollebekk in Ås, Norway. The storage and packaging experiments related to this thesis were conducted at NOFIMA (Norwegian Food, Fisheries and Aquaculture Research Instute) in Ås, Norway, during the spring of 2021. The biochemical analysis was done at NOFIMA and at the Norwegian University of Life Sciences (NMBU) during the fall of 2021.

2.2 History of carrot samples

Carrots and pathogens used for this experiment were provided from the earlier experiment conducted to identify the causal agents of tip rot (master thesis work of Rizan). In brief, four carrot cultivars 'Brillyance'(B), 'Dailyance'(D), 'Namdal'(N) and 'Romance'(R) were grown in pots the previous year (from 12.06.2020 to 14.06.2020) to ensure similar growth conditions for all carrots used (*figure 5A*). The carrots were harvested and stored in cold store near 0°C until inoculation by the candidate pathogens (*figure 5B*). All four cultivars were inoculated on 12.11.20 with one isolate each of *Mycocentrospora acerina*, *Cylindrocarpon destructans*, *Fusarium avenaceum* and *Dictyostelium sp.* and stored at 0 + 1°C, 3 ± 1°C and 6 ±

1°C until they were used in the packaging experiments (*figure 5C*). Carrots inoculated with *Mycocentrospora acerina* and *Cylindrocarpon destructans* that were stored at 0 + 1°C or 3 ± 1°C were used in the packaging experiment. The tip rot development on carrots inoculated with *M. acerina* by the time the carrots were used for the packaging experiment, *(figure 5D).* For details about the tip rot causal agent identification, inoculum production, inoculation procedure, pathogenicity test and tip rot development after inoculation see the master thesis by Mohamad, (2021). Healthy carrots used in the respiration experiment and the pre-trials were stored in plastic bags at 0 + 1°C. Carrots used in the main experiment were either healthy or sick, that is with tip rot symptom.

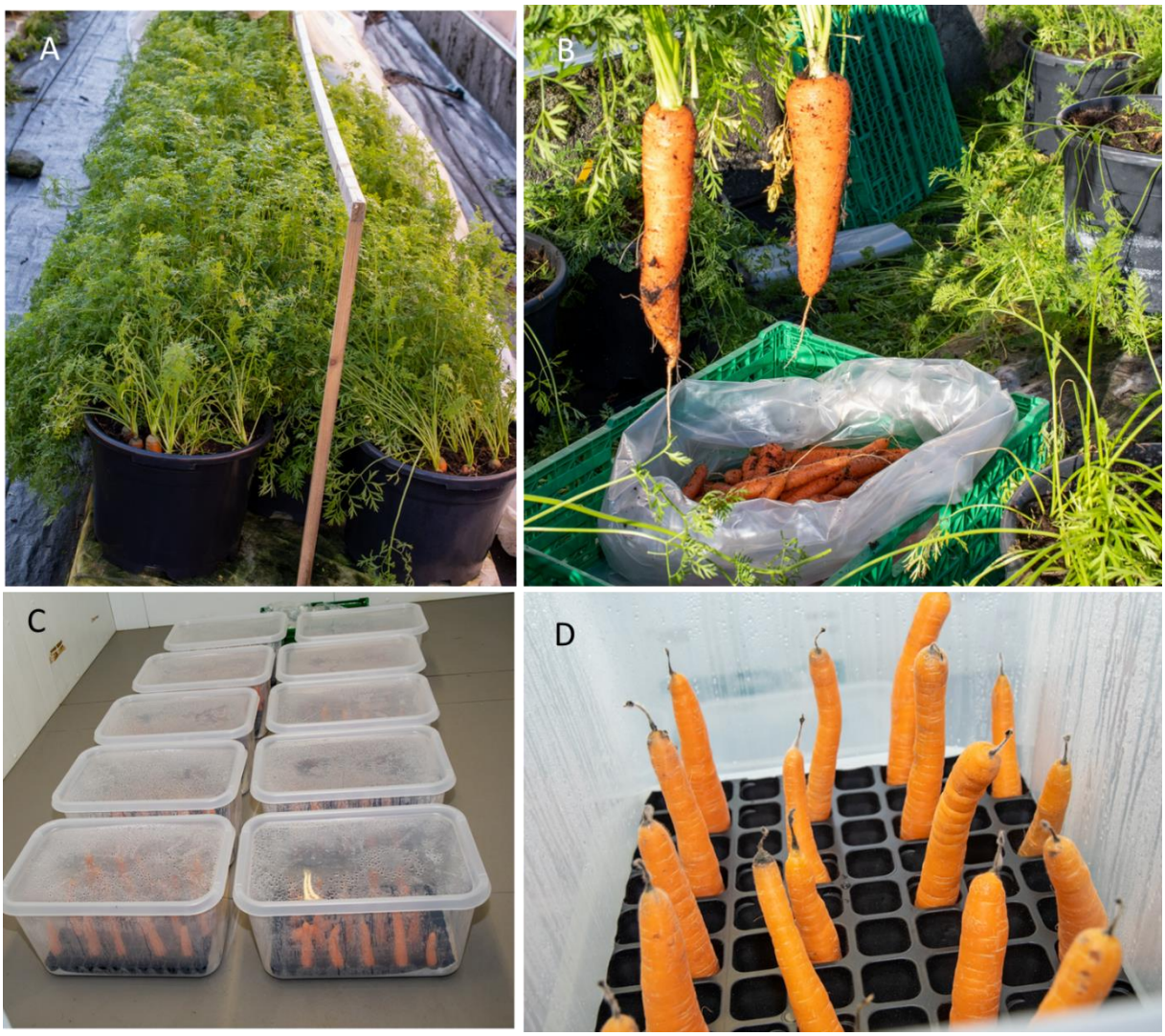

Figure 5. Background information about the carrots used in the packaging experiment: A) Carrot growth in pots, B) Carrots at harvest, C) carrots inoculated by the candidate pathogens and incubated in cold room (3±1°C) and D) Tip rot developmental stage on M. acerina *inoculated by time the carrots were used for the packaging experiment. (Photo: Belachew Asalf).*

2.3 Determination of carrot respiration

2.3.1 Preparations

Healthy carrots of each cultivar were collected from the cold storage on 25.02.2021. The soil and dirt were removed from each carrot by brushing and rinsing in cold tap water (*figure 6*). Carrots were then placed in plastic bags, with the opening folded in to keep them from drying out, and kept in a cold store at 4°C. On 26.02.2021 and 03.03.2021, the carrots were packaged in 850mL (40mm) High Density Polyethylene (HDPE)-trays (Berry Packaging Norway AS, Kristiansund, Norway) top sealed with a barrier film (Wipak, Nastola Finland). The top film was sealed on the trays using a Multivac T200 (Multivac, Wolfertschwenden, Germany). The sealing temperature was 165°C. Five carrots from each cultivar were put in each tray and the weight of the carrots was recorded. All the trays were packaged and moved to a climate chamber and stored at 20°C.

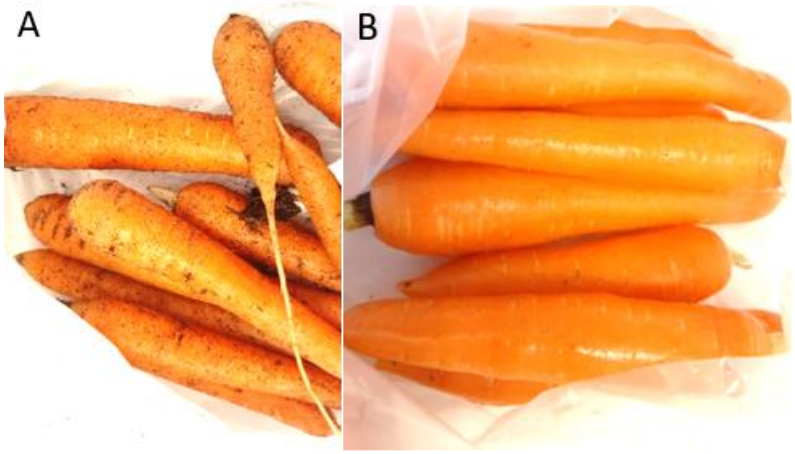

Figure 6. Healthy carrots before and after cleaning. Photo: (Torstein Kvamme, 25.02.21)

2.3.2 Measuring gas content(O_2/CO_2) in the headspace of the packages

The measurements were done according to the methodology described in (Larsen, 2015). The starting atmosphere in the headspace of the packages was air. The gas content (O_2/CO_2) in the headspace of the packages was measured using a Checkmate II O_2/CO_2 analyser (PBI Dansensor, Aalesund, Norway). The gas samples were measured with the help of a needle connected to the gas analyser, through a septum placed on the top film, (*figure 7*). The headspace gas concentration was measured at constant intervals (every second hour) for 10 hours after packaging. For each cultivar, 4 packages (replicates) were measured. The headspace volume in the packages was determined by subtracting the volume of the carrots from the total volume of the packages.

Figure 7. Measurement of gas concentration in the headspace of the packages. The gas analyser measures the gas concentration with the help of a needle connected to the gas analyser, through a septum placed on the top film. Photo: (Torstein Kvamme, 26.02.21)

2.3.3 Predicting optimal number of perforations in the packages

The gas measurements were used to calculate the O_2 consumption rate (RO_2) and the CO_2 production rate (RCO_2) using linear regression (Fonseca et al., 2000; Iqbal et al., 2005). The gas measurement times, total volume of package, the headspace volume, carrot weight, RO_2 and RCO_2 were plotted into a prediction model in microsoft excel (Larsen, 2015). The

prediction model was used to estimate the number of perforations needed in the top film to attain the desired gas concentrations in the headspace of the packages. Only the measurements made on the 26.02.2021 were used to predict perforation for the pre-trial.

2.4 Pre-trial (package experiment using healthy carrots)

In the pre-trial, the optimal number of perforations was determined and adjusted in preparation for the main trial.

2.4.1 Treatments

In the pre-trial, three types of packaging conditions (treatments) were used (High, Low and Bio), (*figure 8*). The High-Density Polyethylene (HDPE)-trays were the same as described above in section 2.2 and packaged with the same top film.

The three treatments were:

i) High: HDPE-tray with a low number of perforations giving high CO_2-concentrations (10-12%CO_2).

ii) Low: HDPE-tray with a high number of perforations giving low CO_2-concentrations (<5% CO_2).

iii) Bio: a 1200 mL (45mm) pulp-tray (Rottneros packaging AB, Sunne, Sweden) in a sealed Polylactid acid (PLA)- pouch (Taghleef Industries spA, San Giorgio di Nogaru (DU), Italy), giving low CO_2-concentrations (<5%CO_2).

Figure 8. Packages for each treatment: A) Bio (Low CO_2); B) Low (CO_2); C) High (CO_2). Four packages (replicates) in each treatment. Each package has four carrots with one of each of the four cultivars ('Brillyance'(B), 'Dailyance'(D), 'Namdal'(N) and 'Romance'(R)). Red dots on the top film indicate additional holes (by acupuncture needle). Photo: (Torstein Kvamme, 22.03.21)

2.4.2 Preparations

For this experiment, one carrot of each of the four cultivars was placed in each tray, four carrots in total. Each carrot was cleaned as described above in section 2.2. Long carrot tips were removed as well as petiole growth from the tops and root hair. Carrots were then placed in plastic bags to keep them from drying out and stored at 4°C over night. The carrots were packaged the following day, on 03.03.2021.

Four packages (replicates) were used for each treatment (High, Low and Bio), i.e, 12 packages in total, (*figure 8)*. The weight of the carrots in each package was measured. The HDPE-trays used in the High- and Low-treatments, were top sealed with a barrier film (Wipak, Nastola Finland) using the sealing machine as described above under section 2.2 . The pulp-trays did not fit the sealing machine and were instead packaged inside PLA-pouches that were closed using an impulse hand sealing machine (Packer poly sealer PBS-400-C, Packaging Aids Ltd., Norfolk, England). To attain higher perforation for the Low CO_2-treatments, additional holes were made in the top film with an acupuncture needle. The holes were made visible

with a marker. The number of holes needed was estimated using the prediction model as described above under section 2.2. After packaging the trays, the packages were moved to the 4°C cold store. The O_2/CO_2-content in the headspace of the packages were analysed using the gas analyser after 12 and 24 hours. The packages were then moved to a 20°C chamber. The O_2/CO_2-content of the headspace in the packages, were then measured daily. Perforation was further adjusted by making additional holes in the high perforation treatments. After four days at 20°C the experiment ended on 07.03.2021.

2.5 Main-trial (Packaging experiment using healthy carrots and carrots with tip rot)

Table 1: Overview for the number of packages in each combination of treatments (types of packaging material and level of Co_2), Healthy carrot (control) and carrots with tip rot (caused by Cylindrocarpon destructans *and* Mycocentrospora acerina*) and storage conditions: Chill where carrots were stored at 4°C for 3 days and 6°C for 18 days or CRTC (Chill-Room Temperature, Chill) is where carrots were stored 3 days at 4°C, then 3 days at 20°C and finally 15 days at 6°C. Each package contained 1 carrot of each of the four cultivars 'Brillyance'(B), 'Dailyance'(D), 'Namdal'(N) and 'Romance'(R).*

Treatments (packaging material and CO_2 - concentration)	Carrots with and without tip rot	Storage temperature	
		Chill	CRTC
HDPE-tray high CO_2 (=10-12%CO_2)	Control	3	4
	C. destructans	3	4
	M. acerina	3	4
HDPE-tray low CO_2 (<5%CO2)	Control	3	3*
	C. destructans	3	3*
	M. acerina	3	3*
Bio: Pulp-tray low CO_2 (<5%CO_2)	Control	3	3
	C. destructans	3	3
	M. acerina	3	3
Total		27	30

* The nine packages from the Low treatment with CRTC conditions were used in the biochemical analysis

2.5.1 Pathogens

For the main experiment, the packages either contained healthy carrots (Control), carrots infected with *C. destructans*, or carrots infected with *M. acerina*, (*table 1)*

2.5.2 Treatment of the carrots

Before packaging, the carrots were washed in cold tap water and cleaned similarly to what was described in the pre-trial. All carrots were measured in length. For carrots infested with *M. acerina* or *C. destructans*, the severity of the tip rot was measured as the length of lesion from the tip of the root, that is inoculation point. The rotten part was removed. After removal of the rotten part, the carrots were measured in length. For carrots with deeper rot inside, the tip of the carrot was adjusted to the depth of the lesion, making the new carrot tip at the end of the lesion. Each carrot was marked with a code to compare the effect of the treatments on tip rot development before and after storage.

2.5.3 Treatments (Packaging material)

The three treatments (High, Low, and Bio) were the same as described above in section 2.3. Based on the findings in the pre-trial, the number of additional holes (with acupuncture needle) for each treatment type was adjusted. The number of holes used in each treatment was: High (2 holes); Low (30 holes); and Bio (10 holes). The different package variants are seen in *figure 9*.

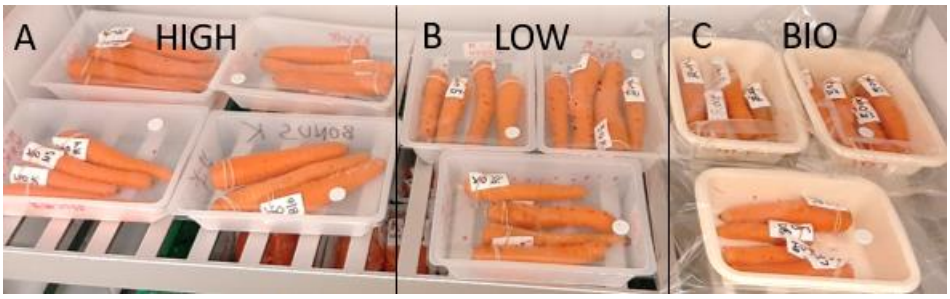

Figure 9. Packages of each treatment: A) High (CO_2); B) Low (CO_2); C) Bio (Low CO_2). Red dots on the top film indicate additional holes (by acupuncture needle) to increase perforation. Photo by (Torstein Kvamme, 31.03.21).

2.5.4 Storage temperature

After packaging of the trays, they were stored at two different storage temperatures (Chill or CRTC) simulating the different steps in the distribution chain, (*table 2*). Storage conditions: Chill: 3 days at 4°C followed by 18 days at 6°C; CRTC (Chill-Room Temperature-Chill): 3 days at 4°C followed by 3 days at 20°C and then 15 days at 6°C. After 21 days of storage the packages opened and further examined.

Table 2. The carrots were either stored at Chill or CRTC storage conditions. Carrots were packaged on the 24.03.2021. Carrots stored in Chill conditions were first stored at 4°C for 3 days and then 6°C for 18 days. Carrots stored in CRTC conditions were first stored at 4°C for 3 days, then 20°C for 3 days followed by 6°C for 15 days. All packages were examined after 21 days of storage, between 14.04.2021 and 16.04.2021. Blue colour indicates storage at low temperature (4°C and 6°C), and red colour indicates storage at high temperature(20°C).

	Packaging		Storage period				Examination
Time	24.03.2021	3 days	27.03.2021	3 days	30.03.2021	15 days	14.04.2021 - 16.04.2021
Chill	4°C	→	6°C	→	→	→	
CRTC	4°C	→	20°C	→	6°C	→	

2.4.5 Measurements of gas-concentration, relative humidity, and temperature during the storage period

The gas content in the headspace of the packages was measured during the storage period, with more frequent measurements during the storage at room temperature. At room temperature the packages were measured every day. After transfer to 6°C the packages were measured on 07.04.21 and on 13.04.21. If the measured O_2-level in the headspace of a package were below 2% during the storage period, the perforation of the package was adjusted by adding more holes (with an acupuncture needle) to improve the gas conditions. The humidity and temperature were measured every 30 minutes with temperature/humidity data loggers (DS 1923 Hygrocron, iButton®) in 15 of the packages, distributed throughout the different treatments and storage conditions. For most of the storage period the packages were stored in the storage room at 6*°C, (figure 10).*

Figure 10: The storage room at 6°C. The packages in the three shelves are from the CRTC condition. The top shelf had packages with non-inoculated control carrots, the middle shelf had packages with carrots infected by Cylindrocarpon destructans *and the bottom shelf had packages with carrots infected by* Mycocentrospora acerina*. The packages from the Chill condition are placed in boxes on the floor. Photo: (T. Kvamme, 31.03.21)*

2.4.6 Examination after the storage period

After 21 days of storage the packages were opened, and the carrots examined in the period 14.04.2021-16.04.2021, (*table 2*). For some of the

packages the smell and taste were described. First, visible signs of growth and rot on the surface was described, then the carrots were sliced in half to examine the rot on the inside. Sprouting in the top, and root hair growth was graded from 1 (little/no growth) to 5 (much growth). The bendability of the carrot was graded between 1 (not very bendable) and 5 (very bendable). Both visible rot on the surface and inside, were measured as length from the tip in millimetre (mm).

2.5 Biochemical analysis

2.5.1 Sampling of the carrots

All carrots from the Low-CRTC treatment were frozen down in liquid nitrogen for biochemical analysis, see (*table 1*). The packages were first opened after the storage period and carrots examined for growth and rot as described in section 2.4. The tip or rotten part was removed, and then the next 2cm of the carrot was used for the samples, (*figure 11)*. The carrot pieces were weighed and placed in liquid nitrogen -196°C for freezing. The carrot samples were kept in liquid nitrogen until it stopped boiling (at which point the sample was completely frozen). The sample was then put in a marked plastic bag (appropriate for storage in freezer) and temporarily stored in dry ice in a styrofoam box (with a lid). All the samples were then stored at -80°C.

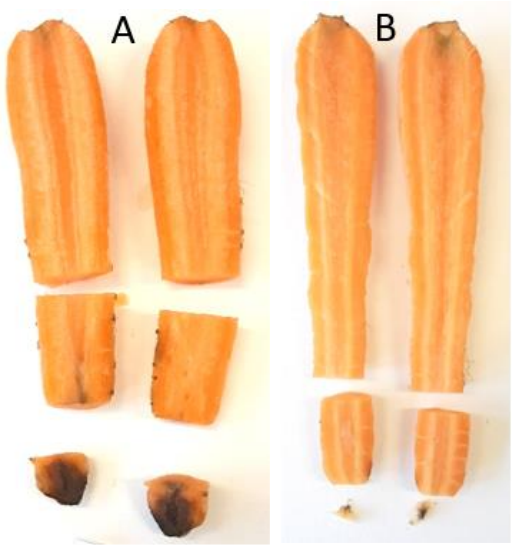

Figure 11. Carrots infected with: (A) Mycocentrospora acerina*; (B)* Cylindrocarpon destructans*. The rotten part in the tip of the root was removed. The next 20mm (middle pieces) of the roots were frozen in liquid nitrogen and used for the biochemical analysis. All 34 carrots in the Low- treatment stored at CRTC conditions were sampled for the biochemical analysis. Photo: (Torstein Kvamme, 14.04.21)*

2.5.2 Freeze drying

The preparations for the chemical analysis were based on the procedures in Rybarczyk-Plonska et al. (2016).

First, the samples were transferred to tubes which were weighed before and after adding the samples. Before and after transfer, the samples and tubes were again kept in a styrofoam box (with a lid) with dry ice to avoid the carrot samples from melting. An aluminium paper lid was folded over the tubes. Six holes were added to the lids using an acupuncture needle to ensure perforation for the freeze drying. All the samples were freeze dried using a Gamma 1-16 LSC Plus freeze dryer (Martin Christ, Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) at -50°C. A light screening cloth was placed over the sample shelf to avoid light contamination

2.5.3 Extraction of polyphenols in carrot

The extraction method was based on Rybarczyk-Plonska et al. (2016), with a small adjustment by using refrigerated methanol.

The samples were ground into a powdery sample using a mortar and pestle. The samples were weighed to 0.200 g in a 15 ml centrifuge tube, and exact weight noted (Rybarczyk-Plonska et al., 2016). The tubes were capped tightly to prevent the powder from taking up moisture. The sample tubes were then stored in at -80°C until extraction. Additional ground sample material was kept in reserve at -80°C, in case new samples needed to be made.

Sample tubes were taken out for extraction and extracted in the period between 08.11.2021 and 11.11.2021. First, 3.00 mL 70% methanol (MeOH) was added to the sample tube using a 5 ml pipette. The samples were gently mixed with a flat spatula and then by swirling the tube. In between pipetting, the tubes were kept in ice in a styrofoam box with a light-shielding cloth. All samples were kept at least 10 minutes on ice. Before centrifugation the tubes were vortexed for 5 seconds. The samples were centrifuged at (4400 rpm, 5346 x *g*) for 15 min at 4°C. The supernatant from each sample tube was transferred to new labelled tubes suitable for storage at -80ºC. The pellet was resuspended with 3.00 mL 70% methanol (MeOH). After a new round of resting, mixing and centrifugation, the second supernatant was combined with the first. The new mixed samples were stored at -80°C, while preparing the rest of the sample extracts.

2.5.4 Analysis for Ferric Reducing Antioxidant Power (FRAP) and total polyphenol content (TPC).

The analysis for Ferric Reducing Antioxidant Power (FRAP) and total polyphenol content (TPC) were done according to the procedures described by J. Volden, 2007.

2.5.4.1 Preparations

After all sample extracts were prepared, they were transported on the 11.11.2021 and stored at -20°C degrees. The following day, 12.11.2021, the samples were analysed for ferric reducing antioxidant power (FRAP) and total polyphenols with a konelab30i (Thermo Electron Corp. Vantaa, Finland). Reagents and solutions were made according to the procedures for FRAP and total phenols analysis with the konelab30i (Volden, 2007a; Volden, 2007b).

The samples were taken out of the freezer and stored in a refrigerator. As the carrot samples still had some pellet in them, they were centrifuged (13 200 rpm) for 4 minutes at 4°C. After centrifugation the tubes were stored at 4°C in the centrifuge. When the konelab30i was ready for analysation of new samples, the samples were transferred from the tubes and into appropriate cuvettes for analysation by the konelab30i.

2.5.4.2 Measurements

The konelab30i made a standard curve using a standard solution, which is used as a comparison for the measurements to calculate the concentrations. The konelab30i reads the absorbance and calculates concentration in the samples.

For the Ferric Reducing Anitoxidant Power (FRAP) assay based on , 200 µl acetate buffer, 20 µl iron trichloride and 20 µl TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) was mixed (Volden, 2007a). From the sample (or standard solution), 8 µl was added. After incubation at 37 ^{0}C for 10 min, the absorbance was measured at 595 nm. This would optimally be measured at 593 nm (Benzie & Strain, 1996).

For the total polyphenol content (TPC) based on Waterhouse (2002), 100 µl Folin-Ciocalteu (FC)- reagent was added to 20 µl sample (or standard solution) and mixed (Volden, 2007b). The mixed sample was incubated at 37 ^{0}C for 60 seconds. Then 80 µl 7.5% (w/v) sodium carbonate solution was added, and the sample mixed again. After another incubation at 37 ^{0}C for 900 seconds, the absorbance was measured at 765 nm (Waterhouse, 2002).

The konelab30i automatically pipetted samples and solutions directly into the cuvettes. The konelab30i measured both FRAP and total polyphenols simultaneously for the samples, and each sample was measured three times each. If the absorbance measurements were out of bounds, the samples were diluted and remeasured by the konelab30i.

The averages for the three measurements were used as concentration values in the samples. For diluted samples, the average of the three diluted measurements were used. FRAP was measured as µmol/L and the total phenolic content as µg/ml. The total phenolic content was calculated as mg/L gallic equivalents (GAE).

2.6 Statistical analysis

The experimental design was a factorial with four factors: i) four cultivars; ii) three packaging material, iii) three pathogen (carrot with tip rot (two pathogens) and healthy carrots (control)) and iv) two storage conditions : Chill and CRTC (Chill-Room Temperature-Chill). The experiment had three to four replications. The data on effect of the treatment combinations on tip rot development (length of lesion) and other parameters were analysed using analysis of variance (ANOVA), General Linear Model of Minitab version 19. The means were compared with Tukey's pairwise comparison of means with a 95% confidence interval in Minitab. FRAP-values and total polyphenol

content (TPC) were analysed using the same method in Minitab® 21.1 (64-bit), with the factors Cultivar and Pathogen.

3 Results----------

3.1 Carrot respiration

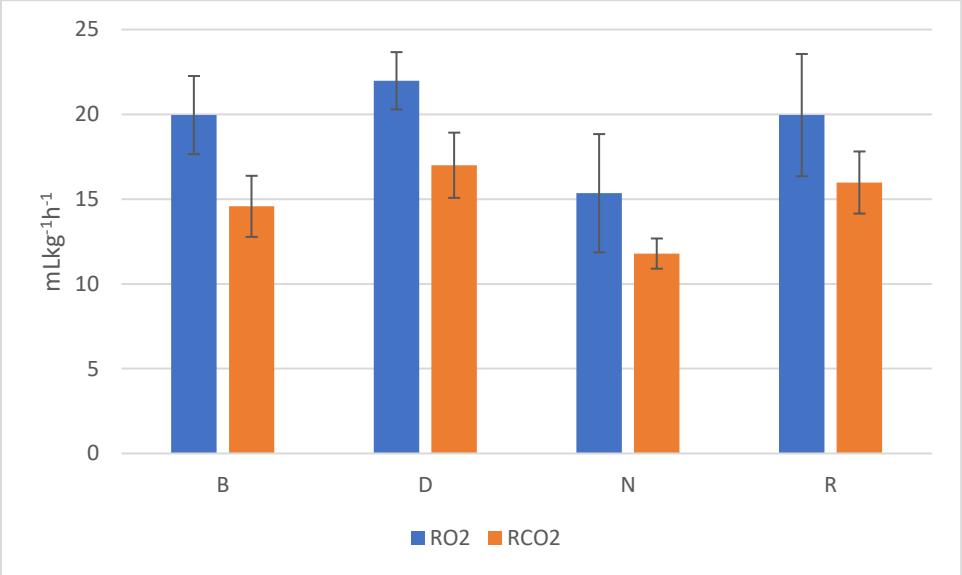

Figure 12. O_2 consumption rate (RO2) and CO_2 production rate (RCO_2) at 20°C for the carrot cultivars 'Brillyance'(B), 'Dailyance'(D), 'Namdal'(N) and 'Romance'(R) stored in an HDPE tray top sealed with a barrier film (Wipak, Nastola Finland). For each cultivar, 4 packages (replicates) were measured. Error bars show the standard error of the mean.

The rate of O_2-conumption (RO_2) and CO_2-production (RCO_2) at 20°C for the different carrot cultivars, (*figure 12*). Individual RO_2 values ranged from 12 to 23 ($mLkg^{-1}h^{-1}$). The cv. 'Dailyance' had the highest RO_2 on average and 'Namdal' had the lowest. Individual RCO_2 values ranged from 11 to 19 ($mLkg^{-1}h^{-1}$). The cv. 'Dailyance' had the highest RCO_2 on average and 'Namdal' had the lowest.

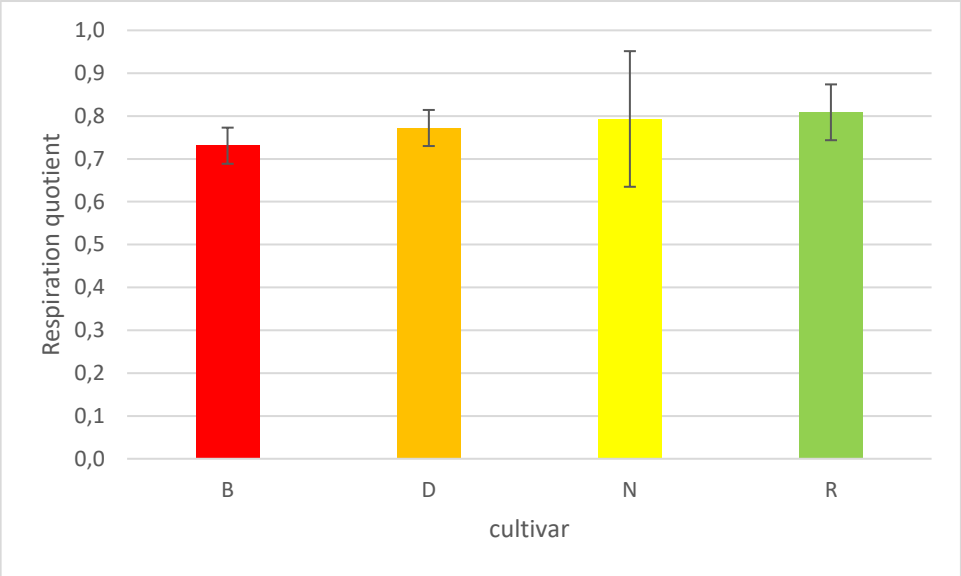

Figure 13. Respiration quotient (RQ) for the carrot cultivars 'Brilliance'(B), 'Dailyance'(D), 'Namdal'(N) and 'Romance'(R). For each cultivar 4 packages were measured. Error bars show the standard error of the mean. $RQ = \frac{VCO_2}{VO_2}$

The respiration quotient (RQ) at 20°C for the different carrot cultivars, (*figure 13*). The lowest and highest individual RQ measurements were found in cv. 'Namdal' and were 0.66 and 0.97. The four cultivars had similar RQs on average, ranging from 0.73 in 'Brilliance' to 0.81 in 'Romance'. The average RQ for all samples was 0.78 with a standard error of the mean at 0.08.

3.2 Pre-trial – changes in gas concentration for different treatments at 20°C

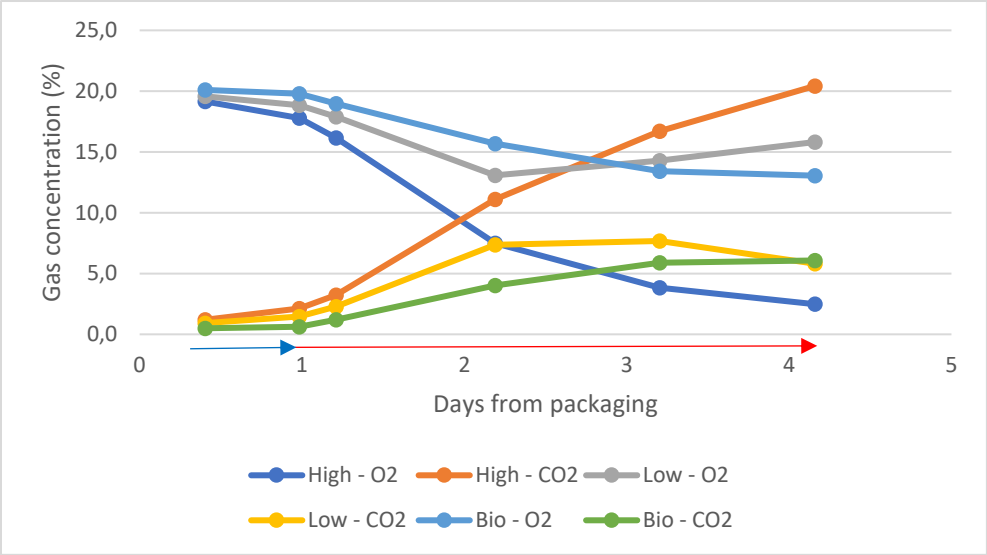

Figure 14. Average changes in the gas concentration (O_2/CO_2) in the headspace of the packages for the three treatments (High, Low, Bio) over time. The blue arrow indicates storage at 4°C and the red arrow indicates storage at 20°C. The three treatments: High (CO_2); Low (CO_2); Bio (low CO_2). For each treatment, 4 packages (replicates) were measured.

The average gas development over time for the three different treatments (High, Low, Bio), (*figure 14*). The highest CO_2 levels were reached in the High-treatment with values between 18 and 23.7% CO_2 at the end of the experiment. The lowest values of O_2 were found in the High-treatment with values >5% O_2 for all packages, and as low as 0.197% O_2 in one of the packages. The Low -and Bio-treatments shared similar development rates and all packages had CO_2 values between 5-10% at the end of the experiment. The O_2-values were between 10-20% with slightly higher values in the Low-treatment compared with the Bio-treatment.

3.3 Main-trial

3.3.1 Temperature

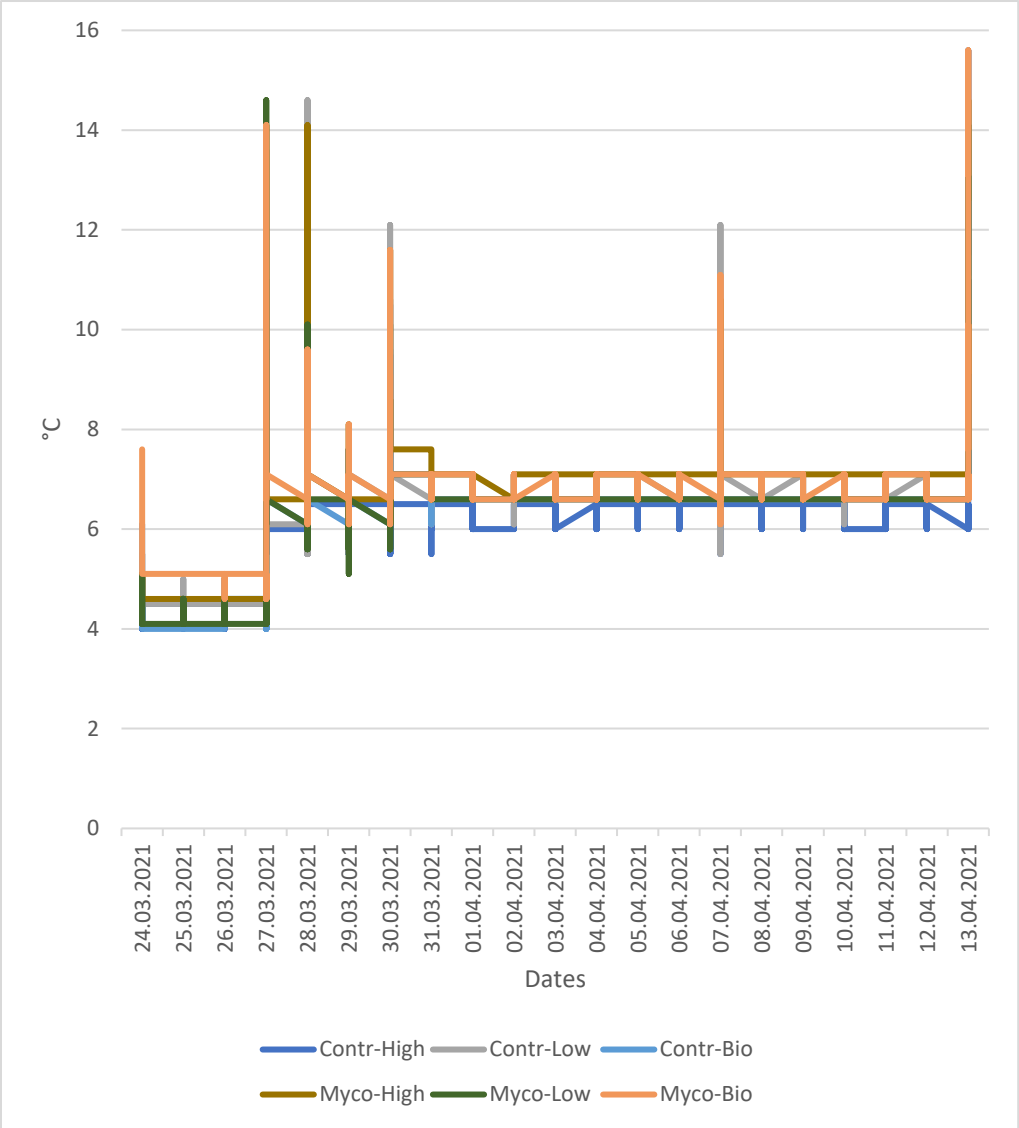

Figure 15. Temperature measurements in Chill storage during the storage period. Measurements in 6 packages with control carrots or carrots infected with Mycocentrospora acerina for all the three treatments. Temperatures were measured in 30 minute intervals. The whole temperature variation for each day is shown in the figure.

The temperature measurements for 6 packages stored in Chill conditions, (*figure 15*). The temperature is quite stable for all the 6 packages measured, with temperatures ranging from 4.5±0.2°C during the first three days, and from 6.7±0.5°C during the rest of the storage period.

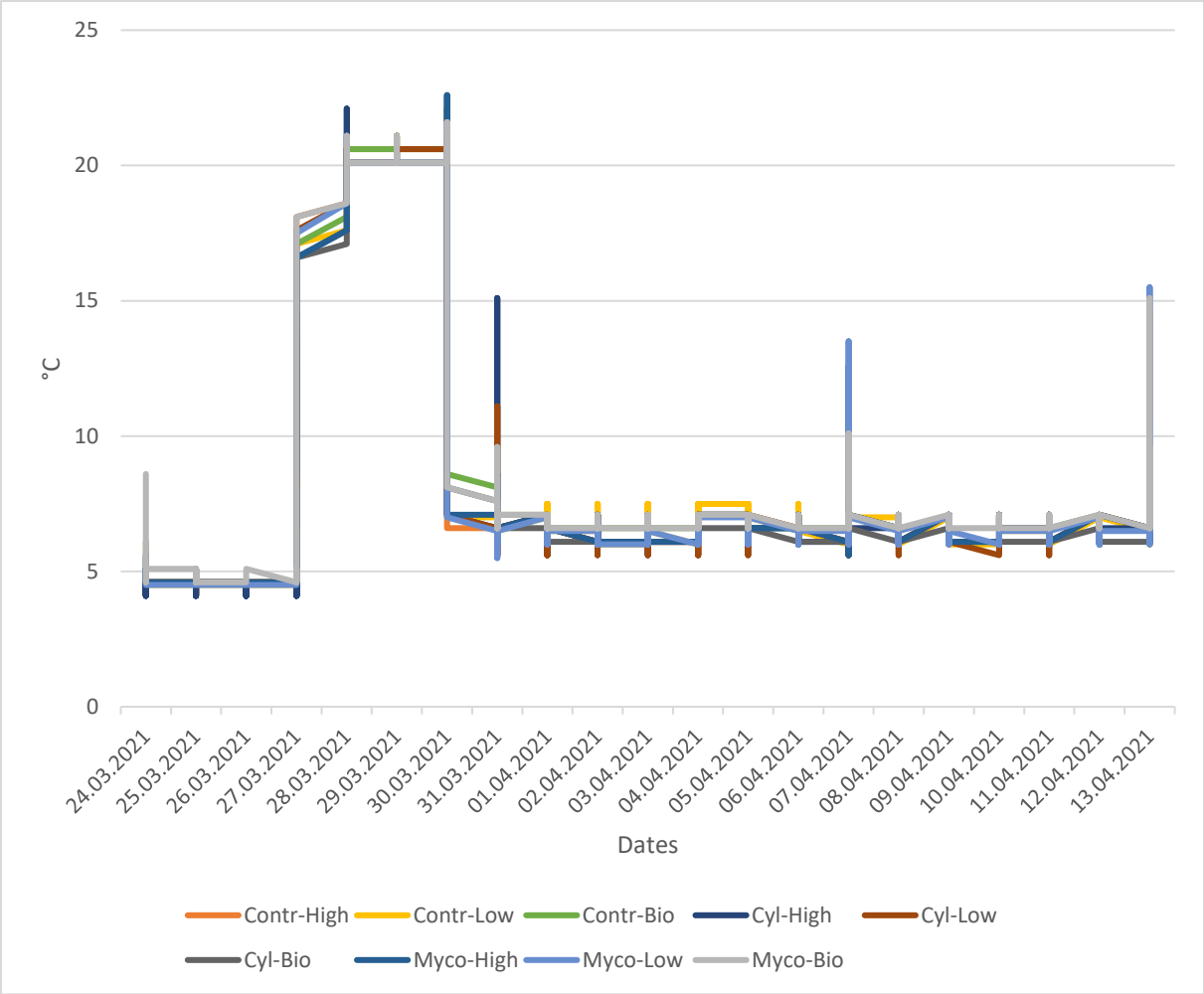

Figure 16. Temperature measurements in CRTC storage during the storage period. Measurements in 9 packages from all treatments and pathogens. Temperatures were measured in 30 minute intervals. The whole temperature variation for each day is shown in the figure.

The temperature measurements for 9 packages in CRTC storage, (*figure 16*). The temperature is quite stable for all treatments and pathogen combinations. For the first three days temperatures were 4.6 ± 0.2°C, and for the next three days in room temperature, the temperatures ranged from 20 ± 2°C. During the rest of the storage period the temperatures were 6.6 ± 0.5°C.

3.3.2 Humidity

The results for the relative humidity measurements are not shown as they were illogical and could not be explained. For most of the packages, the RH was in the range 90-100% during the storage period.

3.3.3 Gas concentration

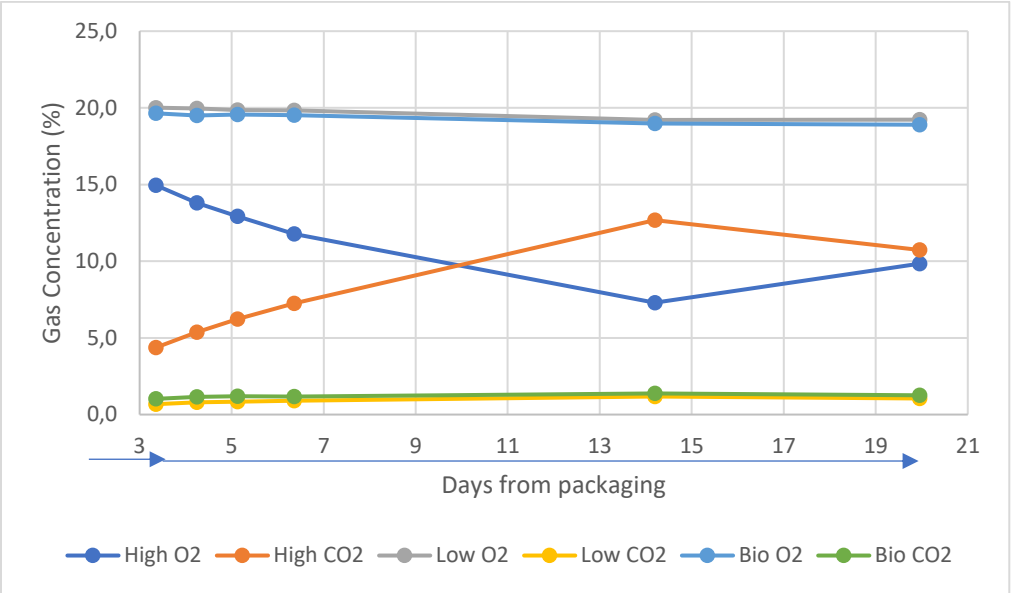

Figure 17. Average changes in gas concentrations (O_2/CO_2) in the package headspace during the storage period for all the 27 packages stored in Chill conditions. Measurements for 9 packages from each treatment (High, Low, Bio). For each treatment, 3 packages were controls, 3 had carrots infected by Mycocentrospora acerina *and 3 had carrots infected by* Cylindrocarpon destructans*. The Low and Bio treatments had overlapping gas concentrations. The packages were moved from 4°C to 6°C on day 3, indicated by a blue arrow.*

The changes in gas concentration (O_2/CO_2) during the storage period for the 27 packages stored under Chill conditions is shown in *figure 17*. Packages stored under chill conditions showed similar trends in the changes in gas concentration (O_2/CO_2) regardless of pathogen.

The 9 packages in the High CO_2-treatment had a clear increase in CO_2-concentration and decrease in O_2-concentration during the storage period. One package infected with *C. destructans*, and one package infected with

M. acerina reached an O_2-concentration below 1% and CO_2-concentration close to 20% on the measurement on day 14. For these two packages, the perforation was adjusted by adding holes with an acupuncture needle, and by day 21 the O_2-concentration was at 16% and CO_2-concentration at 5%. One package with control carrots reached an O_2-concentration below 1% on day 21. On average the CO_2-concentration increased by 6.4% and the O_2-concentration decreased by 5.1% during the storage period, from the first to the last measurement.

Both the Low CO_2-treatment and the Bio-treatment showed similar trends in the gas changes during the storage period. In the Low CO_2-treatment the CO_2-concentration increased by 0.4% and the O_2-concentration decreased by 0.8% from the first to the last measurement. In the Bio-treatment the CO_2-concentration increased by 0.2% and the O_2-concentration decreased by 0.7% from the first to the last measurement.

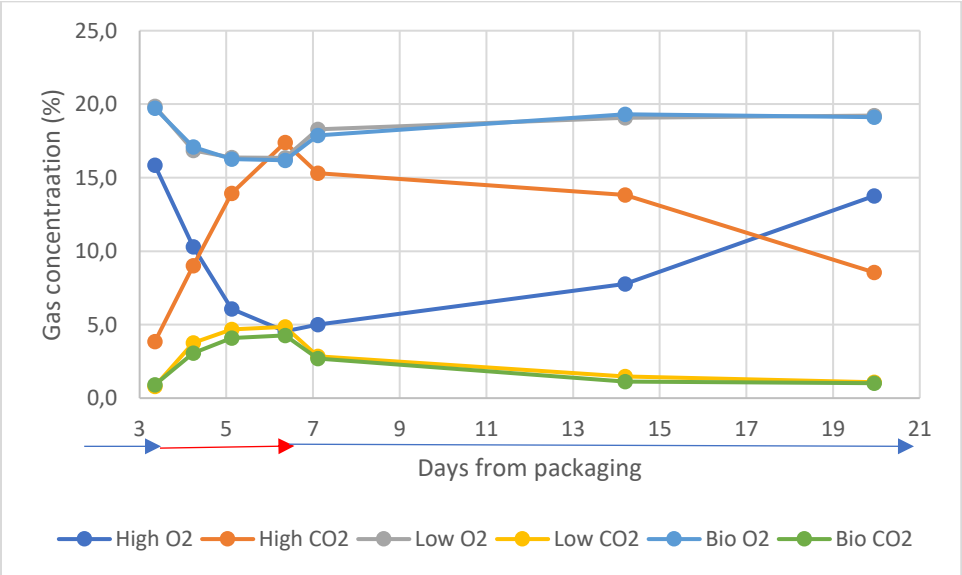

Figure 18. Average changes in gas concentration (O_2/CO_2) in the package headspace during the storage period, stored in CRTC conditions. Half of the packages in each treatment contained healthy control carrots and the other half contained carrots infected with Mycocentrospora acerina*. A total of 20 packages were measured: 8 in High (CO_2), 6 in Low (CO_2) and 6 in the Bio (Low CO_2)- treatment. The Low- and Bio- treatments have overlapping changes in gas concentrations. All packages were first stored at 4°C until day three, then at 20°C for three days, followed by 6°C for the rest of the storage period. Illustrated by blue (4°C and 6°C) and red (20°C) arrows.*

The changes in gas concentration (O_2/CO_2) in CRTC storage for healthy carrots and carrots infected with *M. acerina*, (*figure 18*). Packages in CRTC storage, containing either healthy carrots or carrots infected with *M. acerina*, showed similar changes in gas concentration (O_2/CO_2) during the storage period.

The packages in the High CO_2-treatment had a clear increase in CO_2-concentrations and decrease in O_2-concentration during storage in room temperature, day 3 to 6. For the remainder of the storage period the CO_2-levels slowly decreased, and the O_2-levels slowly increased. From day 3 to day 6, the O_2-concentrations decreased by 11.3% and the CO_2-concentrations increased by 13.6%. From day 6 to day 21, the O_2-concentrations increased by 7.8% and the CO_2-concentrations decreased by 7.1%. The highest CO_2-concentrations were found on day 6, where one package in control had a concentration at 22% and one with *M. acerina* had a concentration at 22.6%.

Both the Low CO_2-treatment and the Bio-treatment showed similar trends in the gas changes during the storage period. During the storage at room temperature from day 3 to 6, the CO_2-concentrations increased by 4% and the O_2-concentration decreased by 3.5%. From day 6 to 21, the CO_2-concentrations decreased by 3.7% and the O_2-concentration increased by 3.1%. Other than the changes in gas concentration during storage in room temperature, the concentrations were stable.

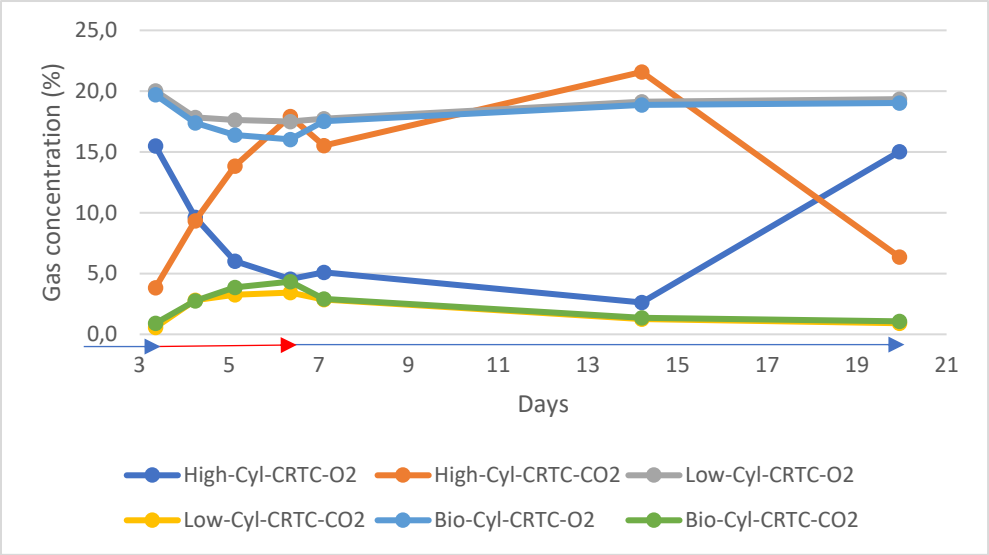

Figure 19: Average changes in gas concentration (O_2/CO_2) in the package headspace during the storage period. Carrots were infected with Cylindrocarpon destructans and stored in CRTC storage conditions. A total of 10 packages were measured: 4 in High (CO_2), 3 in Low (CO_2) and 3 in the Bio (low CO_2)- treatment. Low- and Bio- treatments have overlapping changes in gas concentrations. Packages were first stored at 4°C until day three, then at 20°C for three days, followed by 6°C for the rest of the storage period. Indicated by blue (4°C and 6°C) and red (20°C) arrows.

The changes in gas concentrations (O_2/CO_2) during the storage period for carrots infected with *Cylindrocarpon destructans* stored in CRTC conditions is shown in *figure 19*.

The packages in the High CO_2-treatment had a high increase in CO_2 and decrease in O_2 during storage at room temperature (day 3 to 6). The CO_2 increased by 14.1% and O_2 decreased by 11% during this period. From day 6 to day 14, the CO_2 continued to increase by 3.7% and the O_2-concentration continued to decrease by 1.9%. After acupuncturing in the packages on day 14 to increase perforation, the CO_2 decreased by 15.2% and O_2 increased by 12.4% from day 14 to day 21. In three out of four packages, the O_2-concentration measurement on day 14, was measured below 1%. The CO_2-concentrations on day 14 were 26.3%, 18.3%, 29.4% and 12.3%. From day 6 to day 21, in the fourth package the CO_2-concentration slowly decreased and the O_2 slowly increased

The packages in the Low CO_2-treatment and Bio-treatment had similar changes in the gas concentration during the storage period. During storage in room temperature (day 3 to day 6), the CO_2-concentration increased by 2.9% in Low and 3.4% in Bio. The O_2-concentration decreased by 2.5% and 3.7% during the same period. For the rest of the period in cool storage (day 6 to day 21), the CO_2-concentration decreased by 2.5% in Low and 3.3% in Bio. The O_2-concentration decreased by 1.8% and 3% during the same period.

3.3.4 Examination after storage period

The rot development in all the carrots infected by *M. acerina* at the end of the storage period can be observed in *figure 20*.

Figure 20. Overview of the 19 packages containing carrots infected by Mycocentrospora acerina *after the storage period. Stored in CRTC conditions: A) Low (CO_2); B) High (CO_2); C) Bio (Low CO_2). Stored in Chill conditions: D) Low (CO_2); E) High (CO_2); F) Bio (Low CO_2). Photo by: (Torstein Kvamme).*

3.3.5 Rot on surface

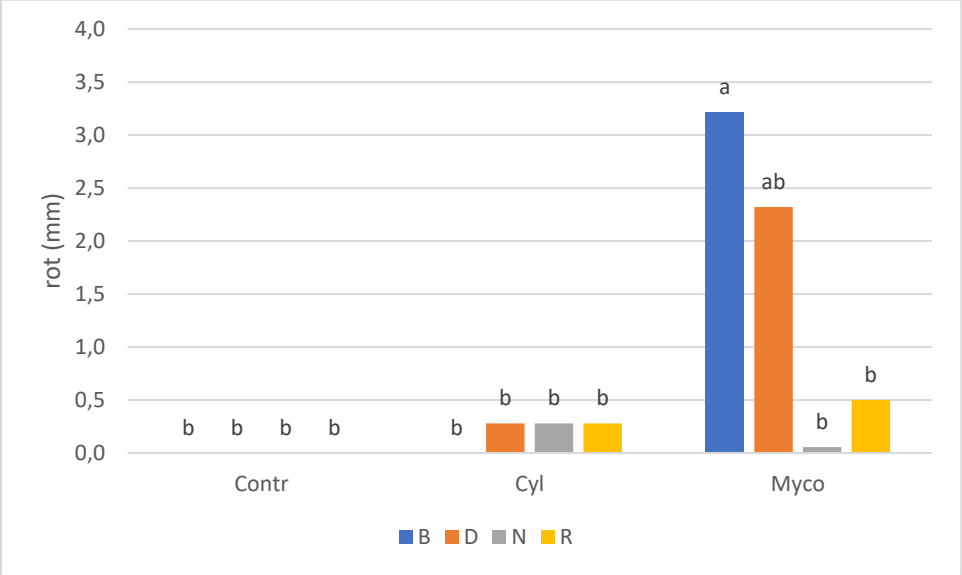

Figure 20. Comparing millimetre rot on the root surface after storage of carrots inoculated with Cylindrocarpon destructans *(Cyl),* Mycocentrospora acerina *(Myco) and non-inoculated controls (Contr) in the four cultivars 'Brillyance'(B), 'Dailyance'(D), 'Namdal'(N) and 'Romance'(R). Approximately 19 carrots of each carrot cultivar and pathogen combination. In total 226 carrots were measured. Means that do not share a letter are significantly different. P=0.006 (significant).*

The cultivar 'Brillyance' showed a significantly higher rot on the surface when infected with *M. acerina*, (*figure 20*). The cultivar 'Dailyance' also showed a higher susceptibility to *M. acerina*, but this was not significant. The rest of the cultivar and pathogen-interactions showed low rot.

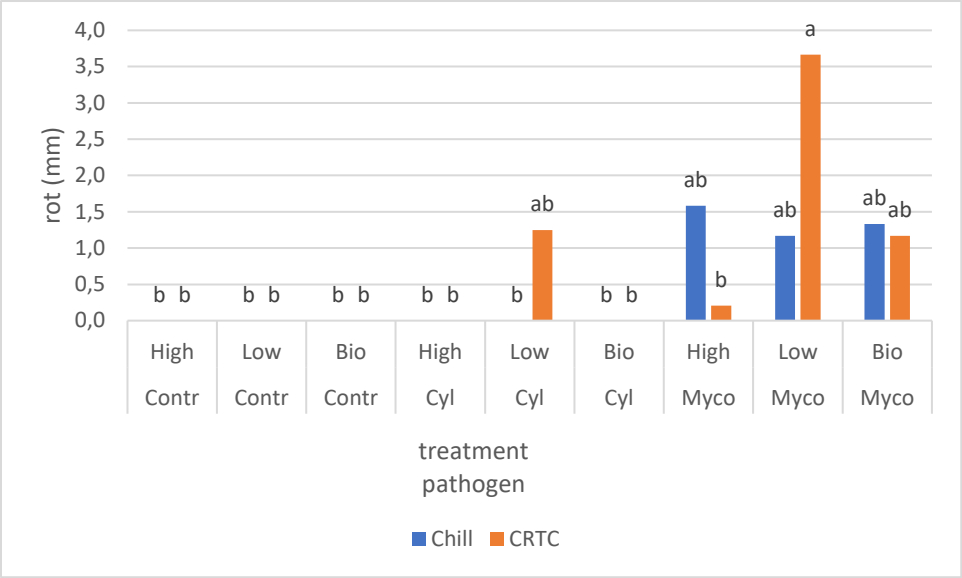

Figure 21. Comparing millimetre rot on the root surface after storage of carrots inoculated with Cylindrocarpon destructans *(Cyl),* Mycocentrospora acerina *(Myco) and non-inoculated controls (Contr) for the three treatmeants High CO_2, Low CO_2, and Bio stored in either Chill or CRTC conditions. Approximately 12 carrots for each combination of pathogen, treatment, and storage condition. In total 226 carrots. Means that do not share a letter are significantly different. P=0.255 (Not significant).*

The interaction of treatment, storage conditions, and pathogen is shown in *figure 21*. The combination CRTC-Myco-Low was significantly higher. Control carrots showed no rot development. *Cylindrocarpon destructans* only had rot development in carrots stored at CRTC conditions and Low CO_2. *Mycocentrospora acerina* had the least rot development in carrots stored at CRTC conditions and High CO_2. The rest of the combinations gave more rot development, but not statistically significant.

3.3.6 Internal rot

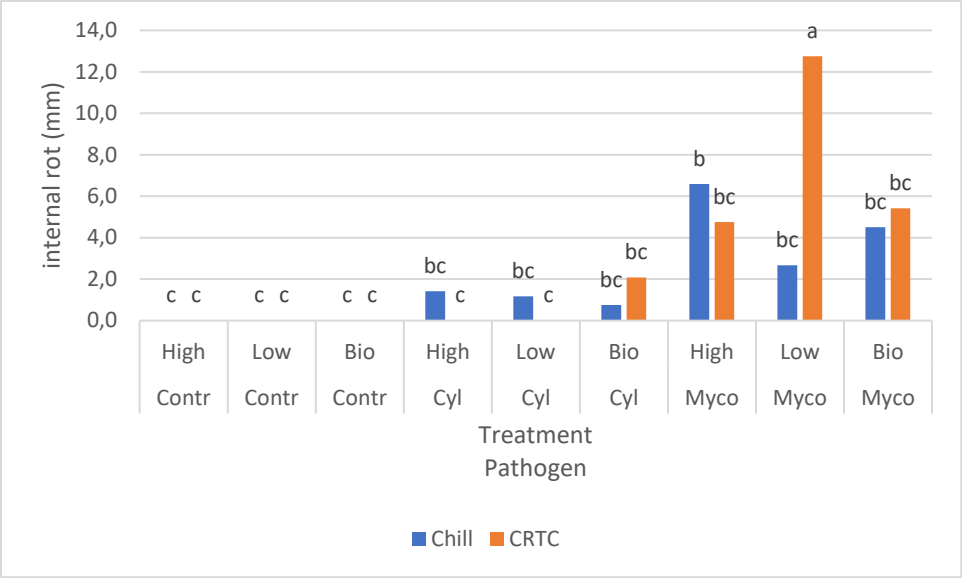

Figure 22. Comparing millimetre internal rot after storage of carrots inoculated with Cylindrocarpon destructans *(Cyl),* Mycocentrospora acerina *(Myco) and non-inoculated controls (Contr) for the three treatmeants High CO_2, Low CO_2, and Bio stored in either Chill or CRTC conditions. Approximately 12 carrots for each combination of pathogen, treatment, and storage condition. The High treatments in CRTC storage had a few extra. In total 226 carrots. Means that do not share a letter are significantly different. P<0.01 (Significant).*

The rot development was highest in carrots stored at CRTC with low CO_2 and infected with *M. acerina*, (*figure 22*). Carrots stored in Chill conditions and low CO_2 has had significantly higher rot than carrots with no rot. The rest of the combinations had rot development, which was not significant. Carrots infected with *C. destructans* only have rot development in the Chill condition for the High and Low CO_2-treatments. The rot was higher in general for carrots infected with *M. acerina* than *C. destructans*. The lowest rot for carrots infected with *M. acerina* was found in Chill storage conditions with Low CO_2. For carrots stored with Bio-treatment, the rot was highest at CRTC storage conditions.

3.3.7 Other observations

Root hair growth showed some trends for small differences in cultivars. Statistical calculations were not done for this parameter.

The petiole growth showed a trend to be lower in the treatment High CO_2 than in Low CO_2 and Bio. The growth was also a bit lower when stored at Chill conditions compared with CRTC. The growth was a bit lower for carrots infected with *C. destructans* compared with control carrots and carrots infected by *M. acerina*. Statistical calculations were not done for this parameter.

The bendability of the carrots was slightly higher in Chill conditions than in CRTC. Carrots infected with *C. destructans* had a slightly higher bendability. Statistical calculations were not done for this parameter.

Callus was only found in carrots infected by *C. destructans* and was present at the start of the experiment. Out of the 76 carrots infected with *C. destructans* used in the experiment, 7 of them had produced callus. The 7 carrots were stored at 3°C in the pathogenicity experiment. New callus formation in the carrots was not observed during the storage period, and the carrots with callus at the end of the experiment, were the same ones as in the start of the experiment.

Grey mould was found on 21 of the total 226 carrots and was distributed over 14 of the 57 packages. Out of these 14 packages, 10 were stored at CRTC conditions, and 4 at Chill conditions.

The taste and smell were not observed for all packages, but the taste seemed to mostly be cultivar-related and no packages with ethanolic smell were observed.

A general observation, both before, during, and after the storage experiment was more severe rot development in *M. acerina*. The rot was deeper as previously described, but also wider in radius.

3.3.8 Total polyphenol content (TPC)

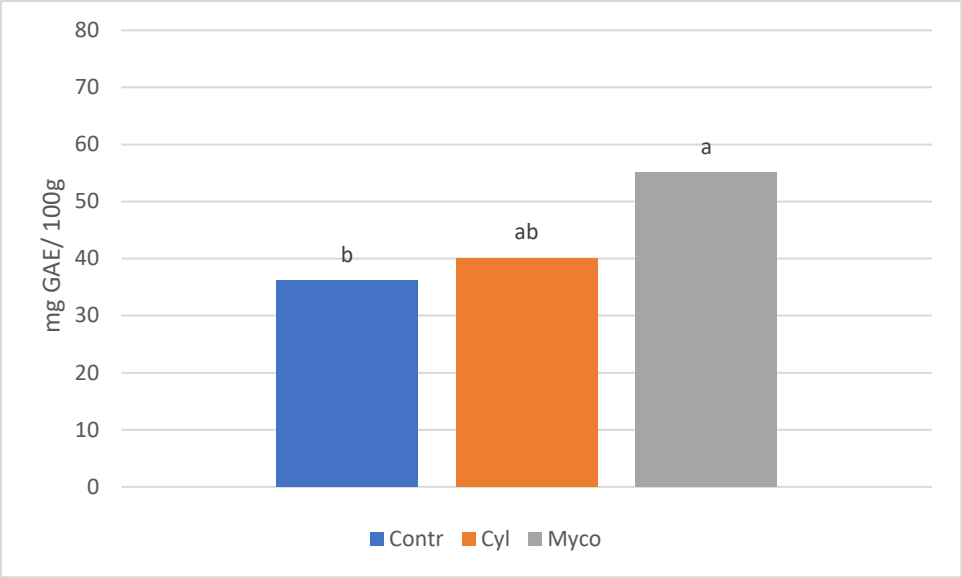

Figure 26: Total polyphenol content (TPC), in mg gallic acid equivalents (GAE) per 100g fresh weight of carrots. The carrot samples were taken from carrots stored in Low (CO_2) - treatment under CRTC storage conditions. Comparison of carrots inoculated with Cylindrocarpon destructans *(Cyl),* Mycocentrospora acerina *(Myco) and non-inoculated controls (Contr). In total 36 carrot samples were measured, with 12 for each pathogen. P= 0.002.*

The TPC was significantly higher in carrots infected with *M. acerina* than healthy control carrots, (*figure 26).* The TPC was higher in carrots infected with *C. destructans* than for control carrots.

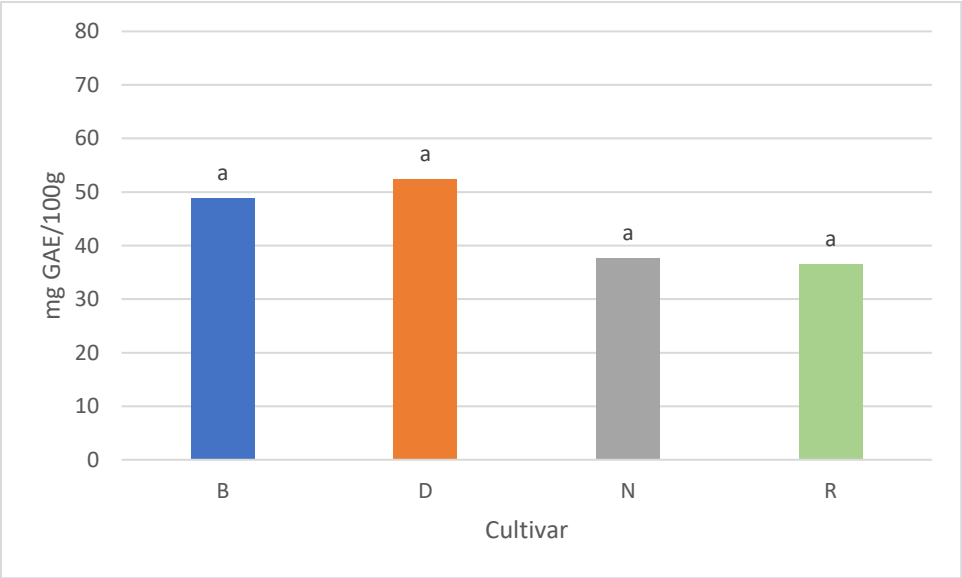

Figure 27: Total polyphenol content (TPC), in mg gallic acid equivalents (GAE) per 100g fresh weight of carrots. The carrot samples were taken from carrots stored in Low (CO_2) -

treatment under CRTC storage conditions. Comparison of the four cultivars 'Brillyance'(B), 'Dailyance'(D), 'Namdal'(N) and 'Romance'(R). In total 36 carrot samples were measured, with 9 for each cultivar. Means that do not share a letter are significantly different. P= 0.125.

There were no significant differences in the TPC of the different cultivars, (*figure 27*). There was however a trend with a higher content in 'Brillyane' and 'Dailyance' compared with 'Namdal' and 'Romance'.

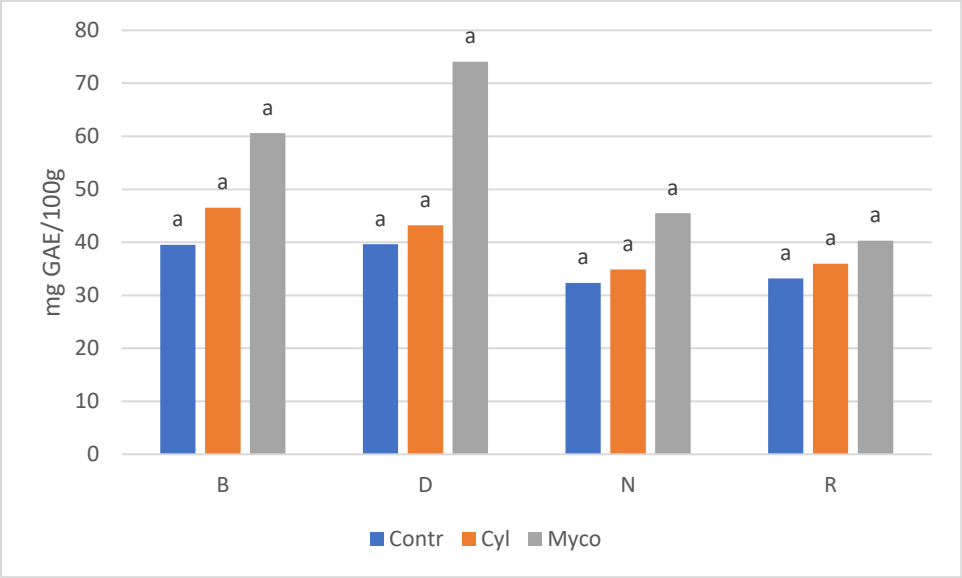

Figure 28: Total polyphenol content (TPC), in mg gallic acid equivalents (GAE) per 100g fresh weight of carrots. The carrot samples were taken from carrots stored in Low (CO_2) - treatment under CRTC storage conditions. Comparison of carrots inoculated with Cylindrocarpon destructans *(Cyl),* Mycocentrospora acerina *(Myco) and non-inoculated controls (Contr) in the four cultivars 'Brillyance'(B), 'Dailyance'(D), 'Namdal'(N) and 'Romance'(R). In total 36 carrot samples were measured, with 3 for each cultivar and pathogen combination. Means that do not share a letter are significantly different. P= 0.877.*

There were no significant differences in the TPC between the different combinations of cultivar and pathogen, (*figure 28*). The total polyphenol content (TPC) does however follow a pattern for all cultivars, with lowest content in control, higher in carrots infected with *C. destructans*, and highest in carrots infected with *M. acerina*. This trend is most clear in cultivars 'Brillyance' and 'Dailyance', but this was not significant.

4. Discussion----------

In this study, the average respiratory quotient (RQ) of the four cultivars 'Brillyance', 'Dailyance', 'Namdal' and 'Romance' was 0.78. The temperature, relative humitiy (RH), and gas concentration changes was measured during simulated storage of healthy control carrots, carrots inoculated by *M.acerina* and for carrots inoculated by *C. destructans*, under three different packaging conditions. Package temperature was found to be highly sensitive to the ambient air temperature, and quickly adjust to the ambient air temperature. Carrots stored under chill storage conditions showed decreased rot development compared with CRTC (chill-room temperature-chill) storage. Packages with low perforation quickly got High CO_2-concentration during storage in room temperature and the elevated CO_2 reduced rot development. The pathogen *M.acerina* gave deeper and more severe tip rot symptoms then *C. destructans*. The pathogen C. destructans was observed to increase the CO_2-concentration in the package headspace. Total polyphenol content (TPC) was found to be higher in carrots infected with pathogens with the highest values observed in carrots infected by *M.acerina.*

4.1 Respiration measurements

The RO_2 was measured between 12 and 23 ($mLkg^{-1}h^{-1}$). The RO_2 found by (Iqbal et al., 2008) was around 10 ($mLkg^{-1}h^{-1}$). The RCO_2 was found to be between 11 and 19 ($mLkg^{-1}h^{-1}$). The RCO_2 found by (Iqbal et al., 2008) was found to be around 17 ($mLkg^{-1}h^{-1}$). The values found in this experiment are similar to the ones found in previous research. In the study mentioned here, only one cultivar was studied. As several cultivars were measured in this study, the variation in the RO_2 and RCO_2 values also got larger. All four cultivars had a higher RO_2 than RCO_2, the opposite of the trend seen in (Iqbal et al., 2008). These differences may be due to differences in package

material or timing of measurements. In this experiment the carrots were packaged inside HDPE-trays and measured every 2 hours 5 times. Whilst (Iqbal et al., 2008) used glass jars and measured respiration rate after 0, 31.5 and 63 hours. The respiration rate itself is affected by temperature and gas concentration (O_2/CO_2), and a locked container will be vulnerable to changes in RR over time.

The lowest and highest individual RQ values ranged from 0.66 to 0.97. The average value for the four cultivars 'Brillyance', Dailyance', 'Namdal' and 'Romance' was 0.78. These values are found to be inside the normal range of 0.7 to 1.3 in aerobic respiration. RQ measurements for whole carrots at 20°C (Iqbal et al., 2008) were measured from 0.4 to 0.7. These measurements were a bit lower than the findings in this experiment, but as the RQ is calculated from the relation between RCO_2 and RO_2, the factors previously discussed for RCO_2 and RO_2 also apply here.

4.2 Changes in gas concentration in treatments at 20°C

The gas concentration (O_2/CO_2) in the three treatment types followed the estimated trends with high CO_2 and low O_2 in the High-treatment, and low CO_2 and high O_2 concentration in the Low- and Bio-treatments. This is similar to the findings by (Seljåsen et al., 2004) where the three package types used, got three different gas concentrations (O_2/CO_2) and where the changes were largest at a high temperature (20°C).

In the experiment, the changes in gas concentration were small during the first day. This was during cold storage at 4°C and after the transfer to 20°C the gas concentration quickly changes. Perforation was adjusted during the experiment, which is very apparent in the Low- treatment where the O_2 concentration increases, and CO_2 decreases from day 2. If the perforation had not been adjusted one would instead see a continuous increase in the concentration of CO_2 and decrease in the O_2.

As packages in the main experiment also were placed in room temperature for 3 days, the goal of this experiment was to adjust the perforation to handle 3 days of increased respiration. The number of needle perforations at the end of this experiment were used in the main experiment, to improve perforation and get desired gas concentration in the packages and avoid gas concentrations (O_2/CO_2) harmful to the carrots.

4.3 Main-trial

4.3.1 Temperature

The temperature measurements were successful and the measured values in the packages followed the desired temperatures in the two storage conditions Chill and CRTC. The temperature spikes can easily be explained by the movement of packages to room temperature during gas concentration measurements. The package temperature was measured every 30 minutes. Notice how all the spikes co-align with gas concentration measurements. In CRTC, the packages were moved to room temperature at about 10 p.m. on the 28.03.2021, and the increase during the 2 last hours is responsible for the high temperature on this date.

This illustrates how quickly the temperature changes inside the package when the ambient temperature change. This makes it important to swiftly transport carrots and packages to appropriate chill storage conditions, to avoid negative effects of higher temperatures. As package temperature quickly rises at higher ambient temperature, there is also a risk of condensation during short stays at a higher ambient temperature, and this should be avoided as this is favourable for pathogens.

4.3.2 Relative humidity

The data for relative humidity did not follow any expected patterns, and measurements were variable and difficult to interpretate. It was initially

thought that the relative humidity would be lowest in the Bio-treatment, as the pulp-trays absorb moisture.

The variation was however largest in the Chill storage condition, and for the High CO_2-treatments. Individual package differences may also have had large effects on the results, as only one package of each set of 3 were measured. This means that if the package was dried out due to a too long stay in room temperature, or if perforation in the package needed large adjustments, this individual package would have a strong influence on the relative humidity result of that package condition. Supporting evidence for this was found in the control stored in High treatment under Chill conditions, which had a drop in RH after the gas concentration measurement on the 07.04.2021.

The Low and Bio-treatments had higher perforation, and it was also suspected that this could lead to lower relative humidity, but this was not observed.

4.3.3 Changes in gas concentration

4.3.3.1 Chill storage

Packages stored in Chill conditions followed the same patterns both for packages containing carrots inoculated by *M. acerina* or *C. destructans*, and for packages with healthy non-inoculated carrots.

The gas concentration (O_2/CO_2) followed the same pattern through the entire storage period for treatments Low and Bio, with the two practically overlapping. In Chill storage, the gas concentrations were stable with a low CO_2 under 1% and O_2 around 20%.

In the High-treatment the CO_2 continuously increased the O_2 continuously decreased during storage. The decrease in CO_2 and increase in O_2 during the last part of the storage period, is due to perforation adjustments with needle perforation in response to harmfully low O_2-concentrations. After

increasing the perforation, the O_2 increases and CO_2 decreases, instead of continuing the same way. The too high CO_2 and too low O_2 in this treatment under Chill-conditions, means that the package may be unsuited for long-time storage of carrots. The package needs a higher perforation to avoid anaerobic conditions and negative effects from the O_2/CO_2- concentration on carrot quality and shelf life. With improved perforation, the package can have a positive effect on shelf life and quality, due to reduced respiration in the carrots and inhibitive effects on disease and rot development.

There were some individual differences in the gas concentration changes during the storage period. This is due to differences in respiration response in the packages. In addition to temperature, the size of the carrots is important for determining respiration rate. For packages with larger carrots, the respiration rate may for instance be higher, and as such the changes in gas concentration also increase. Some cultivar differences were found in respiration rate, but as each package had 1 carrot from each cultivar, this should have reduced the effect of cultivar on the gas changes. The respiration rate increases in carrots in response to mechanical damage. Packages and carrots have been handled carefully to avoid such injuries, however for instance during the respiration measurements, or when needle perforating packages, there were a few cases where the needle also harmed the carrots. This may have been a reason for increased response in some packages. In the Bio- treatment there were a few cases where the PLA-bags got torn in small areas, though these holes were repaired as soon as this was noticed, and it doesn’t seem to have affected the results much. This was also most likely to happen during handling of the packages and would as such be noticed quickly.

4.3.3.2 CRTC storage

For the Low- and Bio- treatments stored in CRTC conditions, the packages followed the same patterns for packages containing carrots inoculated by

M.acerina or *C. destructans*, and for packages with healthy non-inoculated carrots. For the High- treatment stored in CRTC conditions, the packages with carrots inoculated by *C. destructans* showed a different pattern than packages with carrots inoculated by *M.acerina* or non-inoculated control carrots.

For the Low- and Bio- treatments the CO_2 concentration increased to about 5% during the period in room temperature, and then steadily decreased during the Chill-period. The O_2 concentration decreased from about 20% to 16%, and then steadily increased back to 20% during the Chill-period. For these packages, CO_2-levels are perhaps too low to have an inhibitory effect on disease- and rot- development. The O_2/CO_2- concentrations will also not have a negative effect on the carrot quality from for instance anaerobic respiration. However, the carrots will not have positive benefits on shelf life from reduced respiration either, as the O_2-levels are too high to reduce respiration.

For the High-treatment the CO_2-concentration also increased during storage in room-temperature, but the increase was much higher, to about 18%. This happened for all the packages. In the following Chill-period, the packages with carrots inoculated by *M.acerina* or non-inoculated control carrots, had a continuous slow decrease in the concentration of CO_2. For the packages with carrots inoculated by *C. destructans*, the CO_2-conentration continues to increase. On day 14, three of the four packages had high CO_2-concentrations and low O_2-concentrations. After adjusting the perforation in these packages by adding needle perforations, the CO_2-concentration dropped, and O_2-concentration increased in the last period. This is the reason for the differences in gas concentration patterns in.

A possible answer to as to why the packages with carrots inoculated by *C. destructans* could be that the CO_2-increase was higher in the other packages, and perforation was adjusted directly after the room temperature period. However, the increase during the period is similar in all the packages, and from notes made on added perforation in packages, this does not seem

to explain the following gas concentration changes. However, these notes were not thorough enough, and as such this could still be the case. The carrots stored in Chill-condition during the entire storage duration, did however have an increase throughout the whole period, meaning that for the CO_2 to increase during the chill-period of the CRTC storage could be a possible scenario. For almost all the packages, this was however not the case, making this a less likely solution. The fourth package (*Cyl*) where the O_2 did not go below 1%, the package followed the same trend as for the other packages in the High-treatment. This package also had 4 carrots, meaning that it should be similar to the rest of the packages.

Another more likely solution is either that *C. destructans* itself has a higher respiration or that it makes the carrots more stressed, and the carrots get an increased respiration. The temperature increase at room temperature, may have made the conditions more favourable for *C. destructans* and given it an opportunity. For packages with carrots infected by *M.acerina*, the CO_2-concentration decreased after transfer back to chill. Packages with carrots infected by that *C. destructans* had instead an increase in CO_2 after the transfer back to chill. This difference in gas concentration changes during CRTC storage between *M. acerina* and *C. destructans* may indicate a role for *C. destructans* in affecting the gas concentration in packages.

As foul ethanolic taste or smell was not observed in the packages, it is likely that the anaerobic conditions were not present for long periods, and that the adjustment to gas atmosphere (O_2/CO_2) has hindered some of the negative effects of high CO_2 and low O_2-concentrations.

4.3.4 Rot development

Internal carrot rot was observed to go deeper into the carrot, and make a bigger lesion, than the observable rot on the carrot surface. The rot development (both on surface and internal) for *M. acerina* and *C. destructans* followed similar trends for all the treatments and storage

conditions, with a higher rot development for carrots infected by *M. acerina*. There were differences in the cultivars, with 'Brillyance' and 'Dailyance' being the ones with most rot development by *M. acerina* and cultivars 'Namdal' and 'Romance' being the ones with the least rot development. For *C. destructans*, 'Brillyance' was the least susceptible cultivar.

In general, the rot development was higher in CRTC than Chill storage condition with one exception. For packages in the High-treatment, the rot development was inhibited when stored under CRTC storage condition. These packages had higher CO_2-concentrations than their Chill counterparts, as a response to the 3 days of storage in room temperature. This observation was not at a significant level, but there seems to be a clear trend.

The overall effect of calluses on the experiment are small. Only 1 of the 7 carrots had rot development than went past the callus. Three of the calluses succeeded in stopping the rot development in the carrot, and for the remaining three the rot had not even reached the callus itself.

4.3.5 Polyphenols

The total polyphenol content was highest in carrots infected by *M. acerina* followed by carrots infected by *C. destructans*, and lowest in healthy control carrots. This same trend was observed for all four cultivars. The polyphenol content in carrots infected by *M. acerina* was significantly higher than that of the control carrots, increasing the likelihood of a response in the total polyphenols in the carrots, due to the pathogen infection. The four cultivars showed different total polyphenol content, with the cultivars 'Brillyance' and 'Dailyance' having a higher content than cultivars 'Namdal' and 'Romance', though this was not statistically significant. The increase in total polyphenol content in carrots infected by *M. acerina* is highest in the 'Brillyance' and 'Dailyance' cultivars. These were also the two cultivars that had the highest susceptibility and largest rot development.

A possible explanation to the higher rot in carrots infected by *M. acerina* could be due to sampling being moved further up on the root, to use carrot tissue, and not soft rotten tissue. As the total polyphenol content can vary for different parts of the carrot, this could explain the differences seen in the samples, as samples were more likely to be taken further from the tip of carrots infected by *M. acerina* as it caused more severe rot. However, for carrots infected by *C. destructans* the samples were rarely taken further up from the tip, and these still had increased total polyphenol content. Additionally for the samples of 'Dailyance' infected by *M. acerina* a sample with 5mm rot both on the surface and internally had 122mg GAE/100g fresh weight, while the carrot with 10mm rot on surface and 23mm rot internally had 52mg GAE/100g fresh weight. This further strengthens the hypothesis that the carrots infected by *M. acerina* or *C. destructans* have increased total polyphenol content. Polyphenols also play roles in the defence against pathogens, and increased content in response to infection by *M. acerina* or *C. destructans* would not be too farfetched. Polyphenols are also important antioxidants and play an active role in the surface browning of carrots. An increase in polyphenols due to infection, may also make infected carrots more prone to surface browning in packaging halls. The regulative increase in polyphenols may also occur before visible rotting symptoms are present, also making polyphenol content a possible tool for too a certain degree distinguishing between infected and non-infected carrots.

4.4 Further research

The findings on total polyphenol content (TPC) in infected and non-infected carrots showed promise for possibly explaining increased risk of phenolic browning in packing halls and should be further investigated.

As tip rot is a problem consisting of several causal agents, and the increased CO_2- values observed in packages infected by *C.destructans* should be further investigated as this could be key in explaining some of the role *C. destructans* has in the tip rot disease.

5. Concluding remarks

Tip rot disease is a complex disease, and the results in this thesis has helped increase the knowledge on how it develops during storage.

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