Improving storability and quality of Swedish apples

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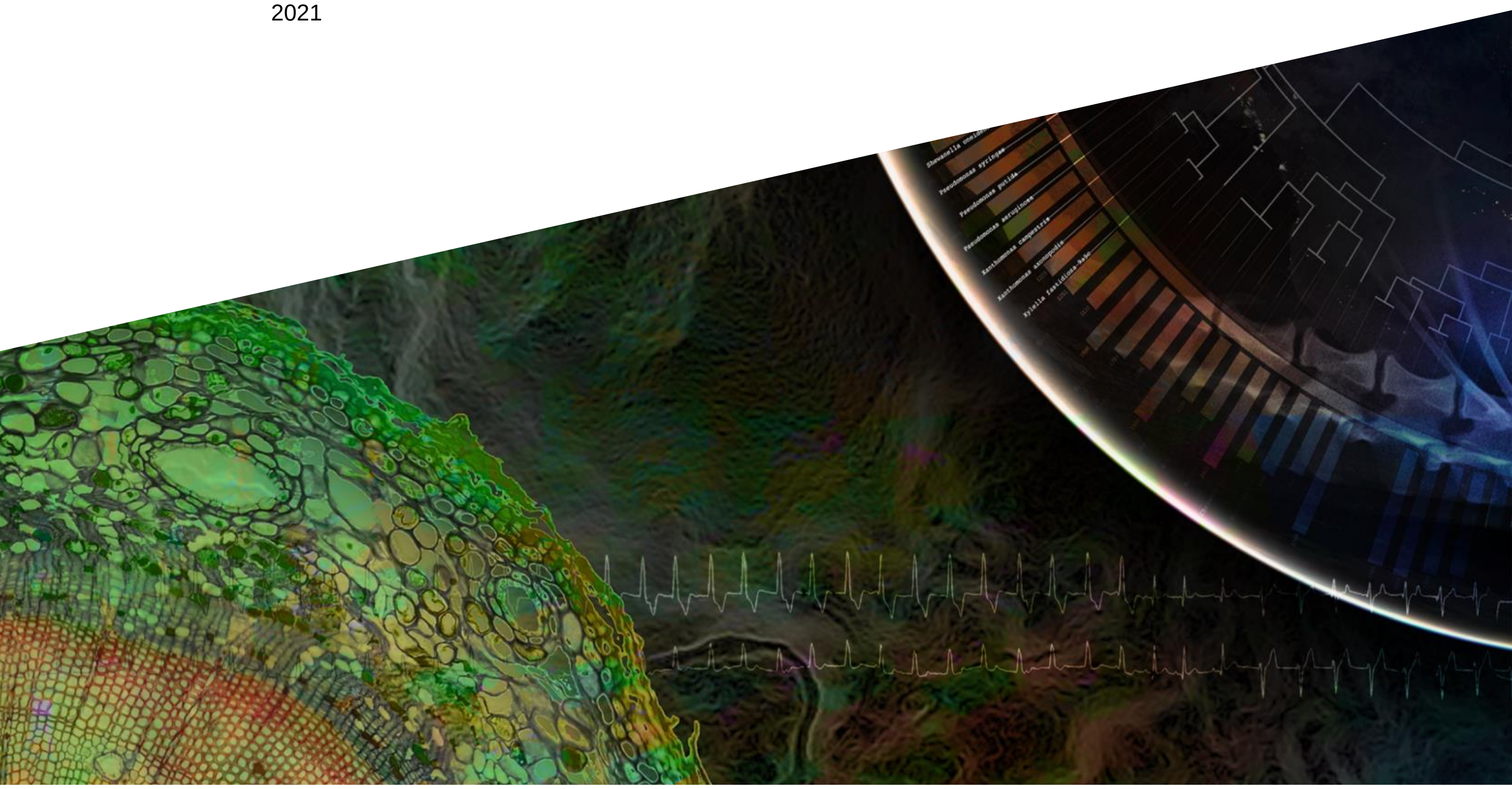

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Table of contents

List of figures

1. Introduction

1.1. Apple - a brief history

The apples we eat today (*Malus x domestica*) are thought to have been selected from the wild apple species *Malus sieversii* in central Asia (Velasco et al., 2010; Cornille et al., 2014), but there is also a major genetic contribution from *Malus sylvestris*, the European crab apple (Cornille et al., 2012). From Asia, apples spread to Greece and the Roman Empire via the Silk Road. The Romans cultivated many different varieties of apple and bred new ones. Furthermore, they developed pruning techniques and had knowledge of the need for fertilizers (Juniper et al., 1998). The Roman Empire brought this knowledge to their conquered areas and from there it spread to neighboring areas such as Scandinavia. At excavations of Scandinavian settlements from as early as before Christ, apples from the species European crab apple (*Malus sylvestris*) have been found. This shows that apples have been part of the diet for a long time in the area (Rohde Sloth et al., 2012). After the collapse of the Roman Empire the developed cultivars were still grown in the former territories, where the varieties spontaneously crossbred over the centuries, giving rise to new cultivars (Juniper et al., 1998). In modern times, breeding goals have focused on resistance to pathogens, increased yields/ha, improved appearance and improved overall quality of the fruit (Crosby et al., 1992; Li et al., 2015). Breeding based on traditional crosses is, however, time consuming as sometimes up to five backcrosses are needed to introduce one resistance gene or to remove undesired traits (Luo et al., 2020).

1.2. World market

Over the last 20 years, China has increased the production of apples and is now the biggest producer in the world (40 million tons a year). Other large producers are USA, Poland, Turkey, and Iran producing 4.6, 4.0, 3.6, and 2.5 million tons per year, respectively. There is a global trend toward a decreasing area of cultivated apples, while the total yields are increasing due to more efficient growing practices (FAOSTAT, 2018). Due to pesticide residues in the fruit, China has not expanded into the EU market (Snyder and Ni, 2017). Instead, countries like USA, Italy, France, New Zeeland, South Africa and Chile provide a large part of the apples consumed in the EU (FAOSTAT, 2018).

1.3. Swedish apples - market share and varieties

The Swedish yearly production of apples during the last 20 years has increased from 16,000 tons to 30,000 tons, due to modernization of the orchards with denser planting of trees as compared to older orchards (Jordbruksverket, 2017; FAOSTAT, 2018). However, this production only covers 20-25% of the demand and therefore, fruit is imported mainly from Italy, Poland, Germany, Chile and Argentina. The import of apples to Sweden amounts to about 85,000 tons per year (Jordbruksverket, 2017).

The most common varieties grown in Sweden are 'Ingrid Marie', 'Aroma' and 'Discovery', but cultivation of newer varieties such as 'Rubinola', 'Frida', 'Elise' and 'Santana' is increasing rapidly (Persson, 2018). Potentially, modern Swedish apple orchards could yield up to 40 tons/ha, but the average yield is lower, between 15 and 20 tons/ha the last 10 years (Ascard et al., 2010; Jordbruksverket, 2018). For comparison, the average yield in the Netherlands is 40 tons/ha and in Belgium 45 tons/ha. Norway and Finland, which are located north of most of the Swedish orchards, have yields of about 10 tons/ha (FAOSTAT, 2018). Differences in climate have probably a large effect on yield. For example, high temperatures during spring can lead to an increase in yield by 25% as compared to colder conditions (Wagenmakers, 1996).

1.4. Possible health benefits from eating apples

Diets rich in fruit and vegetables are beneficial in many ways and have been shown to increase the life span and health of people (Critselis and Panagiotakos, 2019). Apples contain a large number of phytochemicals, such as phenols and flavonoids. Different studies have linked phenols to a reduced risk of diseases like diabetes type 2 (Boyer and Liu, 2004; Adyanthaya et al., 2010). A high intake of flavonoids was associated with lowered mortality in ischemic heart disease, as well as a lower risk of cardiovascular disease and lung and prostate cancer. Furthermore, symptoms of asthma and type 2 diabetes were also reduced (Knekt et al., 2002).

Another important group of components in apples is fibers, such as pectin. Fiber in the diet stabilizes the blood sugar level, which in turn might reduce the risk of diabetes. Risk of cardiovascular diseases might also decrease as some fibers bind to cholesterol (Dhingra et al., 2012).

1.5. Quality of apple fruit

Quality is a concept that is difficult to define, as it does not necessarily mean the same to different stakeholders in the production and distribution chain. To the end consumer, quality often means acceptability. Higher quality means that the consumer is more likely to buy the product. To the researcher, quality can refer to parameters that can be measured, monitored and used to estimate maturity of the fruit to predict the optimal harvest time (Shewfelt, 1999). Fruits picked too early or late in relation to optimum maturity stage at harvest are more likely to get storage disorders and diseases, resulting in low quality (Vanoli and Buccheri, 2012). Internal quality attributes such as firmness, starch, soluble solid content, acidity and antioxidants are important quality parameters for both consumers and researchers (Musacchi and Serra, 2018).

EU has trade norms for fruit and vegetables sold within and between EU's member states, as well as for products imported from outside EU. For apples, there are specific norms (Jordbruksverket, 2019). The minimum requirements state that the fruits should be intact, healthy, clean, free from pests and pest damage, free from water core, free from excessive moisture and free from any unusual odors. There are also quality norms that divide fruits into different quality classes; extra, class 1, and class 2. The highest class, i.e. extra, includes apples typical for the variety with an intact shaft. Fruit flesh should be healthy and only minimal damages are accepted. For class 1 only minor damages such as small bruises or slight defects in shape and color are accepted. The shafts do not need to be intact as long as there is no additional damage to the fruit. Apple varieties are further classified in four color groups (A,B,C,D) depending on red coloration. Apples of color group A must have red coloration on at least 75 % of the skin, group B 50 %, and group C 33 % whereas no red surface is needed in group D. There are also norms on maturity, stating that the apples must be ripe or mature enough to be able to continue ripening (Jordbruksverket, 2019).

The weight, size and shape of fruits are important quality measurements. All of these are dependent on pollination, as insufficient pollination can lead to smaller or misshapen fruits. Insufficient pollination occurs when the number of pollinators visiting the flowers is too low or when not all stigmas of the flower are pollinated. The part of the apple with pollinated stigmas will grow more vigorously making the fruit lopsided (Way, 1978; Garratt et al., 2014). Frost is another factor that can damage young fruitlets, causing russeting (Way, 1978).

Fruit color can be used as a quality factor as the background color goes from green to yellow as the fruit ripens. Background color can be assessed by comparing the fruit to a color chart (McGlone et al., 2002). Another way to measure color is by using a chromameter, which shows color lightness (L), red to green color (+a to –a) and yellow to blue (+b to –b) (Commission International d'Eclairage; (Lancaster et al., 1994). Some of the color comes from anthocyanins, which is a group of compounds that contribute to the antioxidant activity of the apples. Apples also contain vitamin C (Drogoudi et al., 2008). The antioxidant activity is a quality parameter that is important to people who want to eat healthy.

Firmness is another important quality parameter. In ripening fruits, the firmness decreases. This is due to changes in the pectin content in the cell wall matrix (Koricanac et al., 2020) and activity of cell wall degrading enzymes acting on the different cell wall components (Goulao et al., 2008; Wei et al., 2010). During ripening the levels of polysaccharide degrading enzymes increase in apple tissue. These enzymes digest certain carbohydrates in the cell wall of the fruit cell (Ross et al., 1994; Wei et al., 2010). In ripening fruit there are also changes in bonding between polymers, which can cause cell separation and further softening of the fruit (Brummell, 2006). Firmness is often measured using a penetrometer with an 11.1 mm plunger, which is pressed against opposite sides of the fruit (McGlone et al., 2002).

Although taste preferences differ between individuals, both within and between countries, the balance between acidity and sweetness is generally important (Bonany et al., 2013). During ripening, sugar and acids are broken down. The changes in acidity and sweetness can be used as quality parameters to keep track on how the fruit develops during storage, and during shelf life after storage (Koricanac et al., 2020). Soluble solids content (SSC) is measured by a refractometer, and titratable acid (TA) is measured by titration (McGlone et al., 2002). The main acid in apples is malic acid (Roth et al., 2007). Apple aroma compounds, like volatile 2-metylbutyl acetate, are important quality traits, since they can be affected by storage method and storage time. Longer storage in cold rooms tends to result in lower levels of these compounds (Roth et al., 2007).

To evaluate fruit quality based on firmness, SSC and acidity is problematic, as you have to use destructive methods, i.e. you have to cut the fruit to be able to make the measurement, which limits the amount of samples that can be measured. Moreover, the methods are time consuming and there is a risk that they show wrong values due to handling errors (Noh and Lu, 2007).

1.6. Fruit development

Development of fruitlets start shortly after crosspollination, i.e. the pollination of the flower by pollen from a compatible crab tree or another cultivar. After the flower is fertilized, cells start to divide in the fruitlet (Bain and Robertson, 1951). For about 40 days the fruit grows fast due to cell division (Denne, 1960). During this period there is a high level of respiration (Pearson and Robertson, 1953). The second stage of apple fruit development is cell enlargement (Bain and Robertson, 1951). This period lasts from the end of the cell division stage until harvest (Denne, 1960). Cell enlargement can in turn be broken down into three phases. During the first stage of cell expansion, the increase in volume of the cells is due to enlargement of the vacuoles. In the second stage, cells start to increase in size more rapidly and the third stage starts when intercellular spaces increase in size (Warrington et al., 1999). During cell division and cell enlargement there is a high respiration rate that increases with higher temperature (Pretorius et al., 2011).

By thinning flowers at the right time, both cell division and cell enlargement can be increased, leading to larger fruits (Denne, 1960). Fruits with small and tightly packed cells are firmer than fruits with bigger cells (Volz et al., 2004). Lower temperatures during cell division and cell enlargement leads to smaller fruits due to lower growth rate (Warrington et al., 1999).

Just before ripening starts, respiration drops to the preclimacteric minimum, i.e. the respiration rate is at its lowest level. The fruit has then reached a specific stage of maturity, which means that if it is picked, it will continue to ripen off the tree. After the preclimacteric minimum and as the fruit starts to ripen, respiration rises to the climacteric maximum (Millerd et al., 1953). The development is very fast at this stage and ethylene levels rise sharply (Peirs et al., 2001).

The senescent phase is the third and final stage in fruit development. Here the fruit ages and there are changes in the tissue due to increased respiration and depletion of carbohydrates (Blackman and Parija, 1928; Gasser and von Arx, 2015). The changes during senescence are usually irreversible and eventually lead to the death of the cells of the fruit (Sacher, 1973). Respiration decreases while ethylene levels initially rises during senescence, only to fall sharply in the end (Blackman and Parija, 1928; Lieberman et al., 1977). Senescence is an active process in the fruit with changes in enzymatic activity, protein content and hormone activity (Sacher, 1973).

1.7. Maturity and ripeness

It is important to keep a distinction between maturity and ripeness when discussing apple development. As the fruit develops, it eventually reaches physiological and horticultural maturity. At physiological maturity, the fruit can continue to develop even after picking. Horticultural maturity means that the fruit has developed enough to result in a product acceptable to the consumer. Ripeness on the other hand means that the fruit develops its characteristic flavor and visual traits and reaches optimum eating quality. Riper apples tend to taste better, but if the fruits are harvested before they reach optimal color, sweetness and firmness, it is easier to store and transport them without damaging them (Kader, 1999).

As fruit matures, it goes from green to yellow (Cardenas-Perez et al., 2017). The reduction of green color is due to the breakdown of chlorophyll in the fruit (Rutkowski et al., 2008). There is also an increase in the concentration of anthocyanin the last weeks before harvest, resulting in red coloring (Iglesias et al., 2008). The decrease in firmness as the fruit matures is due to different enzymes breaking down the cell wall (Wei et al., 2010). The starch in the fruit is gradually converted into sugar as the fruit matures (Kovacs and Eads, 1999). During ripening, the decrease in chlorophyll concentration and firmness continues. As malic acid concentration decreases, also acidity is decreasing. Carbohydrates are lost through respiration, but at a slow rate that does not affect taste (Knee, 1971)

2. Maturity indices

2.1. Non-destructive methods

Days after full bloom is an index used to estimate harvest time (Luton and Hamer, 1983). This index is, however, not reliable as it is heavily influenced both by weather and location (Narasimham et al., 1988).

Ethylene production and respiration rate show distinctive patterns during maturation and ripening, and can be measured to determine the maturity of the fruits (Millerd et al., 1953; Peirs et al., 2001). Respiration and ethylene production can be measured by gas chromatography – mass spectrometry (GC-MS) with great precision (Song and Bangerth, 1996). Portable gas analyzers are available on the market. Gases can also be analyzed by other methods, such as the gas analyzer "Sidor" (Sick Maihak GmbH) shown in Figure 1. It uses the infrared spectral area to optically analyze the level of the gas. The sensor is called NDIR, nondispersive infrared sensor (Werner, 2008).

Figure 1 Measuring respiration in 'Saga'. Photo: J. Sjöstrand

I_{AD}–measurements by a DA-meter is a more recent method to determine maturity. Using spectroscopy, it is possible to assess the amount of chlorophyll under the skin of the fruit. This method was developed for peaches and nectarines and measures the breakdown of chlorophyll during ripening. An index is calculated as the difference in absorption at 670 nm and 720 nm. These wavelengths were chosen as 670 nm is near the chlorophyll a absorption peak and 720 nm is used as the background of the spectrum. The result is an "Index of absorbance difference" or I_{AD}. This method was later developed into a portable instrument called DA-meter, alluding to Δabsorbance, i.e. the difference in absorbance described above. I_{AD} was shown to correlate well with sugar content, titratable acids and firmness of the fruit (Nyasordzi et al., 2013). However, the rate of chlorophyll degradation depends on the cultivar as well as the production area. Therefore, it is important to find optimal values for each variety and regional weather condition (DeLong et al., 2014; DeLong et al., 2016). As I_{AD}–measurements are non-destructive, it is possible to follow the maturity progress in one specific apple. It is a fast and direct method, which can help the grower to pinpoint when to start harvesting for optimal storability. It could also show growers when the fruit is too ripe for long-term storage (DeLong et al., 2014). Not only can I_{AD} predict storability, but also physiological disorders and rot since pathogen and physiological disorder severity is linked to certain maturity stages (Watkins et al., 2000; Farneti et al., 2015; Knutsen et al., 2015).

Figure 2 DA-meter measuring I_{AD} in 'Aroma'. Photo: I. Tahir

In recent years, methods using near infrared spectroscopy (NIR) have been developed. These have the advantage of using hand held tools, but need to be calibrated for each type of fruit or in some cases cultivar within a fruit species (Jannok et al., 2014).

2.2. Destructive methods

Today, Brix, firmness and starch degradation are common ways to determine maturity. Brix is a measure of total soluble solids content and is measured by a refractometer (Costamagna et al., 2013; Jannok et al., 2014). Firmness is usually measured by a penetrometer on either side of the fruit after peeling the areas (Blankenship et al., 1997). The probe is 11.1 mm in diameter, and shows firmness in kilograms (Reid et al., 1982).

Figure 3 Firmness test on 'Ingrid Marie'. Photo: J. Sjöstrand

With an iodine solution the starch degradation can be measured, as iodine reacts with starch and colors it blue. A slice of apple is cut across the core and dipped in iodine solution for a couple of minutes (Reid et al., 1982). After the iodine has reacted with the starch, the color of the slice is compared to a color chart. The darker the color, the higher content of starch (Menesatti et al., 2009). Methods to assess iodine coloration have been automated. Figure 4 shows starch degradation assessment by the Amilon device (Isolcell, Italy), which compares the stained area of apple slices with the references to the right.

Figure 4 Starch degradation assessment using the Amilon device (Isolcell, Italy)

A Streif index is calculated from the three indices Brix, firmness and starch degradation, by the equation

Streif index=F/(SSC*SD)

where F stands for firmness, SSC for Brix value and SD for starch degradation (Streif, 1996). As the apples ripen, the Streif index decreases (Peirs et al., 2001). The Streif index is specific for a variety and does not vary much depending on weather conditions or growing practices. The combination of the three indices in the Streif index makes it more robust than if the indices are used separately, as, for example, SSC can show varying results depending on weather conditions (DeLong et al., 1999).

In Sweden, most apples are stored in ultra-low oxygen storage (ULO) which means that the fruit is stored in rooms with an atmosphere containing less than 2% oxygen. Swedish apples should generally have a Streif index between 0.22 and 0.18 at harvesting for optimal storability in ULO-storage. Fruit stored in cold storage should have a Streif index between 0.17 and 0.11. A Streif index below 0.11 indicates that the fruit is too mature and can only be stored a short time (Tahir, 2014). The disadvantages with the Streif index are that the method is time-consuming and uses destructive measurements. It is, however, a reliable and consistent measure during the period before harvest and has been used successfully in for example Poland, the Netherlands and Germany for over 20 years (DeLong et al., 1999).

When analyzing ethylene often internal ethylene is measured. A gas sample is taken from the core of the apple with a syringe, and the gas is then inserted into a GC-MS. This is one of the most reliable methods of determining maturation (Kitsaki et al., 1999).

3. Storage

The rate of ripening can be regulated by controlling respiration rates, which in turn can be adjusted by temperature, humidity and atmosphere. By reducing temperature and managing O_2 and CO_2 levels, the respiration rate of stored fruit is lowered (Fidler and North, 1968), and fruit storability can be increased even more (Dilley, 2010). Unknowingly, people have been experimenting with storage in modified atmosphere for centuries by burying apples or keeping them in unventilated storage rooms. Jacques Etienne Berard conducted the first scientific study on modified atmosphere for storage of fruit in the early 1880s. He discovered that fruit uses oxygen and produces carbon dioxide when maturing, and that fruit stored in rooms with low levels of oxygen did not ripen as fast as other apples (Dalrymple, 1969).

3.1. Controlled atmosphere/Modified atmosphere

Controlled atmosphere (CA) storage means that fruit is stored in rooms with decreased levels of O_2 and increased CO_2. This slows respiration and ethylene biosynthesis, thereby slowing maturation and senescence, which in the end results in longer storage time (Wright et al., 2015). If the concentration of CO_2 and O_2 is not optimal and the level of oxygen gets too low, apples might be damaged by anaerobic respiration (Knee, 1973; Wright et al., 2015).

The point at which the apples start the anaerobic respiration is called the anaerobic compensation point (ACP) (Aubert et al., 2015). The ACP can vary from one year to another within a cultivar (Gasser and von Arx, 2015). Another important term is lower oxygen limit (LOL), which is when the respiration is at the lowest possible level without causing anaerobic respiration. The optimal storage conditions are just above the LOL (Yearsley et al., 1996). ULO is a development of CA (Brackmann et al., 1993). During ULO conditions the level of O_2 is <1 kPa (Balla et al., 2008).

3.2. Dynamic controlled atmosphere (DCA)

The development of DCA (dynamic controlled atmosphere) can be divided into three steps. During the first step, focus was on finding a technology to maintain constant levels of O_2 and CO_2 as well as temperature. During the second step, it became possible to control gas levels and temperature more precisely. During the third and current step, storage conditions can be dynamically adjusted based on produce response (Prange et al., 2013). Stress responses of the fruit such as chlorophyll fluorescence or ethanol production are monitored throughout the storage (Prange et al., 2002; Zanella and Sturz, 2015). Ethanol is produced in the cells of the fruit if oxygen levels are too low to maintain aerobic respiration and instead fermentative respiration takes place. The level of produced ethanol can then be measured in the headspace of the container the fruit is stored in. Ethanol production is a sign of stress in the fruit and might also lead to off flavors (Bessemans et al., 2016; Zanella and Sturz, 2015). The lowest possible oxygen level without causing harm to the fruit is applied to maximize the positive effects. With DCA it is possible to, at least, halve the oxygen concentration in the storage room as compared to ULO storage without causing physiological disorders in the fruit (Brizzolara et al., 2017; Mditshwa et al., 2018).

3.3. Dynamic controlled atmosphere - chlorophyll fluorescence (DCA-CF)

The stress response in the fruit during storage can be measured by changes in chlorophyll fluorescence using sensors on a few selected fruits (Zanella et al., 2005). When the fruit is stressed by oxygen deficiency, chlorophyll fluorescence increases sharply. When sensors detect this spike in fluorescence, the oxygen level is increased until the fluorescence returns to the initial value (Prange et al., 2002). If this increase in oxygen level is done fast enough and to a sufficient degree, there will be no signs of anaerobic respiration (Zanella et al., 2005). This method is called DCA-CF (dynamic controlled atmosphere - chlorophyll fluorescence) (Mditshwa et al., 2018).

During the last two decades, different trials involving DCA-CF have been performed to investigate the effects on fruit quality. In a trial with the cultivar 'Topaz', DCA-CF showed quality improvement in a number of ways as compared to traditional CA. Browning disorders went down by 67%, while lenticel rot decreased by 18%. Moreover, DCA-CF storage decreased softening of the fruit and the fruit retained higher levels of titratable acids and soluble solids (Lafer, 2010). In 'Granny Smith' apples, a reduction of superficial scald was noticed in apples stored under DCA-CF monitoring and this method is widely used in South Tyrol to control superficial scald (Zanella et al., 2005; Lafer, 2010).

A possible problem with DCA-CF is that the monitoring is done using only a few measuring points, e.g. six apples, which are supposed to represent all the fruits in the batch (Bessemans et al., 2016; Anese et al., 2020). In an experiment it was shown that LOL is higher on the sun-exposed side than on the shaded side of the apple in both 'Cortland' and 'Honeycrisp' (Wright et al., 2012). It has been known for a long time that the sun-exposed side of the apple contains more carbohydrates (Leonard and Dustman, 1943). It also has a higher respiration rate and a higher amount of xanthophyll, which means that it is more active than the shaded side (Ma and Cheng, 2003; Chen et al., 2009). Chlorophyll content on the other hand, is lower on the sun-exposed side of the fruit than on the shaded side (Ma and Cheng, 2003). Another possible problem is that chlorophyll is degraded as apples ripen (Mditshwa et al., 2018). Therefore, the few selected apples and their former placement in the tree may affect fruit response and, in turn, the storage atmosphere to a great extent. Fluorescence can also occur due to other stresses, such as ammonia gas leaks (Prange et al., 2012).

3.4. Dynamic controlled atmosphere - ethanol (DCA-ET)

A variant of DCA is DCA-ET (dynamic controlled atmosphere - ethanol), which monitors ethanol levels (Zanella and Sturz, 2015). This method has only been tested on a few cultivars. The method is sometimes called DCS, which is short for dynamic controlled system (Mditshwa et al., 2018). A drawback of DCA-ET is that ethanol easily dissolves in water and that apples can reabsorb and break down ethanol. Readings might therefore not be completely reliable (Bessemans et al., 2016).

3.5. Dynamic controlled atmosphere – respiration quotient (DCA-RQ)

A new promising method called DCR (dynamic control of respiration) measures CO_2-accumulation divided by O_2 uptake (Van Schaik et al., 2015). This method is sometimes called DCA-RQ where RQ stands for respiration quotient (Mditshwa et al., 2018).

To have airtight storage rooms is important, as leakages of gas will give inaccurate values for the respiration quotient. Furthermore, when O_2 levels and respiration are reduced there are very small differences and the measurement errors increase (Bessemans et al., 2016). The low levels of oxygen in the atmosphere during DCR also lowers the release of ethylene from the stored apples (Mditshwa et al., 2018).

4. Storage diseases and disorders

4.1. Pathogens

Common storage rots in the Nordic climate are *Penicillium expansum, Neofabraea spp*, *Colletotrichum spp.*, *Monilinia spp.*, and *Botrytis cinerea* (Maxin et al., 2012). *Phacidopycnis washingtoniensis*, or rubbery rot, is a pathogen new to Europe that has been found in northern Germany (Weber, 2011).

The symptoms of *P. expansum* are watery light brown lesions. There is a distinct difference between affected areas and unaffected areas. Spores are white at first, but turn to a bluish green color later, hence the common name blue mould. Infected fruits have an earthy and musty odour (Vico et al., 2014). *Penicillium expansum* has the ability to produce the mycotoxin patulin, which is both cytotoxic and genotoxic (Zhong et al., 2018; Assuncao et al., 2019). Inoculum can develop from debris present in the bins used to transport apples to storage. After storage, infected fruits can spread spores to healthy fruits through the dump water (Spotts and Cervantes, 1986). The pathogen infects apples mainly through wounds, but can also infect the fruit via the stem (Rosenberger et al., 2006). Culling of infected fruit is an important measure to reduce damages (Zhong et al., 2018).

Figure 5 Apple infected with Penicillium expansum, cultivar 'Rubinstar'. Photo: J. Sjöstrand

Neofabraea spp., or Bull's eye rot, infects apples through lenticels or wounds during the growing season, but then lies dormant until the fruit is in storage (Köhl et al., 2018). Symptoms usually become visible some months after the harvested apples have been put in storage and include slightly sunken, brown and firm lesions with a dark or light centre (Cameldi et al., 2017). There is no clear distinction between the infected area and the healthy area. Older lesions often have cream-colored spore masses coming from the lesions (Spotts et al., 2009). The pathogen is present in leaf litter, fruit mummies and cankers in the orchard and infects the fruit from there (Köhl et al., 2018). The optimal temperature range for mycelial growth is between 18 and 20°C meaning that damages can develop fast during shelf life. Low temperatures slow down mycelial growth (Hortova et al., 2014).

Colletotrichum spp. are important pathogens in the Nordic climate, which infect the fruit with appressoria, and are causing bitter rot (Peres et al., 2005; Borve and Stensvand, 2007). Like *Neofabraea*, bitter rot is present in the orchard in buds, leaf litter and fruit mummies (Peres et al., 2005). Hot and humid weather can cause severe spread of the disease in the orchard, but in colder climate, the disease usually emerges during storage (Jones et al., 1996; Borve and Stensvand, 2007). Symptoms can be diverse, but generally include sunken, brown and circular areas. In the case of *C. acutatum*, orange conidial masses are a distinguishing mark that sets it apart from *Neofabraea* spp. (Borve and Stensvand, 2007). Recent studies have shown that what was believed to be two species is actually two species complexes, *C. acutatum* and *C. gloeosporioides*. These differ in virulence, where the *C. gloeosporioides* species complex is more aggressive than the *C. acutatum* species complex. However, *C. acutatum* generally has a higher tolerance to certain fungicides and also tends to release more spores. To differentiate the two species complexes, the fungi are cultured on agar and colony shape, color and size are evaluated. In addition, the size and shape of the conidia are used for identification (Munir et al., 2016). Minimizing losses to *Colletotrichum* spp. is done by harvesting the fruit early. More mature fruit is more susceptible to the pathogen due to softer flesh (Ahmadi-Afzadi et al., 2013; Borve et al., 2013).

Figure 6 Apple infected with Colletotrichium acutatum, cultivar 'Rubinola'. Photo: I. Tahir

Monilinia fructigena, or brown rot, is spread from infected fruits or other colonized material in the orchard (Falconi and Mendgen, 1994; Holb, 2008). As the number of infected fruits increases, spores become more abundant (Holb, 2008). Two other species, *M. laxa* and *M. fructicola,* can also infect pome fruits, although *M. fructigena* is the most common in apples (Holb, 2003). To harvest at optimal harvest time is an important measure to minimize infections, as both an early or late harvest increase damages due to brown rot (Valiuskaite et al., 2006). *Monilinia* spp. infect fruit mainly through wounds, and therefore an important measure is to protect the fruit from damage (Xu and Robinson, 2000).

Figure 7 Fruit mummy with severe brown rot (Monilinia fructigena), cultivar 'Ingrid Marie'. Photo: J. Sjöstrand

Grey mold (*Botrytis cinerea*) causes significant losses during post-harvest storage (Weber et al., 2018). The pathogen may infect fruit from mummified infected fruits left from last year's harvest. The infection starts already at flowering, however symptoms do not appear until the fruit is close to maturity (Tronsmo and Raa, 1977). Infection usually starts in a wound of the fruit, and at first, it can be seen as extra coloration around the infected area (Tronsmo and Raa, 1978; Staples and Mayer, 1995). As the infection progresses, the affected area turns soft and rotten, and lastly brown and dry. Infected fruits on trees develop fast and usually fall early. Infection can spread in storage (Tronsmo and Raa, 1978).

4.2. Physiological disorders

Damages may develop due to unfavourable conditions during the preharvest or postharvest period. This could be due to nutritional imbalance, climate factors or unfavorable storage conditions (Kays, 1999; Watkins et al., 2004).

Soft scald is a condition characterized by band-like browning of the skin and flesh of the fruit. There is a clear line between affected and unaffected areas (Brooks and Harley, 1934). In general, a later harvest tend to increase the presence of soft scald, but after a certain point the risk of soft scald decreases (Watkins et al., 2005; Prange et al., 2011). Unsuitably low temperature during storage is another factor that has been linked to an increased presence of soft scald (Watkins et al., 2005). Losses can be as high as 30% of the fruit (DeLong et al., 2004). There is evidence that the condition is metabolic, as controlled atmosphere during storage and addition of ethylene blocker 1-methylcyclopropene (1-MCP) both have been shown to decrease the incidence of soft scald (Fan and Mattheis, 1999; Blankenship and Dole, 2003; DeLong et al., 2006). Although methods to reduce the incidence of soft scald have been found, the cause of it is not yet fully understood (Tong et al., 2003).

Figure 8 Soft scald on 'Santana' apples. Photo: J. Sjöstrand

Soggy breakdown, which is also known as low temperature breakdown (LTB), is another physiological disorder that is induced by low temperature during storage. As for soft scald, lower temperatures as well as a harvest date later than the optimal harvest date greatly increases the incidence of LTB (Watkins et al., 2005). One theory is that soggy breakdown and soft scald might be different symptoms of the same disorder (Plagge et al., 1935). The two disorders develop under similar circumstances and have some symptoms in common, such as brown spongy flesh and alcohol flavour (Plagge et al., 1935; Watkins et al., 2004), but a possible connection between them has yet to be confirmed. Other symptoms of LTB include browning and softening of the fruit flesh beneath the skin. Internal symptoms of LTB are often hard to detect in intact fruits, but sometimes symptoms like loss of gloss in the fruit skin occur, which leads to a dull surface (Plagge et al., 1935).

Bitter pit is a physiological disorder that develops during fruit development and storage (Ferguson and Watkins, 1989). The disorder manifests itself as small pits forming mainly in the calyx end of the fruit. As the pits age they dry out and turn brown (Ferguson and Watkins, 1989). The damage is due to collapse of cell walls (Smock and Van Doren, 1938). It has long been known that low levels of calcium are linked to the development of bitter pit (Delong, 1936). Spraying with different calcium salts during growing is a way to decrease the incidence of bitter pit. Another method is to dip harvested fruits in a calcium solution (Turner et al., 1977). If apples are harvested before the optimal harvest time, there is an increased risk of developing bitter pit (Prange et al., 2011).

Superficial scald is an important postharvest disorder which is characterized by dark, diffuse discoloration of the peel (Bain and Mercer, 1963; Fan and Mattheis, 1999). As chilling can initiate the condition (Watkins et al., 1995), slowly cooling weather before harvest reduces the occurrence of scald by conditioning the fruit to the cool storage conditions. If the harvest has to be done during a hot season, the fruit can be conditioned by a slow decrease of temperature in the storage to decrease the risk of scald (Marc et al., 2020). An early harvest can increase the incidence of scald, as can low levels of calcium (Dallenbach et al., 2020). α-farnesene is a compound found in the cuticle of apples that has been linked to superficial scald. It is likely the oxidation of α-farnesene, and the resulting compounds, that cause the symptoms of superficial scald (Fan and Mattheis, 1999; Rowan et al., 2001). It is, however, not known how these compounds cause damage (Lurie and Watkins, 2012). The incidence of superficial scald is affected by fruit maturity and cultivar, and post-harvest treatments with diphenylamine lowers the prevalence of the disorder (Huelin and Coggiola, 1968; Dallenbach et al., 2020). Diphenylamine is an antioxidant which prevents oxidation of α-farnesene (Huelin and Coggiola, 1970a; Zanella, 2003). (Watkins et al., 1995). Proper ventilation or controlled atmosphere with low O_2 can in some cases reduce superficial scald (Huelin and Coggiola, 1970b; Wang and Dilley, 1999).

5. Current study

The total consumption of apples in Sweden is about 110 million kg per year. In 2016 only about 25% of that was Swedish fruit (Jordbruksverket, 2017). This is an increase from 2010 when the corresponding figure was about 20%, but there is still room for further increases as many Swedish consumers prefer Swedish cultivated fruit (Fernqvist et al., 2011; Jordbruksverket, 2017).

The increase in Swedish production was possible due to new orchards with denser planting, which produce more fruit per hectare (Jordbruksverket, 2017). However, compared to countries in central or southern Europe yields are low (FAOSTAT, 2018).

During the summer months, the proportion of Swedish apples sold is lower than for the rest of the year (Jordbruksverket, 2017). To ensure that Swedish produced apples get a larger market share, it is important to store the fruit in a way that makes it possible to sell fruit all year around. To be able to store the fruit in an optimal way we also have to be sure that we harvest the fruit at the optimal harvest date.

Apples are lost due to decay from pathogens and disorders, which tend to increase if the fruit is not harvested at the optimal harvest time (Borve et al., 2013). In Sweden, the Streif index is the common maturity index used to predict harvest time and storability (Tahir, 2014). As this index was developed over 20 years ago, the reasons for using the method are well substantiated (Streif, 1996; Tahir, 2014). There are however drawbacks to the Streif index, as the measurements are time consuming and destructive and based on small samples (DeLong et al., 1999). With a non-destructive type of assessment, such as the I_{AD}-measurement, more apples could be sampled without losing part of the produce (DeLong et al., 2014). A more extensive sampling could lead to a more uniform ripeness assessment and hence a better storability.

In 2018, the Swedish government established the education program "Industridoktorander inom livsmedelsområdet (LivsID). The project "New storage methods of apple, adapted for Nordic conditions" is a part of this program and the goal is to develop better storage methods and a more precise prediction of harvest date (Passoth, 2020). Using a DA-meter, larger samples of fruit are tested which could lead to a more uniform quality and better storability. I_{AD}, maturity and how they affect physiological disorders, as well as rot caused by pathogens, are investigated. Trials where I_{AD} is compared to traditional indexes, such as firmness, sugar content, starch degradation, and Streif index are conducted. Currently, Swedish apples are stored in cool rooms or under ULO-conditions. In ULO storage the levels of carbon dioxide and oxygen are static. By using the DCA regime, it is possible to adjust the atmosphere based on specific stress signals from the fruit (Gasser and von Arx, 2015). During DCA storage, quality parameters, such as firmness, decrease slower than during traditional CA. This means that the fruit might withstand fungal attacks in a better way, which in turn leads to improved storability (Lafer, 2010; Ahmadi-Afzadi et al., 2013). Optimal storage conditions (Gasser and von Arx, 2015; Bessemans et al., 2016) and DA-values for storage (DeLong et al., 2014; DeLong et al., 2016) have been determined for many cultivars but not much is known about Swedish-grown cultivars. By determining the optimal storage conditions and DA values for our domestically grown cultivars there is a possibility to improve the quality of Swedish apple fruit, and lower the loss, which in turn would help achieve the goal of Swedish apples all year round. Furthermore, growers would benefit from being able to sell apples for a longer period after harvest as the fruit quality deteriorates slower under the DCA storage (Lafer, 2010). Apples of high quality all the year round would increase the market share and consequently the revenues of the Swedish apple industry.

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