Predicting strawberry shelf life based on input quality

GreenCHAINge Fruit & Vegetables WP3, BO-29.03-001-010

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Contents

Summary

The general objective in GreenCHAINge Fruit & Vegetables Work package 3 (GreenCHAINge), is to pave the way towards a supply chain with a high and constant strawberry quality. This report focuses on two main aspects: how initial quality at harvest influences strawberry quality and to what extent non-destructive measurement techniques can accurately determine and predict strawberry quality.

At harvest, strawberries are picked at a certain level of ripeness, which is generally determined based on colour. The strawberry quality at the point of harvest is termed as its "initial quality". Initial strawberry quality is directly influenced by several issues related to growth, such as weather, picker and time of year. Other issues, such as order volume and market price, can also affect initial quality by changing the boundaries of harvest selection. The effect of variations in these issues on the initial quality and the shelf life in the chain is currently unknown.

Beyond the harvest moment, strawberry quality changes over the course of its shelf life. While the ripening process will initially bring the fruits towards a ready-to-eat state, it will also make them more vulnerable to lose quality due to mechanical injury, decay, water loss and physiological deterioration. A well-established cold chain will strongly slow down these processes, but cannot prevent them altogether.

As indicated, the aim of this study is to get insights in the effects of the ripening stage at harvest on the quality in the supply chain and in the use of non-destructive measuring methods to determine strawberry quality. To study the effects of initial quality, strawberries from the cultivars 'Elsanta' and 'Lusa' were harvested at different ripening levels during different times in their respective seasons and were stored for various times, after which their quality was assessed. Parallel to these experiments, different non-destructive techniques were tested to assess their capacity to determine and predict strawberry quality. After assessing the influences of strawberry cultivar, growth season, harvest ripeness and storage time and the use of PTR-ToF-MS and near infra-red spectroscopy, we can conclude that:

- Growth season influences strawberry diameter
- Harvest ripeness influences all quality parameters apart from calyx decay
- Storage time strongly influences colour, firmness and fruit decay
- Spectral analysis using VIS-NIR showed that firmness can be determined non-destructively
- VIS-NIR shows promise for predicting firmness and fruit decay

This document is the result of a study as part of GreenCHAINge. This study was executed from January 2015 until March 2019 by researchers of Wageningen Food & Biobased Research (WFBR), who performed an objective and independent study for Driscoll's Europe and Bakker Barendrecht, who partly financed this project.

1 Introduction

1.1 Background

The GreenCHAINge project is a project financially supported by the industry and the Dutch government (Topsector horticulture public private partnership) comprising different sub-projects (work packages) focussing on different fruit and vegetable products. One of the sub-projects, work package 3, is dedicated to soft fruit and is carried out with and by Driscoll's BV, Bakker Barendrecht BV and Wageningen Food and Biobased Research (WFBR).

A major goal of the soft fruit project is to contribute to the understanding of strawberry quality and as such pave the way towards controlling quality to supply high and constant strawberry quality. Strawberries lose quality due to mechanical injury, decay, water loss and physiological deterioration (Nunes et al., 1995). One of the key aspects that influence the quality in the supply chain is the so-called initial quality or quality at harvest. Based on literature and expert knowledge it can be expected that the initial quality is strongly dependent on the ripening level of the fruit at harvest. The ripening stage at harvest follows a normal distribution. Growers set limits on the ripening stage of the fruits at the harvest moment. These limits should be constant and fixed but due to several issues such as weather, picker, time of the year, order volume and market price they may be changed consequently affecting the quality. While extremes are generally avoided, everything in between may vary. The effect of these variations on the initial quality and the shelf life in the chain is currently unknown. Additionally, it is also unknown to what extent the level of quality can be maintained or improved when adjusting the quality limits.

Fruit ripening is a complex process that is influenced by the action of hormones, the production of pigments, the metabolism of sugars and formation of volatiles. Ripening stage is therefore characterised by different physiological parameters such as firmness, colour, amount of specific sugars, organic acids, ratio sugars-acids and several volatiles. Some of these parameters may have a strong relation with the quality development within the distribution chain and therefore be used as a marker for shelf life.

A second very relevant aspect determining quality is the strawberry variety. The differences between cultivars (phenotype) are generally very well-known. How a variety interacts with all those aspects that influence quality is however more complex and difficult to predict. The study on the effect of ripening limits/stages should therefore be combined with the "cultivar" aspect.
A large part of the sub-project was dedicated to these subjects. Therefore a number of experiments was set up together with the companies involved in the project to answer these questions and improve our understanding of the relation between ripening stadium, initial quality and shelf life for a number of cultivars. Finally the use of non-destructive methods to measure relevant initial quality parameters was also addressed in this research.

This document reports the work carried out within this ripening, quality and shelf life research, that has been conducted fully independently by Wageningen Food & Biobased Research.

1.2 Objective

The main goal of this study is to get insight in the effect of the ripening stage at harvest on the quality in the supply chain, in other words what are the consequences of narrowing/widening the ripening limits towards the unripe and over ripe stage. This effect will also depend on the variety, the growth season and on the growth year. Another relevant aspect of this work is to identify which parameters of the initial quality are most related to shelf life. This should deliver relevant information, i.e. focus on what is relevant to measure within the supply chain. Finally the possibility to measure the most relevant aspects with non-destructive methods was also explored.

1.3 Report structure

This document is intended to report the extensive work carried out in this part of the work package to the partners involved in the work package, but also as a reference for future work on this field. This research has been carried in a number of separate experiments and therefore the report is structured as such. Each experiment is reported in a separate chapter. Finally the main conclusions are drawn in the final chapter.

2 Effects of season and ripening level at harvest on storage quality

2.1 Introduction

Strawberry (Fragaria x ananassa Duch.) is a popular fruit, renowned for its rich aroma and taste. The fruit is produced worldwide, and totals over 9.2 Mt per year (FAOSTAT, 2019). Classified as a 'soft fruit' (Manning, 1993), strawberries are very sensitive to handling and have a relatively short shelf life (Kader, 1991; Pérez et al., 1999). During their shelf life, strawberries are generally qualified based on their colour, firmness, calyx decay and fruit decay (Collins and Perkins-Veazie, 1993; Nunes et al., 1995; Villarreal et al., 2008). However, they are also qualified on their aroma and taste (Pelayo et al., 2003; Kim et al., 2013). Ripe strawberry fruits produce hundreds of different aroma volatiles, culminating into a complex aroma that is cultivar specific (Ulrich et al., 1997; Forney et al., 2000; Schwieterman et al., 2014). Current standard techniques to determine strawberry quality characteristics are destructive. However, non-destructive techniques that may be of interest to determine strawberry quality are PTR-ToF-MS for volatile-detection (Jordan et al., 2009; Cappellin et al., 2012) and near infra-red spectroscopy (NIR) (Shao and He, 2008; Sánchez et al., 2012; Amodio et al., 2017).

In order to assess the effects of season, ripeness and storage time on the quality of strawberries, strawberries were harvested at three time-points in their respective season. During harvest, a selection was made based on colour to select strawberries that were unripe, ripe and overripe. This difference was very apparent, as unripe strawberries were a very light red and showed white shoulders and or tips, ripe strawberries were bright red all over the fruit surface and overripe strawberries were a darker red overall. One day after harvest and arrival at WFBR, the strawberries were analysed based on volatile production, quality, colour, Near Infrared (NIR) spectroscopy and firmness.

2.2 Material and methods

Plant material
Strawberries (Fragaria × ananassa) variety Lusa were obtained from Driscoll's of Europe (Prinsenbeek, the Netherlands) and variety Elsanta from Bakker Barendrecht (Ridderkerk, the Netherlands). The strawberries had been grown under uniform growth conditions per variety during spring 2016 in the Netherlands. Ripeness levels were determined by eye based on colour during harvest. Harvested strawberries were placed directly into punnets that were subsequently placed in boxes. Directly after harvest, the boxes were transported to Wageningen Food & Biobased Research (Wageningen, the Netherlands), where the punnets were randomized per ripeness level, labelled and stored at 4°C and 80% RH. On days 1, 5 and 9 after harvest and labelling, respective samples were placed at 20°C and 80% RH for 6 hours prior to the measurements.

Volatile analysis
To determine volatile organic compound (VOC) production of the strawberries, the strawberries in a punnet were carefully transferred into an amber Duran GLS 80 wide mouth laboratory bottle of 2000 mL (DWK Life Sciences GmbH, Mainz, Germany) with septa mounted in the lids. After flushing with clean and filtered air for 1.5 min, the jars were closed, moved and after 1.2 h of headspace accumulation sampled using a PTR Qi-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria). The PTR-ToF-MS had a drift voltage of 900V at 60°C and 3.8 mbar, resulting in an E/N of 121 Td. Sampling flow rate was 60 mL/min and the mass range was 20 – 512 m/z. Samples were taken from the jars by direct injection into the PTR-ToF-MS drift tube through a heated (110°C) peek inlet connected to a syringe needle. PTR-ToF-MS data was analysed using the program PTRwid (Holzinger, 2015), after which noise reduction was achieved by averaging over 20 consecutive and stable ToF

spectra of the same sample, followed by baseline removal. Subsequently data were normalized for accumulation time and punnet weight. Peak identification was done within PTRwid.

Weight & Visual quality

Strawberry punnet weight was recorded using a MS6002TS balance (Mettler-Toledo GmbH, Giessen, Germany). Visual quality was scored by assessing the punnet on appearance and smell, after which calyx quality and skin decay were assessed for each individual strawberry in a punnet. Appearance was evaluated by looking at the closed punnet from above, using a 4-point scale where 3, 2, 1 and 0 represent 0 - 8.25, 8.25 - 16.5, 16.5 - 25 and >25% defects, respectively. Smell was assessed by smelling the punnet directly after removing the punnet cover if available and scored on a 3 point scale where 0, 1 and 2 represent no, light and strong/persistent off-odours, respectively. Calyx quality was assessed by scoring each strawberry in a punnet individually on a 4 point scale where 0 represents fresh green; 1, yellow and/or one leaf brown; 2, multiple (up to 50%) leaves brown and 3 > 50% of the leaves brown. Decay of the outer skin was assessed on all strawberries in a punnet by scoring each individual strawberry on a 6 point scale; 0, no bruises; 1, single dry bruise; 2, single wet bruise or multiple dry bruises; 3, multiple wet bruises; 4, minor mould or rot; 5, > 50% mould or rot. Scores of individual strawberries were averaged per punnet.

Colour analysis

Strawberry colour was assessed on 15 strawberries per punnet using image analysis of both sides of the strawberries. Calyxes were removed prior to acquiring the image. Images were acquired using an LED light cabinet (Designed by WFBR and build by IPSS Engineering, Wageningen, the Netherlands) containing a RGB camera (MAKO G-192C POE, Allied Vision, Stadtroda, Germany). The RGB images were calibrated using a 24 patch colour checker card (Color checker classic, X-rite Europe GmbH, Regensdorf, Switzerland). Image analysis was done using multi-threshold colour image segmentation in the HSV colour space (in-house software tool developed at WFBR, Wageningen, the Netherlands) to single out the strawberry background and assess the strawberry Hue colour values.

VIS-NIR

From the same 15 strawberries per punnet used for colour analysis, VIS-NIR spectra were recorded. To record the spectra a parallel setup was used, consisting of a MSC 521 VIS NIR and a MCS 511 NIR (Zeiss, Oberkochen, Germany) with a KL 1500 electronic light source (Schott AG, Mainz, Germany). The shoulder of individual strawberries were placed against the probe and the spectra acquired. Spectra were averaged per punnet and the averaged spectra pre-processed by mean normalization using Unscrambler X v10.5 (CAMO AS, Trondheim, Norway). Correlation to other physiological parameters was done using Partial Least Square Regression (PLSR) within Unscrambler X.

Firmness

From the same 15 strawberries per punnet used for colour and NIR analysis, firmness using a Firm Tech FT7 (UP GmbH, Ibbenbüren, Germany). Strawberries were placed on the turn-table and one by one subjected to a threshold and maximum force of 75 and 250 g, based on which the width and the compression force per depth (g mm^{-1})were determined.

Sugars

The same 15 strawberries used for colour, NIR and firmness analysis were each divided in half, the halves of the 15 strawberries grouped into two batches and each batch cut to small pieces for sugar and organic acid analysis. Samples were frozen in liquid nitrogen and immediately ground using an IKA A11 basic analytical mill (IKA, Staufen, Germany) under liquid nitrogen. Ground samples were stored at -80 °C. From the frozen material for sugar analysis, samples of 250 mg were prepared, to which 5 mL of ethanol was added and vortexed thoroughly. Subsequently, samples were incubated in an 80 °C rotary water-bath for 2 hours, after which the samples were vortex again and placed on ice. Following this, the samples were centrifuged for 10 minutes at 8500 x g and 4°C. Supernatant was collected and 1 mL samples of this were dried using a Vacufuge concentrator 5301 (Eppendorf, Hamburg, Germany) at 30°C for 4 hours and left overnight. The pellet was re-suspended in 1 mL MQ water by vortexing vigorously for 30 s, placing the samples for 10 minutes in an ultrasonic bath and vortexing once more for 30 s. Samples of 400 µL were diluted with 40 µL internal standard (2.00 mg L^{-1} deoxygalactose, Acros organics) and MQ water to a total volume of 4 mL and filtered using a 0.2

µm Sartorius Minisart hydrophilic syringe filter. Samples were ran on an HPLC (Dionex ICS5000, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Marathon autosampler (Spark Holland), Dionex GS50 pump and a Dionex PED-2 detector operating in pulsed amperometric mode. Carbohydrates were separated at 25 °C on a CarboPac1 column (250 × 2 mm ID) equipped with a guard column and calibrated using 2.55 mg L^{-1} xylose, 15.00 mg L^{-1} glucose, 12.50 mg L^{-1} fructose and 2.53 mg L^{-1} sucrose. Sample volume was 10 µL, which was eluted using 45 mM NaOH at a flow rate of 0.25 mL min^{-1} over a total of 40 min. Samples were analysed using Chromeleon 7.1 (Thermo Fisher Scientific) and processed using Microsoft Excel.

Organic acids
From the frozen material for organic acid analysis, samples of 5 g were prepared, to which 20 mL of 1 M sulfuric acid was added. Each sample was mixed using a T25 Ultraturrax with a S25 N probe (IKA, Staufen, Germany) and centrifuged for 30 min at 3250 x g and 4 °C. Per sample, supernatant was collected and 900 µL prepared for further analysis, to which 100 µL internal standard (pthalic acid) was added. These samples were filtered using a 0.2 µm Sartorius Minisart hydrophilic syringe filter into an HPLC vial and ran on an HPLC system equipped with a Novapak C18 1 cm ID column at 30°C and a 486 Tunable Absorbance detector (Waters, Milford, MA, USA). Organic acids were calibrated using 5.970 mg L^{-1} oxalic acid, 6.148 mg L^{-1} citric acid, 6.084 mg L^{-1} malic acid, 5.828 mg L^{-1} succinic acid and 6.026 mg L^{-1} fumaric acid. Sample volume was 20 µL, which was eluted from the column using an isocratic flow of 444 µL sulfuric acid in 2 L MQ water. Total runtime was 35 min, during which the UV-detector detected for 20 min at 210 nm, followed by 15 min at 280 nm. Samples were analyzed using EmpowerTM 2 Chromatography Data Software (Waters, Milford, MA, USA) and processed using Microsoft Excel.

Data Analysis
Data analysis was done using Microsoft Excel for data collection and Unscrambler X v10.5 (CAMO AS, Trondheim, Norway) for multivariate analyses.

2.3 Results and discussion

Volatile development during storage
Since strawberries, as a soft fruit, are very susceptible to damage and volatile release is greatly facilitated when fruit is damaged, we started with volatile measurements. To this end, punnets of strawberries were carefully emptied into large 2 L wide mouthed Duran bottles. Subsequently, the bottles were moved to the PTR-ToF-MS and analysed 1.2 hour after closure. Production of individual volatiles was averaged per storage day from 5 bottles per variable. Volatile production was shown as the logarithmic of the counts per second (cps) per strawberry weight over time (log [cps g^{-1} h^{-1}]). In order to intuitively compare the production of the individual volatiles between cultivars, seasons, ripening stage and time of storage, a conditional formatting was applied per volatile over all variables (Figure 1). Since the production of volatiles with a mass / charge ratio over 175 Da was very low, the masses listed range from 17 – 175 Da. Strikingly, the abundance of individual volatiles was lower during the mid-season. However, experience in other projects has learned that the PTR-ToF-MS has shown variation over time, regardless of using the same settings. Nevertheless, at this point in time, this variation only seems to have occurred between multiple the seasons and appeared relatively stable over the time-span of storage (up to 9 days). As such, we can still compare the volatile production between cultivars, ripening stages and times of storage.

Early in the season, Lusa strawberries produced a wider variation of volatiles compared to Elsanta. On the other hand, Elsanta showed a relatively high production of a select number of volatiles. The two cultivars each produced different volatile compositions. These data illustrate that the cultivars Elsanta and Lusa have different volatile production patterns. From unripe to overripe strawberries, both Elsanta and Lusa showed increased production of volatiles, although the increase was most noticeable as the storage time increased. Indeed, storage time seemed to be the most dominant factor that increased the production of the actively produced volatiles. The effect of storage time was relatively small in unripe strawberries and became larger with the increased ripeness of strawberries.

Interestingly, a very similar pattern is also visible in the increase in decay and the loss of firmness. This could suggest that the weakened cell structures and the increased occurrence of wet bruises result in an increased release of volatiles in general, more so than an evolution in the production of different volatiles.

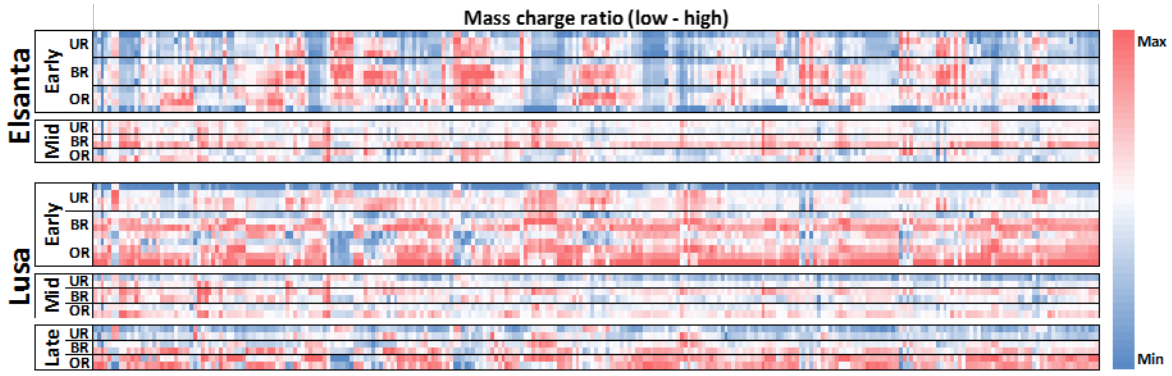


Figure 1: Relative volatile production of strawberries at different ripening stages in different seasons using PTR-ToF-MS. Strawberries of cultivars Elsanta and Lusa were picked relatively Early, halfway (Mid) or Late in the season and selected to be unripe (UR), ripe (BR) or overripe (OR). Each ripening stage shows days 1, 4, 7 or 8, and 10 (Early) or day 1 and 7 (Mid and Late) of the storage period. Mass charge ratios are organized from low to high from left to right, respectively. Data represent logarithmic values of PTR output per kg per hour (counts per second kg^{-1} h^{-1}). Conditional formatting was applied per mass charge ratio over all four variables with red and blue depicting the highest and lowest volatile production, respectively.

Overall quality during storage

Directly after volatile analysis, strawberries were assessed for the quality parameters “appearance”, “off-odour”, “calyx-decay” and “fruit decay”.

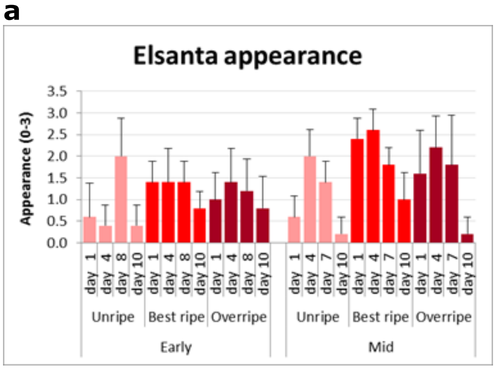
Appearance, described as the first impression of the quality of the strawberries within the punnet on a scale of 0-3, at 0.6 was relatively low for unripe Elsanta strawberries from early in the season (Figure 2a). A main reason for this was the number of white on the strawberries, which is generally recognized as unripe and less flavourful strawberries. During storage, the appearance improves somewhat up to 2, but after 10 days of storage is reduced to 0.4 again. Ripe Elsanta strawberries start out mediocre at 1.5 and remain constant until after 10 days the appearance decreases to 0.8. Overripe strawberries scored 1.0 after the first day of storage, which improved to 1.4 on day 4, but after this decreased to 0.8 on day 10. In the middle of the season, unripe strawberries still scored low 1 day after harvest, but on day 4 already scored 2.0, after which the appearance declined again. Ripe strawberries, at a 2.5 appearance score, scored better compared to early in the season, but this score declined after the 4th day down to 1.0 after the 10th day of storage. Overripe strawberries started out at 1.5 on day 1, increased to 2.2 on day 4 and declined sharply after 7 days to 0.2. For Lusa, early season unripe strawberries, at 2.3, scored higher in appearance than Elsanta strawberries and remained relatively constant during storage. Ripe Lusa strawberries started out on this same level, but quickly declined to around 1.0 after this initial level. Overripe Lusa strawberries had a low appearance overall during the early season. In the middle of the season, Unripe Lusa strawberries scored relatively high at 2.5, which increased to 3.0 after 4 days of storage, at which it remained. Ripe strawberries showed a similar rise, but their appearance declined afterwards to 1.3 after 10 days. Overripe strawberries started out slightly lower at 2.0 and after 4 days on this level declined to 0.6 after 10 days of storage. During late season, unripe Lusa strawberries had an appearance varying between 2.0 and 2.5, whereas ripe strawberries started out at 1.0 and, after a 0.8 increase on day 4, decreased down to 0.5 on day 10. Overripe strawberries during this season at 0 had a very poor appearance. Overall, the decline in appearance during storage was mainly due to the obvious ripening stages of the strawberries and the visible decay in the calyx.

Off-odours, described as any non-typical strawberry scents and classified based on the strength and pungency of these scents on a scale of 0-3, for early season Elsanta strawberries started out relatively insignificant during the first 4 days of storage (Figure 2c). On days 7 and 10 of storage the off-odour score increased to 0.5 in all ripeness levels. In the middle of the season, off-odours were near insignificant in unripe and ripe strawberries throughout the storage time. Overripe strawberries however showed an increased level of off-odour between 0.2 and 0.8 between day 4 and 10 of storage. For Lusa, during the early season all ripeness levels showed minor development of off-odour as storage progressed beyond day 4 (Figure 2d). In the middle of the season, off-odours did not occur much. Late in the season, ripe and overripe Lusa strawberries showed serious off-odours (1.0-2.0) at days 7 and 10 of storage.

Calyx decay was described as yellowing and browning of the calyx on a scale from 0-3. For early season Elsanta strawberries, which had relatively small calyxes, all ripeness levels showed the same pattern (Figure 2e). After 1 day of storage, all calyxes already showed some yellowing and or browning on individual calyx leaves at score 1.0, which had increased only marginally on day 4 of storage. Afterwards, calyx decay increased strongly to 1.8 on day 7 and 2.3 on day 10. In the middle of the season, calyx decay still started out the same as the early season and increased slowly to 1.3 after 7 days of storage. After 10 days of storage calyx decay had increased more sharply to 1.8. In Lusa strawberries, which had a much larger calyx, calyx decay also behaved very similar for all ripeness levels (Figure 2f). Early in the season, average calyx decay after 1 day of storage was 0.3 and afterwards increased slowly to 0.9 after 10 days of storage. In mid-season, calyx decay showed 0.7 on day 1 and gradually increased to 1.6 on day 10 of storage. During late season, unripe Lusa strawberries showed a calyx decay of 1.0 after 1 days of storage, which increased to 1.8 after 7 and 10 days of storage. Ripe and overripe strawberries showed calyx decay values that were 0.1 and 0.2 points lower, respectively, for all measuring days.

Fruit decay was described as the extend of dry bruises, wet bruises and rotten spots. For Elsanta, early in the season, unripe strawberries had a decay score of 1.0 and showed mainly individual dry bruising, which remained constant during storage (Figure 2g). Ripe strawberries showed more dry bruising and also some wet bruising already after the first day of storage (score 2.0), which increased slightly to 2.1 after 7 or 10 days of storage. Overripe strawberries started out with wet bruises (score 2.2) and this number increased as storage continued to 2.9 after 10 days. In the mid-season, unripe Elsanta strawberries started out with the occasional dry bruise (score < 0.9) and this did not increase during storage. Ripe strawberries already showed wet bruising at score 2.0, which did not increase much during storage. Overripe Elsanta strawberries started out similarly as ripe strawberries and increased their number of wet bruises slightly during storage from 2.1 to 2.7 after 1 and 10 days, respectively. Lusa strawberries during the early season started out with a decay score of 0.5 after 1 day in unripe strawberries, which had increased to 1.0 after 7 days of storage (Figure 2h). Ripe strawberries started out at 1.1 and increased to 2.1 after 10 days of storage. Overripe strawberries started out at 2.0 and increased to 2.9 after 10 days of storage. During the mid-season, Lusa strawberries behaved the same as the early season. During the late-season, unripe strawberries showed more bruising at 1.3, which increased to 1.9 during storage. Ripe strawberries showed considerable wet bruising at 2.2, which increased to 3.0 during storage. Overripe Lusa strawberries started out with multiple wet bruises at 3.0 and showed considerable development of rot at 4.0 as storage progressed.

a



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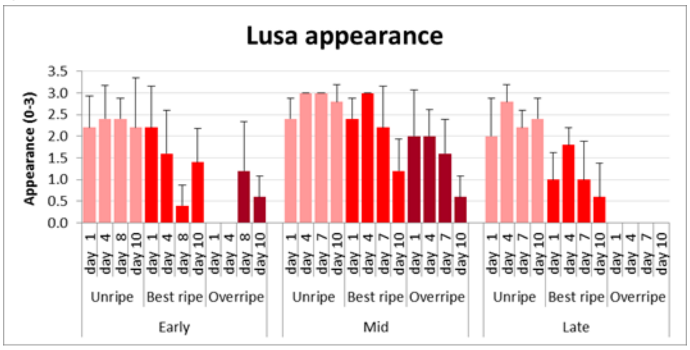

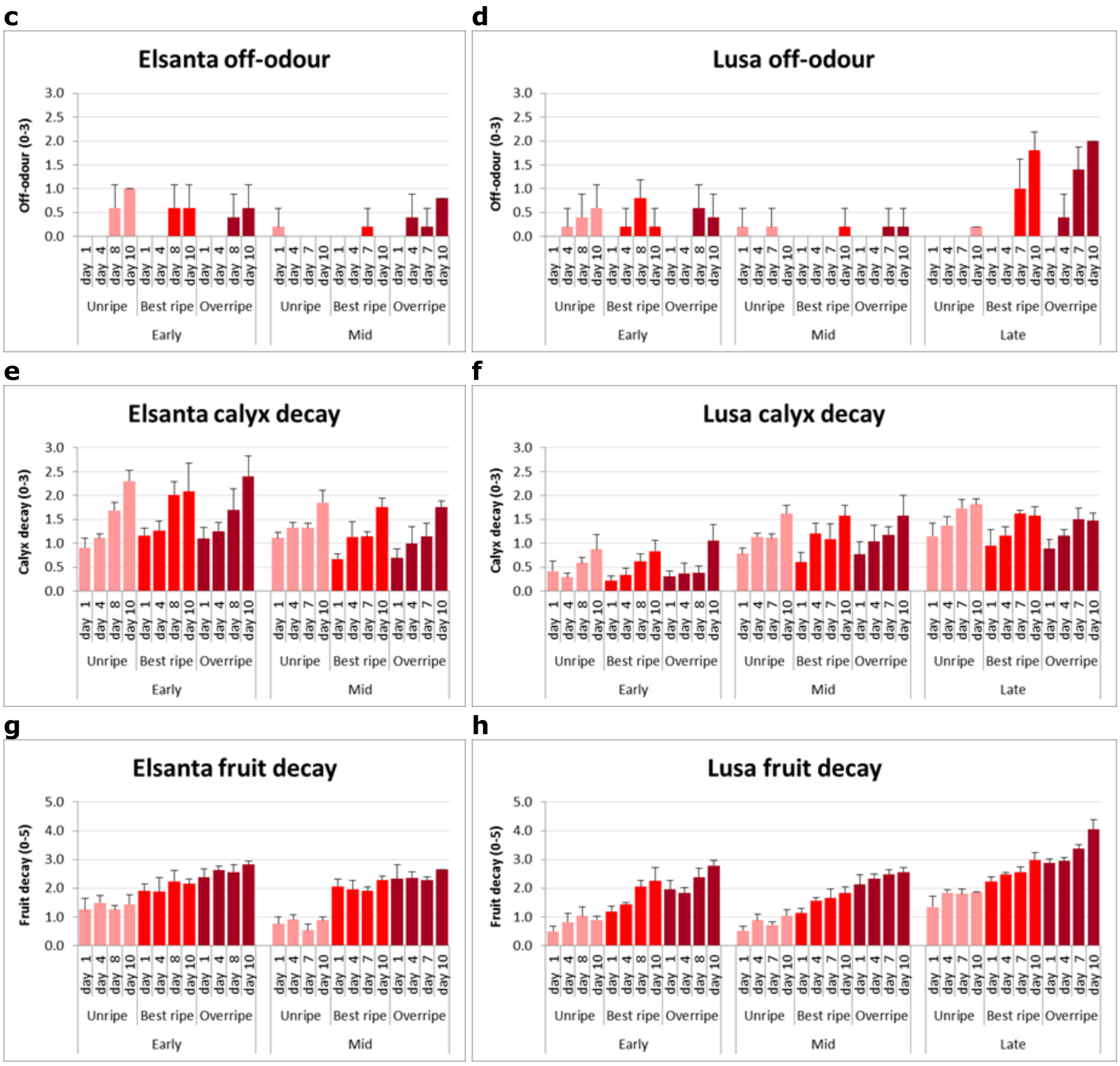

Figure 2: Quality of strawberries at different ripening stages in different seasons during storage. Visual quality was assessed by appearance (a-b), odour (c-d), calyx (e-f) and decay (g-h). Strawberry varieties used were Elsanta (a, c, e and g) and Lusa (b, d, f and h). Strawberries were harvested early, halfway (mid) and late in their respective seasons. Ripening levels were unripe (light red), best ripe (bright red) and overripe (dark red). Storage behaviour was assessed after 1, 3, 6 or 7 and 9 days at 4 °C. Data represent means ± 95% CI.

Colour development during storage

Colour, as analysed through image analysis, was described in two ways. First, the amount of white shoulders and tips were quantified by determining the relative fraction of white fruit surface. Second, the colour of the surface classified as red was determined in degrees Hue, on a circular scale from 0-360 degrees. While the Hue scale does not describe the lightness or darkness of the colour, it does show changes in the essence of the colour; from the more fresh reds (10 °Hue) to reds that contain more purple (345 °Hue, here plotted as -15 °Hue). In other words, as the colour of strawberries deepen from light fresh red in unripe strawberries to darker purplish red in overripe strawberries, this translates to Hue values from 10 to -15 °Hue.

Regarding the white shoulders, early-season Elsanta strawberries showed a considerable amount of white fruit surface, at an average of 4% (Figure 3a). During storage, less white surface was apparent, 1% after 10 days. Elsanta strawberries that were classified as ripe also showed the occasional white at 1% of the total surface after 1 day of storage, but after 4 days no white was found among the fruit. Overripe strawberries showed no white shoulders. Mid-season unripe Elsanta strawberries only showed 1.0% of white 1 day after storage, which after longer storage was near absent. Ripe and overripe Elsanta strawberries showed no white shoulders. Lusa strawberries showed no or very limited white surface regardless of season, ripening stage or storage time (Figure 3b).

Strawberry colour of early-season unripe Elsanta strawberries decreased from 10.0 to 6.0 °Hue on days 1 and 10, respectively (Figure 3c). Ripe strawberry colour decreased from 5.0 to 2.5 °Hue and the colour of overripe strawberries decreased from 1.0 to 0.0 °Hue on days 1 and 10, respectively. Mid-season Elsanta strawberry colour decreased from 8.0 to 4.0 °Hue and from 0.0 to -2.0 °Hue for unripe and ripe strawberries, respectively. Overripe Elsanta strawberries had a Hue of -4.0 °, which remained relatively constant during storage. Lusa strawberry colour early in the season was 4.5 °Hue in unripe strawberries, which had decreased to -2.0 ° Hue after 10 days of storage (Figure 3d). Ripe strawberries was -3.5 °Hue on day 1 and decreased to -7.0 °Hue at day 10 of storage. Overripe strawberries on day 1 had a Hue of -6.0 °, which had decreased to -9.0 on day 10. Mid-season Lusa strawberries showed a very similar pattern to early season strawberries. Late-season Lusa strawberries that were unripe had a 2.0 ° Hue on day 1, which decreased to -6.0 °Hue on day 10. Ripe strawberries had a Hue of -6.0 ° on day 1, which had decreased to -10.0 °Hue at day 10. Overripe strawberries had a Hue of -11.0 ° on day 1, which decreased to -15.0 °Hue on day 4, at which it remained.

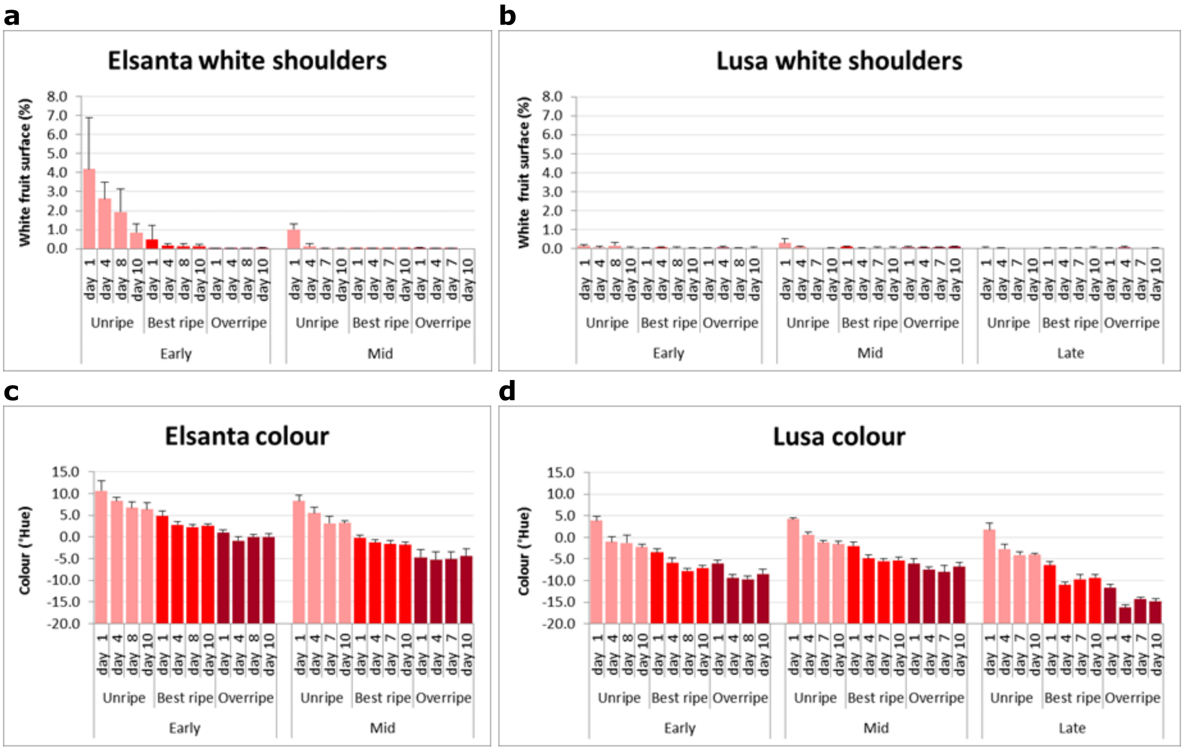

Figure 3: Colour analysis of strawberries at different ripening stages in different seasons during storage. Colour analysis was assessed by white fruit surface (a-b) and colour analysis (c-d). Strawberry varieties used were Elsanta (a and c) and Lusa (b and d). Strawberries were harvested early, halfway (mid) and late in their respective seasons. Ripening levels were unripe (light red), best ripe (bright red) and overripe (dark red). Storage behaviour was assessed after 1, 4, 7 or 8 and 10 days at 4 °C. Data represent means ± 95% CI.

Firmness and strawberry width development during storage

After all non-destructive measurements described above, including NIR measurements (described later), the average firmness and width were assessed per punnet. Early-season unripe Elsanta strawberries were relatively firm at 162 g mm^{-1}, which decreased to 140 g mm^{-1} after 7 and 10 days of storage (Figure 4a). Ripe strawberries decreased from 135 g mm^{-1} on day 1 to 115 g mm^{-1} on day 10 and overripe strawberries decreased from 113 g mm^{-1} on day 1 to 102 g mm^{-1} after 10 days of storage. Mid-season Elsanta strawberries were less firm and did not decrease in firmness during storage, with unripe, ripe and overripe strawberries having firmnesses of 140, 98 and 90 g mm^{-1}, respectively. Lusa strawberries during the early-season that were unripe had a firmness of 175 g mm^{-1} on day 1 and decreased to 138 g mm^{-1} on day 10 of storage (Figure 4b). Early season ripe strawberries were 130 g mm^{-1} on day 1 and decreased to 100 g mm^{-1} after 10 days of storage. Overripe strawberries during the early season were 100 g mm^{-1} on day 1 and decreased to 80 g mm^{-1} after 10 days of storage. Mid-season Lusa strawberries had very similar firmness levels as early-season strawberries. Late season Lusa strawberries on average had a 20 g mm^{-1} lower firmness

compared to mid-season strawberries. Overall, in both Elsanta and Lusa, firmness did not change much between seasons and decreased with increasing ripeness level of the strawberries.
The widths of both Elsanta and Lusa strawberries did not change significantly during storage. Early–season Elsanta strawberries were 34, 35 and 36 mm for unripe, ripe and overripe strawberries, respectively (Figure 4c). Mid-season, the widths were 29, 30 and 30 mm for unripe, ripe and overripe Elsanta strawberries, respectively. In Lusa strawberries, during the early season, the widths were 33, 34 and 36 mm for unripe, ripe and overripe strawberries, respectively (Figure 4d). Mid-season strawberries were 32, 33 and 35 mm for unripe, ripe and overripe strawberries, respectively. Late-season Lusa strawberries were 29, 30 and 31 mm for unripe, ripe and overripe strawberries, respectively. Overall, widths of Elsanta and Lusa strawberries decreased during their respective seasons and increased slightly with increased ripening level.

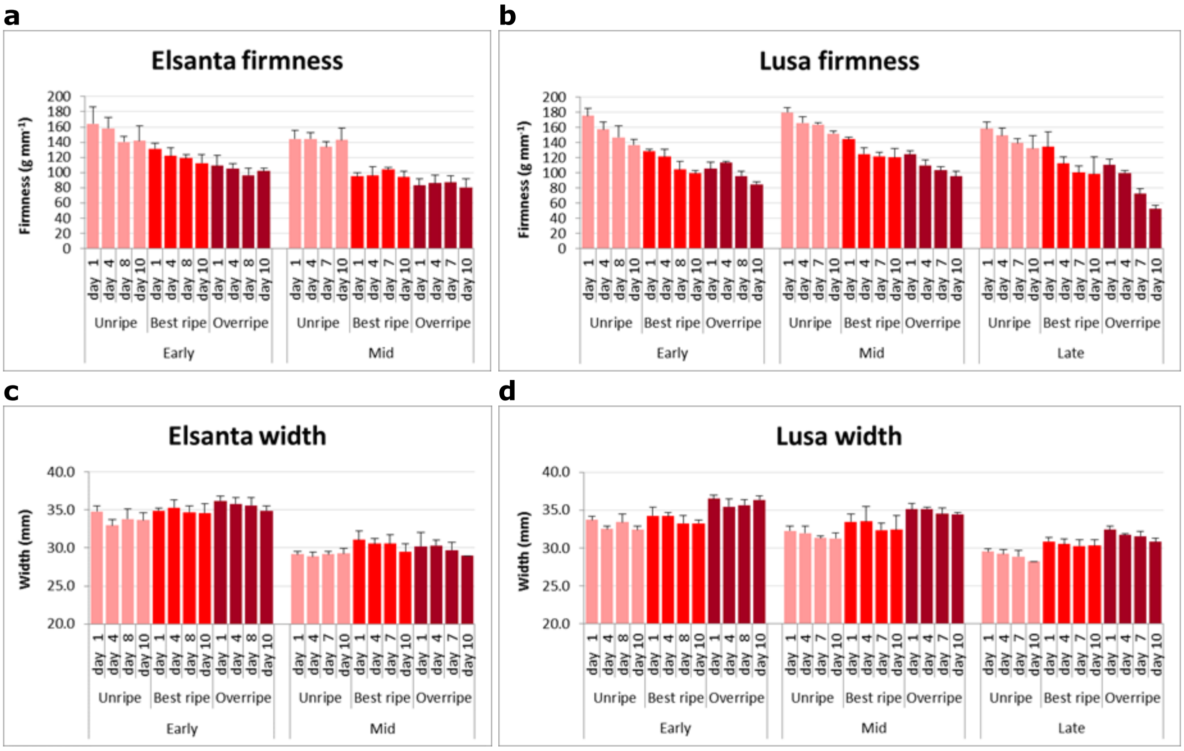

Figure 4: Firmness and width of strawberries at different ripening stages in different seasons during storage. Firmness (a-b) and width (c-d) were recorded for strawberry varieties Elsanta (a and c) and Lusa (b and d). Strawberries were harvested early, halfway (mid) and late in their respective seasons. Ripening levels were unripe (light red), best ripe (bright red) and overripe (dark red). Storage behaviour was assessed after 1, 3, 6 or 7 and 9 days at 4 °C. Data represent means ± 95% CI.

Sugar and organic acid development during storage

One day after harvest, directly following the determination of firmness and width, samples were taken for sugar and organic acid analysis. These samples were not taken from strawberries that were stored longer. Sugar analysis was done to determine the predominant fruit sugars glucose, fructose and sucrose, as well as xylose, which in cut flowers has been connected to post-harvest age (Woltering and Van Meeteren, 2016). For Elsanta, early-season unripe strawberries contained 10 mg g^{-1} glucose, whereas ripe and overripe strawberries contained 11.5 and 11.8 mg g^{-1}, respectively (Figure 5a). Mid-season unripe, ripe and overripe strawberries contained 11.9, 14.0 and 15.2 mg g^{-1} glucose, respectively. Fructose levels in early-season strawberries were 11.5, 13.5 and 13.8 mg g^{-1} for unripe, ripe and overripe Elsanta strawberries, respectively. Mid-season, these levels were 13.9, 15.9 and 17.3 mg g^{-1} fructose in unripe, ripe and overripe strawberries, respectively. Elsanta sucrose levels were 7.9, 7.7 and 6.3 mg g^{-1} during the early-season and 12.8, 14.0 and 14.4 mg g^{-1} during the mid-season for unripe, ripe and overripe strawberries, respectively. For Lusa, strawberries that were unripe, ripe and overripe contained 9.0, 8.9 and 9.9 mg g^{-1} glucose in the early-season, 9.7, 11.0 and 12.0 mg g^{-1} in the mid-season and 7.9, 8.5 and 9.9 mg g^{-1} in the late season (Figure 5b). Fructose levels in unripe, ripe and overripe strawberries were 10.5, 10.4 and 11.7 mg g^{-1} in early season; 11.5, 12.6 and 14.0 mg g^{-1} in the mid-season and 9.2, 10.0 and 11.8 mg g^{-1} in the late season. Sucrose levels in unripe, ripe and overripe strawberries were 11.7, 11.6 and 12.8 mg g^{-1} in the early season; 12.1, 13.7 and 14.0 mg g^{-1} in the mid-season and 9.5, 10.3 and 12.6 mg g^{-1} in the late-season.

Xylose levels in Elsanta were 0.60, 0.70 and 0.69 mg g^{-1} for the early-season and 0.62, 0.74 and 0.82 mg g^{-1} in the mid-season (Figure 5c). For Lusa, xylose-levels were 0.52, 0.54 and 0.53 mg g^{-1} in early-season; 0.47, 0.52 and 0.58 mg g^{-1} in mid-season and 0.46, 0.53 an d0.60 mg g^{-1} in the late season for unripe, ripe and overripe strawberries, respectively.

Overall, glucose and fructose levels show a similar pattern, with fructose being slightly more abundant. Elsanta contains higher sugar levels than Lusa for all sugars measured. The exception to this is sucrose, which in early-season Elsanta was lower than in Lusa. Early season strawberries contain less sugars than the mid-season strawberries, but in Lusa slightly more than the late-season strawberries. Also here, early-season Elsanta contained much less sucrose, up to 6.5 mg g^{-1}, while otherwise sugar levels varied around 2.3 mg g^{-1} between seasons. Xylose levels in Lusa strawberries were very similar between seasons and did not differ between ripeness levels during the early season.

In Elsanta, sugar levels increased with the ripeness levels for all sugars, except early-season sucrose, in which case it decreased. In Lusa, sugar levels also increased with the ripeness levels of the strawberries, except in early-season unripe and ripe strawberries, which showed similar values. Organic acids that were determined were citric acid, malic acid, oxalic acid and succinic acid, in order of predominance. In Elsanta strawberries, the level of citric acid was relatively high at 0.96 mg g^{-1} during the early season in unripe strawberries, but was 0.77 and 0.80 mg g^{-1} in ripe and overripe strawberries (Figure 5d). During the mid-season, unripe strawberries contained 1.2 mg g^{-1} citric acid, whereas ripe and overripe strawberries contained 0.9 and 0.8 mg g^{-1}, respectively. The level of malic acid during the early season was 0.4 mg g^{-1} in all ripeness levels, but during mid-season was slightly higher at 0.47, 0.52 and 0.55 mg g^{-1} in unripe, ripe and overripe strawberries, respectively. Oxalic acid levels during the early season were 0.27 mg g^{-1} in all ripeness levels and during mid-season was 0.18 mg g^{-1} in unripe and ripe strawberries and 0.25 in overripe strawberries. Succinic acid levels were relatively low and did not differ much between either season or ripeness level.

In Lusa strawberries, the level of citric acid was lower than that of Elsanta, at 0.77 mg g^{-1} in early-season unripe strawberries (Figure 5e). Ripe and overripe strawberries during this season both contained 0.62 mg g^{-1} citric acid. Mid-season, unripe, ripe and overripe strawberries contained 0.83, 0.78 and 0.62 mg g^{-1}, respectively and in late season these values were 0.80, 0.69 and 0.60 mg g^{-1} citric acid, respectively. Malic acid levels in Lusa were 0.40 mg g^{-1} during the early season for all ripeness levels, 0.43, 0.40 and 0.39 for unripe, ripe and overripe levels during mid-season and 0.43, 0.41 and 0.33 mg g^{-1} for these levels during the late-season, respectively. Oxalic acid levels in Lusa resided between 0.25 and 0.30 mg g^{-1} for all seasons and seemingly decreased slightly from unripe to overripe. Succinic acid levels in Lusa, at 1.6 mg g^{-1} were higher than in Elsanta and during the mid- and late-season were slightly higher at 0.20 mg g^{-1}.

Overall, organic acid levels did not vary much between season, but citric acid decreased strongly from unripe to ripe strawberries and to a lesser extent from ripe to overripe strawberries.

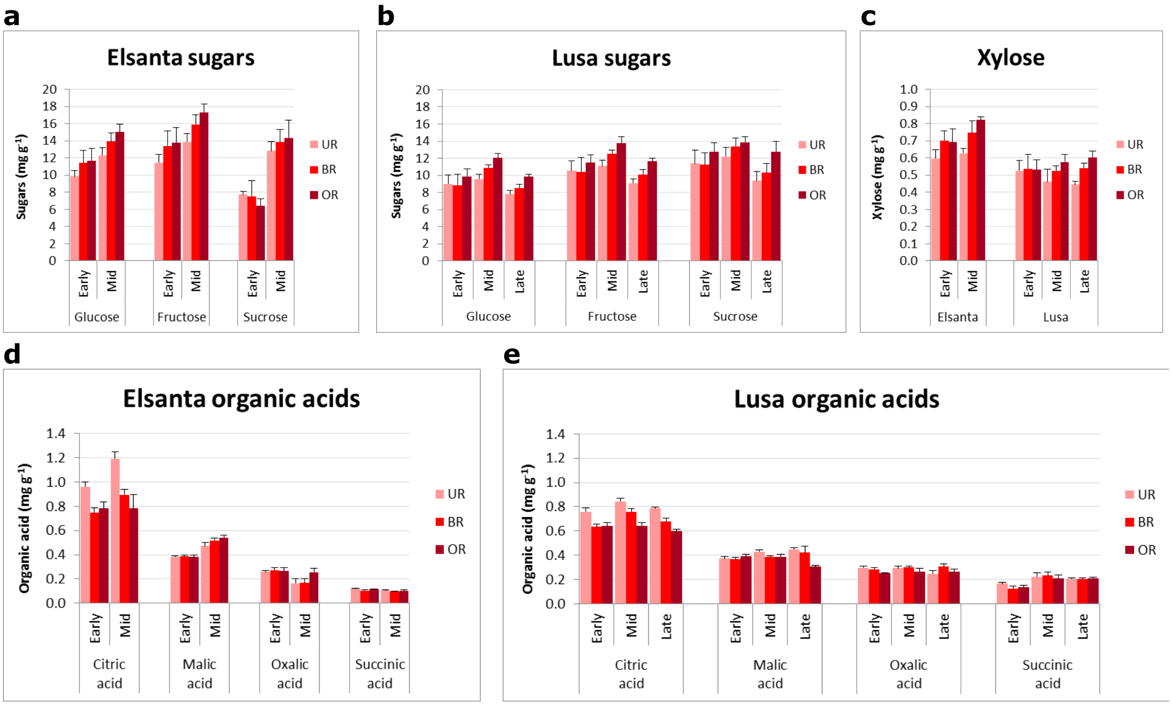

Figure 5: Sugar and organic acids contents of strawberries at different ripening stages in different seasons during storage. Sugars glucose, fructose and sucrose (a and b), xylose (c) and organic acids (d and e) are shown for strawberry varieties Elsanta (a,c and d) and Lusa (b,c and e). Strawberries were harvested early, halfway (mid) and late in their respective seasons. Ripening levels were unripe (UR, light red), best ripe (BR, bright red) and overripe (OR, dark red). Data represent means ± 95% CI.

Volatile production and VIS-NIR relations to physiological parameters

In order to assess whether the measured quality and physiological parameters can be predicted with the volatile pattern, we performed a partial least squares (PLS) multivariate analysis. Since the PTR-ToF-MS data showed some technical variation between seasons (Figure 1), we decided to only use the data from the early seasons of Elsanta and Lusa. For Elsanta strawberries, this analysis showed that the measured physiological parameters firmness, colour, fruit decay, white shoulder percentage and citric acid content showed significant correlation to the general volatile pattern (Figure 6a). More specifically, strawberries with relatively high production of most volatiles had lower values in firmness, colour (°Hue), white shoulder % and citric acid content and showed higher values in fruit decay.

Furthermore, firmness and fruit decay values obtained after 10 days of storage grouped near the firmness and fruit decay values as measured on day 0. However, no clear relations between individual volatiles and physiological parameters were apparent in this dataset. For Lusa strawberries, the analysis showed a strong polarisation of volatile-production that positively correlated with fruit decay and negatively correlated to firmness and colour (Figure 6b). Firmness and fruit decay values after 10 days of storage grouped less close to their day 0 counterparts than they did in Elsanta. Also in Lusa, no clear relations between individual volatiles and physiological parameters were apparent. Altogether, these results imply that higher production of volatiles group with the more ripe strawberries, which are less firm, less acidic, have a deeper red colour and increased fruit decay. Here we have to consider that increased fruit decay means more surface damage and that this could facilitate volatile release from the strawberries.

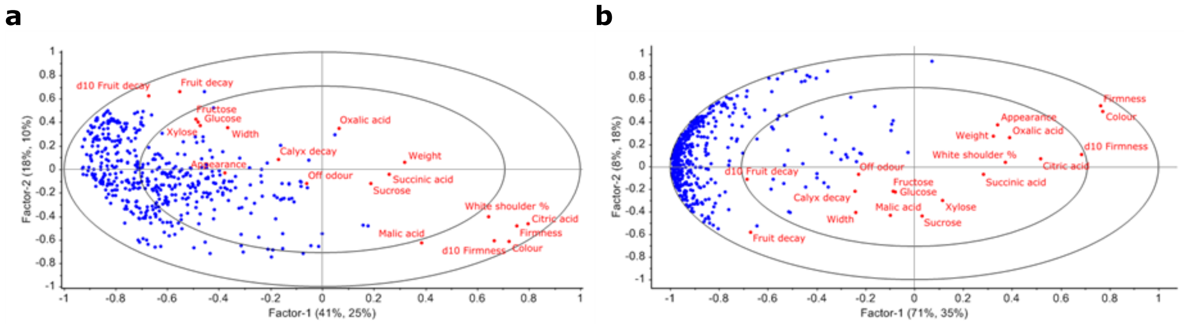

Figure 6: PLS analysis of strawberry volatiles in relation to physiological parameters. Strawberry varieties used were Elsanta (a) and Lusa (b) for early season harvests. Blue dots represent different volatiles as measured using PTR-ToF-MS on 1, 4, 7 and 10 days after harvest. Red dots represent various quality and physiological parameters as measured on the same strawberry samples as used for the PTR-ToF-MS data. The exceptions to this are glucose, fructose, sucrose, xylose, citric acid, malic acid, oxalic acid and succinic acid, which were measured only on day 1 and d10 firmness and d10 fruit decay, which were day 10 averages and connected to the day 1 data to assess potential predictive capacities of the volatile readings.

Besides the multivariate analysis described above, we also performed a multivariate analysis to assess the relation between the quality parameters and the average NIR spectra per punnet. For Elsanta, most wavelengths grouped towards higher values in firmness, colour and white shoulder % and lower values in fruit decay, e.g. less ripe strawberries (Figure 7a). For Lusa, this was not the case, although the relation between firmness, colour, citric acid and fruit decay that was observed in the previous analyses was once more very prominent (Figure 7b).

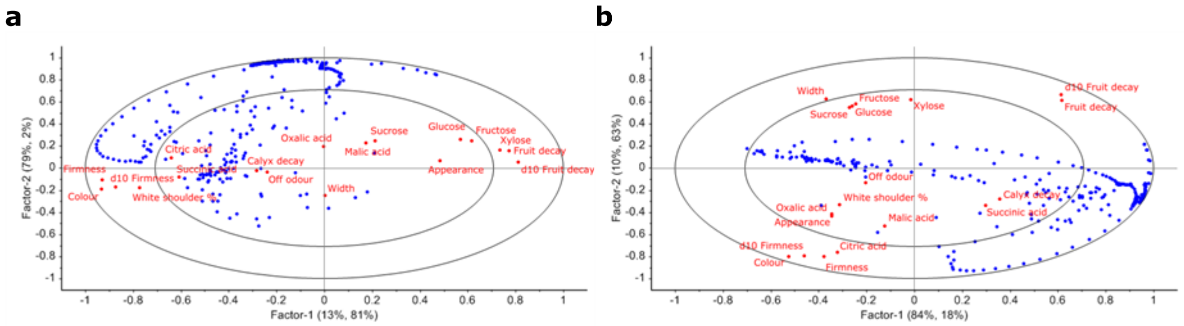

Figure 7: PLS analysis of strawberry NIR readings in relation to physiological parameters. Strawberry varieties used were Elsanta (a) and Lusa (b) for early season harvests. Blue dots represent different wavelengths as measured using NIR spectroscopy on 1, 4, 7 and 10 days after harvest. Red dots represent various quality and physiological parameters as measured on the same strawberry samples as used for the NIR readings. The exceptions to this are d10 firmness and d10 fruit decay, which were day 10 averages included to assess potential predictive capacities of the NIR readings.

Overall, Elsanta and Lusa showed similar patterns, but differed in the levels of these patterns. While Elsanta strawberries showed higher calyx decay, had more white shoulders, were more firm and contained higher levels of sugars and citric acid, Lusa strawberries produced more volatiles and had a deeper red colour (Figure 1-5). Regarding seasonal variation, Elsanta and Lusa responded similarly, with the exception that Lusa early and mid-season strawberries were not significantly different regarding the physiological parameters. In general, later season strawberries showed slight differences, such as a deeper red colour, reduced firmness and width, had slightly less calyx decay, but showed more fruit decay. Sugars and acids showed a slightly different pattern, with mid-season strawberries having slightly higher levels of glucose, fructose, sucrose and citric acid. For Elsanta, sucrose levels during mid-season were much higher, but since this result differs strongly from the glucose and fructose patterns and from the results in Lusa (Figure 5a-b), we should be careful in considering this observation.

Starting ripeness levels behaved similar for both Elsanta and Lusa, with the more ripe strawberries having reduced white shoulders, firmness, width and citric acid content and increased red colour, glucose, fructose, xylose and volatile production, as well as increased fruit decay prior to storage (Figure 1-5). During storage, red colour intensified in all ripeness levels, although the rate at which this occured decreased with the starting level of ripeness (Figure 3c-d). Calyx decay was the same at all ripeness levels, but increased during storage (Figure 1e-f).

Appearance and off-odour values depended on multiple factors which in turn depended on characteristics related to both unripe (levels of whiteness) and overripe strawberries (fruit decay). While appearance scoring was important from a consumer perspective, these scores fluctuated during the storage study due to the different characteristics and as a result became difficult to model. Better would have been to separate these scores into their respective parameters, e.g. white shoulder % and fruit decay, and model these separately. Assessing the predictability of the physiological parameters using volatiles and NIR during this experiment did not yield any useful results, except that some of the physiological parameters showed strong relations between themselves. For example, firmness, colour and fruit decay seemed to correlate very strongly (Figure 6 and 7). Furthermore, averages of firmness and fruit decay from similar strawberry samples that were stored for 10 days grouped relatively close to their day 1 values, suggesting a correlation, which in turn would imply a linear relation.

Correlation firmness and fruit decay
Based on the cross-correlations between firmness and fruit decay (Figure 6 and 7), we decided to visualize the influence of the seasonal and ripeness levels per strawberry batch during storage (Figure 8). For Elsanta, both early- and mid-season strawberry batches showed strong relations between firmness and fruit decay, with unripe, ripe and overripe strawberries having progressively lower firmness and higher fruit decay scores (Figure 8a). The relation between early- and mid-season batches differed only slightly, with the average firmness of mid-season strawberries being approximately 20 g mm^{-1} lower. While not shown in the figure, the four points within each season and ripeness level followed a consecutive order, from day 1 to 10, with strawberries from day 1 being more firm and showing less decay. For Lusa, very similar relations as those in Elsanta were found between ripeness levels within the seasons (Figure 8b). However, early- and mid-season strawberries showed nearly the same pattern between firmness and decay, while late-season strawberries were less firm and showed more decay compared to those in earlier seasons. Overall, these data showed that the average fruit decay score correlates strongly with the firmness levels and follows very similar patterns within each season.

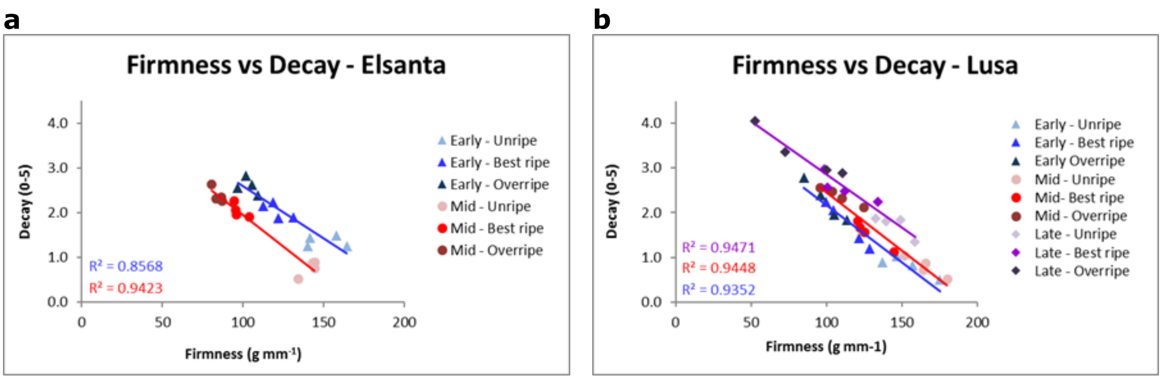

Figure 8: Correlations between average firmness and average decay values at different ripening stages in different seasons during storage. Strawberry varieties used were Elsanta (a) and Lusa (b). Strawberries were harvested early (blue triangles), halfway (mid; red circles) and late (purple diamonds) in their respective seasons. Ripening levels were unripe (light), best ripe (bright) and overripe (dark). The 4 points within a season/ripening cluster represent the mean values of days 1, 4, 7 or 8 and 10, respectively.

The strong linear relation between firmness and decay, which lined up strongly between ripeness levels and storage times, suggests a certain level of predictability. For example, the data suggest that firmness levels on day 1 can predict the firmness and fruit decay after 10 days of storage. However, since the data shown in these figures were averages of 5 punnets per data point, we have to be careful in extrapolating these results to punnets and individual strawberries.

Additionally, since firmness and decay are measureable on the skin surface, it should be possible to detect them using VIS-NIR spectroscopy. During this experiment, VIS-NIR spectroscopy had been performed as well, but was modelled to predict too many physiological parameters simultaneously. In order to verify these findings, a repeat experiment was set up including VIS-NIR measurements in a more targeted approach to predict strawberry shelf life as judged from their firmness, colour and fruit decay (Chapter 4).

3 Monitoring 2016 and 2017

In order to assess the variation during the production process of strawberries and how this variation correlates to variation in shelf life, a large scale monitoring experiment was set up within the participating companies. Variables within this experiment are strawberry variety (Elsanta, Murano, Scarlett and Lusa), growth systems (soil, substrate, greenhouse, tunnel, open air, table top, rain cover and combinations thereof), growers, locations and production moments. While most quality assessment was done by the involved companies, a number of batches were transported to WFBR for additional quality assessment and to determine shelf life. Quality at WFBR was assessed by volatiles, visual quality (appearance, odour, calyx and decay) and colour. The resulting dataset was used to assess links between a wide number of chain aspects and the actual product quality.

Following the results from both the season and ripening experiment and the monitoring in 2016, we decided to continue the monitoring phase, but to exchange the volatile analysis. Instead we included NIR and firmness analysis to further assess the relations between firmness, decay and NIR in commercially sourced strawberries. The experimental setup for the sampling during the monitoring phase was similar to that in 2016. Firmness and NIR were performed as described earlier (Chapter 1). The resulting dataset was used to assess links between a wide number of chain aspects and the actual product quality.

The results from the two years of monitoring can be read in the report "Monitoring strawberry quality" (Pereira da Silva, 2019).

4 Use of VIS-NIR to assess and predict firmness and decay

4.1 Introduction

During the experiment on assessing the influence of season and ripening stage of strawberries (cvs. Elsanta and Lusa) during storage, a correlation was found between strawberry decay and firmness (chapter 2.3). Furthermore, while NIR results were not shown to predict firmness, they were theorized to do so. During the monitoring project phase in 2017, additional NIR, firmness and fruit decay data were collected, which supported the earlier findings. In this phase, 4 different cultivars had been used; Elsanta, Lusa, Scarlet and Murano, which had been grown under various growth systems, growers, locations and production moments. The different cultivars showed varying tightness in correlation between firmness and VIS-NIR and it was concluded that part of the VIS-spectrum played an important role in the correlation with firmness. These results could imply that the relations between tissue softening and colouration differs between the 4 cultivars. However, due to the large variations in growers, locations, production moments and sample sizes, it was difficult to assess the exact cause of the difference in cultivar performance.

In this chapter, we aimed to confirm our earlier results on the correlation between firmness and fruit decay and to assess whether VIS-NIR could indicate and predict firmness and fruit decay. Furthermore, we hypothesized that colour development and tissue softening were not synchronized in the same manner in different cultivars. To test this hypothesis, we measured 3 cultivars (Elsanta, Lusa and Zara) at 2 different production moments (Prod. 1 and Prod. 2) from the same growers and production fields. Strawberries were assessed for visual quality, NIR, colour and firmness and stored for 1, 3 and 6 days at 8 °C and 90% RH. Multivariate analysis on these results was used to assess the relations between VIS-NIR, firmness, colour and fruit decay.

4.2 Material and methods

Plant material

Strawberries (Fragaria × ananassa) of the varieties Lusa and Zara were obtained from Driscoll's of Europe (Prinsenbeek, the Netherlands) and of the variety Elsanta from Bakker Barendrecht (Ridderkerk, the Netherlands). The strawberries had been grown under uniform growth conditions for either Lusa and Zara or Elsanta during the late spring of 2018 in the Netherlands. Strawberries of each cultivar were harvested at two different production moments (Table 1). Ripeness levels were determined by eye based on colour during harvest. Harvested strawberries were placed directly into punnets that were subsequently placed in boxes. Directly after harvest, the boxes were transported to Wageningen Food & Biobased Research (Wageningen, the Netherlands), where the punnets were randomized per ripeness level, labelled and stored at 4 °C and 80% RH. On days 1, 5 and 9 after harvest and labelling, respective samples were placed at 20 °C and 80% RH for 6 hours prior to the measurements.

Table 1: Harvest dates of the different production moments of strawberry cultivars, Elsanta, Lusa and Murano.

	Prod. 1 (yyyy-mm-dd)	Prod. 2 (yyyy-mm-dd)
Elsanta	2018-05-16	2018-06-06
Lusa	2018-05-16	2018-05-30
Zara	2018-05-30	2018-06-06

Weight & Visual quality
Strawberry punnet weight was recorded using a MS6002TS balance (Mettler-Toledo GmbH, Giessen, Germany). Visual quality was scored by assessing the calyx quality and skin decay for each individual strawberry in a punnet. Calyx quality was assessed by scoring each strawberry in a punnet individually on a 4 point scale where 0 represents fresh green; 1, yellow and/or one leaf brown; 2, multiple (up to 50%) leaves brown and 3 > 50% of the leaves brown. Decay of the outer skin was assessed on all strawberries in a punnet by scoring each individual strawberry on a 6 point scale; 0, no bruises; 1, single dry bruise; 2, single wet bruise or multiple dry bruises; 3, multiple wet bruises; 4, minor mould or rot; 5, > 50% mould or rot. Scores of individual strawberries were averaged per punnet.

Colour analysis
Strawberry colour was assessed on 15 strawberries per punnet using image analysis of both sides of the strawberries. Calyxes were removed prior to acquiring the image. Images were acquired using an LED light cabinet (Designed by WFBR and build by IPSS Engineering, Wageningen, the Netherlands) containing a RGB camera (MAKO G-192C POE, Allied Vision, Stadtroda, Germany). The RGB images were calibrated using a 24 patch colour checker card (Color checker classic, X-rite Europe GmbH, Regensdorf, Switzerland). Image analysis was done using multi-threshold colour image segmentation in the HSV colour space (in-house software tool developed at WFBR, Wageningen, the Netherlands) to single out the strawberry background and assess the strawberry Hue colour values of the red, white and green regions of the strawberry skin.

VIS-NIR
From the same 15 strawberries per punnet used for colour analysis, VIS-NIR spectra were recorded. To record the spectra a parallel setup was used, consisting of a MSC 521 VIS NIR and a MCS 511 NIR (Zeiss, Oberkochen, Germany) with a KL 1500 electronic light source (Schott AG, Mainz, Germany). The shoulder of individual strawberries were placed against the probe and the spectra acquired. Within this experiment, involving individual Elsanta, Lusa and Zara strawberries, the 2nd derivative was calculated from the VIS-NIR spectra and subjected to a Savitzky-Golay differentiation for smoothing. Correlation to other physiological parameters was done using Partial Least Square Regression (PLSR).

For the combined VIS-NIR dataset, involving Elsanta and Lusa data from 2016-2018, the 2nd derivative was calculated from the VIS-NIR spectra and averaged per punnet. These average spectra were then subjected to a Standard Normal Variate (SNV) transformation to centre and scale their data. Following this, a Savitzky-Golay differentiation was applied for smoothing. Correlation to other physiological parameters was done using Partial Least Square Regression (PLSR).

All transformation and analysis techniques described in this section were performed using Unscrambler X v10.5 (CAMO AS, Trondheim, Norway).

Firmness and width
From the same 15 strawberries used for colour and NIR analysis, firmness and width were measured using a Firm Tech FT7 (UP GmbH, Ibbenbüren, Germany). Strawberries were placed on the turn-table and one by one subjected to a threshold and maximum force of 75 and 250 g, based on which the width and the compression force per depth (g mm^{-1}) were determined.

Data and statistical analyses
Data were processed using Microsoft Excel and statistical analyses were done using Unscrambler X v10.5 (CAMO AS, Trondheim, Norway).

4.3 Results and discussion

Visual quality of the strawberries was scored based on calyx and fruit decay. Production 1 of Lusa strawberries delivered no overripe strawberries until later during the storage period of that production. Therefore these strawberries were not stored more than 1 day.

Calyx decay for Elsanta during the first production period did not differ much between the different ripeness levels, residing between 1.5 and 2.0 (Figure 9a). During the second production period, while unripe Elsanta strawberries had a similar calyx quality, ripe and overripe strawberries showed a calyx decay between 2.0 and 2.3. Lusa strawberries showed a similar calyx decay values during both production periods (Figure 9b). The exception here being unripe strawberries during the second production period, which showed similar calyx decay as the ripe and overripe strawberries during that period. Zara strawberries during the first production period showed an increase in calyx decay during storage from 1.4 on day 1 to 1.8 on day 9 (Figure 9c). During the second production period, Zara strawberries did not show this increasing pattern and instead showed calyx decay values ranging between 1.3 and 1.7.

Fruit decay for Elsanta during production 1 was around 1.0 for unripe, 1.8 for ripe and 2.1 for overripe strawberries and did not change during storage (Figure 9d). During production 2, fruit decay of Elsanta was around 1.4 for unripe strawberries throughout storage, but for ripe strawberries was at 2.0 until after day 6 it increased to 3.2 on day 9. Overripe Elsanta strawberries started at a fruit decay of 1.8 on day 1, which increased to 2.1 on day 6 and thereafter to 2.8 on day 9. Lusa strawberries showed a more gradual increase in fruit decay during storage (Figure 9e). During production 1, fruit decay increased from 1.4 to 1.9, 1.8 to 2.1 and 2.5 to – for unripe, ripe and overripe strawberries, respectively. During production 2, fruit decay increased from 1.1 to 1.2, 1.8 to 2.0 and 2.0 to 3.0 for unripe, ripe and overripe strawberries, respectively. Zara strawberries showed a different pattern, which increased gradually, but strongly during storage (Figure 9f). Here fruit decay during production 1 increased from 1.1 to 2.7, 1.8 to 3.5 and 2.2 to 4.3 for unripe, ripe and overripe strawberries, respectively. During production 2, fruit decay was slightly lower overall ripeness levels and increased from 0.9 to 2.1, 1.3 to 3.0 and 1.9 to 4.1 in unripe, ripe and overripe strawberries, respectively.

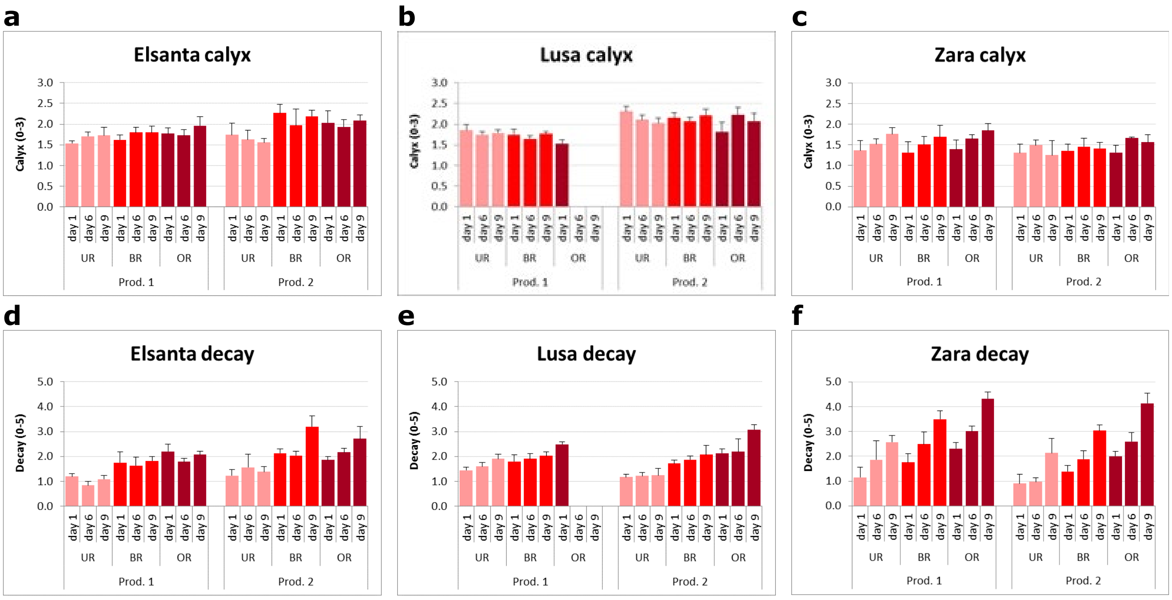

Figure 9: Visual quality of strawberries at different ripening stages in different production periods during storage. Visual quality was assessed for calyx quality (a-c) and decay (d-f). Strawberry varieties used were Elsanta (a and d), Lusa (b and e) and Zara (c and f). Strawberries were harvested at two production periods: 1 and 2. Ripening levels were unripe (light red), best ripe (bright red) and overripe (dark red). Storage behaviour was assessed after 1, 6 and 9 days at 4 °C. Data represent means ± 95% CI.

Colour development

As in chapter 2.3, colour was described as the amount of white shoulders and tips and as the colour of the surface classified as red in degrees Hue, on a circular scale from 0-360 degrees. The observed colours during this experiment ranged from bright reds (5 °Hue, here plotted as 365 °Hue) to more purple reds (352 °Hue) in unripe and overripe strawberries, respectively.

Regarding the white shoulders, the first production period of Elsanta showed less than 5% fruit surface in unripe strawberries on day 1, compared to 3 and 2% in ripe and overripe fruits, respectively (Figure 10a). After 9 days of storage, the amount of white surface was below 1% in all ripeness levels. The second production showed a different picture, in which unripe Elsanta strawberries showed 15% white surface on day 1, which was 5% after 9 days of storage. Ripe and overripe strawberries showed 4 and 3% white surface on day 1, which was 2 and 1% on day 9. Lusa strawberries, during the first production, showed 5, 2 and 0% white on day 1 for unripe, ripe and overripe strawberries, respectively (Figure 10b). After 9 days of storage, these values were 0.5 and 0%. During the second production, unripe strawberries on day 1 showed 12% white fruit surface, compared to 2% for ripe and overripe strawberries. Already after 6 days, the average Lusa white fruit surfaces were around 3% for unripe, 1% for ripe and 0.5% for overripe strawberries and these levels were also observed after 9 days of storage. Zara showed more white shoulders, during production 1 starting at 20% white fruit surface in unripe strawberries and ending at 10% after 9 days of storage (Figure 10c). Ripe and overripe strawberries showed 4 and 2% white surface on day 1, respectively, about one percent lower on day 6 and 4 and 5% for ripe and overripe strawberries on day 9. Production 2 was very similar to production 1.

Red colour development during storage in Elsanta strawberries from production 1 changed from 362 to 360 in unripe, 355 to 354 in ripe and 353 to 352 °Hue in overripe strawberries from day 1 to day 9 (Figure 10d). Production 2 was very similar, except for unripe strawberries that changed from 364 to 358 °Hue during the storage period. Lusa strawberries changed most of their colour between days 1 and 6 and not significantly afterwards (Figure 10e). For production 1, the red colour shifted from 363 to 358, 358 to 355 and 352 to – °Hue for unripe, ripe and overripe strawberries between days 1 and 6 of storage. As before, overripe strawberries were not present for Lusa during production 1. During production 2, between days 1 and 6 of storage, the red colour shifted from 366 to 362, 357 to 357 and 352 to 351 for unripe, ripe and overripe strawberries, respectively. After 9 days of storage, the colour showed slightly higher values for all ripeness levels. Zara strawberries showed a similar pattern, the red colour shifting from 365 to 363 in unripe, 358 to 358 in ripe and 354 to 353 in overripe strawberries, between days 1 and 6 of storage (Figure 10f). Also here, the values on day 9 were slightly higher. Production 2 showed very similar values as production 1.

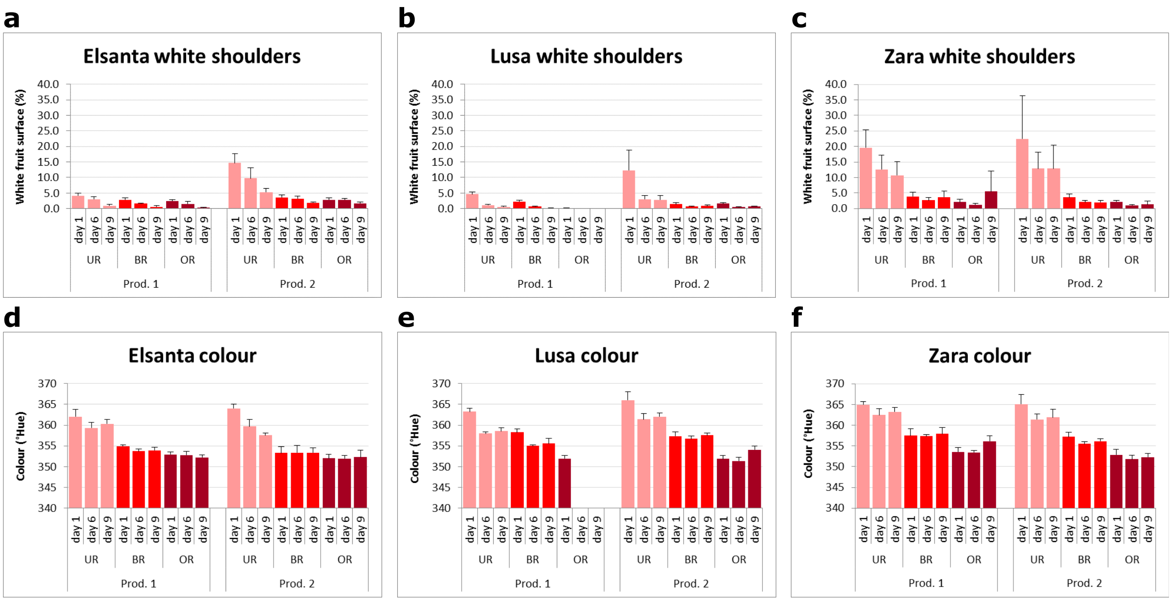

Figure 10: Colour analysis of strawberries at different ripening stages in different production periods during storage. Colour analysis was assessed by red surface fraction (a-c) and colour analysis (d-f). Strawberry varieties used were Elsanta (a and d), Lusa (b and e) and Zara (c and f). Strawberries were harvested at two production periods: 1 and 2. Ripening levels were unripe (light red), best ripe (bright red) and overripe (dark red). Storage behaviour was assessed after 1, 6 and 9 days at 4 °C. Data represent means ± 95% CI.

Firmness in unripe Elsanta strawberries from production 1 showed little change, from 190 g mm^{-1} on day 1 to 185 g mm^{-1} on day 9, while the firmness of both ripe and overripe strawberries changed from 175 to 145 g mm^{-1} during storage (Figure 11a). During production 2, strawberries changed between days 1 and 9 from 200 to 180 for unripe, 140 to 120 for ripe and 120 to 120 g mm^{-1} for overripe strawberries, respectively. For Lusa, firmness of unripe strawberries changed from 250 to 210 g mm^{-1} between days 1 and 9 of storage, compared to 210 to 170 for ripe strawberries, while overripe strawberries showed a firmness of 150 g mm^{-1} after 1 day of storage (Figure 11b). During production 2, Lusa firmness changed from 260 to 200, 205 to 155 and 180 to 130 g mm^{-1} in unripe, ripe and overripe strawberries, respectively. For Zara, in production 1, firmness changed from 310 to 235, 260 to 170 and 255 to 135 g mm^{-1} for unripe, ripe and overripe strawberries, respectively (Figure 11c). In production 2, these values were 310 to 245, 250 to 195, 235 to 150 g mm^{-1} for unripe, ripe and overripe strawberries, respectively.

Widths of Elsanta strawberries increased with increased ripeness levels, from ~27 mm in unripe to 30 mm in overripe strawberries (Figure 11d). Production 1 had slightly wider strawberries, about 1 mm than production 2. Lusa strawberry widths during production 1 were ~31 mm for unripe and ~33 mm for ripe and overripe strawberries (Figure 11e). During production 2, widths were ~27, 30 and 32 mm for unripe, ripe and overripe strawberries, respectively. Zara strawberry widths during production 1 were similar for all ripeness levels, at ~35mm. During production 2, these values were ~34, 37 and 38 mm for unripe, ripe and overripe strawberries, respectively. However, as can be observed from the error bars, Zara strawberries showed a large variation in width; some punnets contained less than 10 large strawberries and others more than 25 smaller ones.

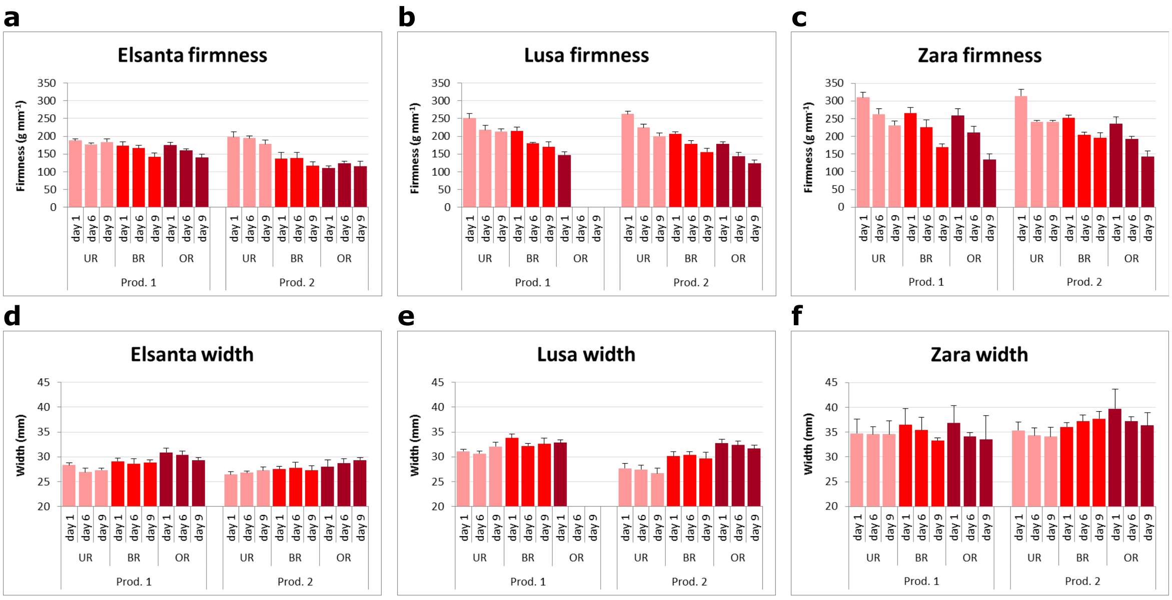

Figure 11: Firmness and width of strawberries at different ripening stages in different production periods during storage. Strawberry varieties used were Elsanta (a and d), Lusa (b and e) and Zara (c and f). Strawberries were harvested at two production periods: 1 and 2. Ripening levels were unripe (light red), best ripe (bright red) and overripe (dark red). Storage behaviour was assessed after 1, 6 and 9 days at 4 °C. Data represent means ± 95% CI.

Elsanta strawberries started relatively soft and lost relatively little firmness during storage compared to Lusa and Zara. Colour of ripe Elsanta strawberries was relatively close to that of the overripe strawberries. Furthermore, ripe Elsanta strawberries from production 2 showed a relatively high fruit decay and a relatively low firmness. Zara strawberries showed more white shoulders and were more firm compared to Lusa and Elsanta (Figures 10a-c and 11a-c). Interestingly, compared to Elsanta and Lusa, Zara showed a much more rapid fruit decay during storage (Figure 9d-f).

Aside from these specific observations, there was a striking mirroring in pattern between fruit decay and firmness, as had been suggested from chapter 2.3.

To assess the plausible relationship between firmness and fruit decay, as well as its linearity and predictability, we plotted the respective values of individual punnets and colour-coded the points based on production and ripeness. The results show that the relationships are not as strong as observed in chapter 2.3. However, since the results from chapter 2.3 were based on averages of 5 punnets per day and the correlations in the current experiment are based on individual punnets, we would not expect similarly high values. Elsanta strawberries show a negative relation between firmness and fruit decay, which could be construed linear (r2 = 0.44; Figure 12a). However, punnets belonging to ripe and overripe strawberries from production 1 and to ripe strawberries from production

2 showed a relatively high fruit decay, as was also observed from Figure 9a. Lusa strawberries showed a relatively linear relationship (r2 = 0.60; Figure 12b), whereas Zara strawberries showed an even stronger relationship (r2 = 0.67; Figure 12c). Looking beyond the overall linearity and focussing more on the individually coloured groups, it can be observed that for Elsanta and Lusa many of the groups show small clouds with a similar direction, but which differs from the overall trend line. These small clouds represent individual punnets from the three storage days and the direction of these clouds represents the relationship between firmness and fruit decay during storage. In other words, the model between firmness and fruit decay during storage could be made stronger by incorporating a factor that relates to the starting points of the individual clouds. However, since we had only a limited dataset, this was incorporated here.

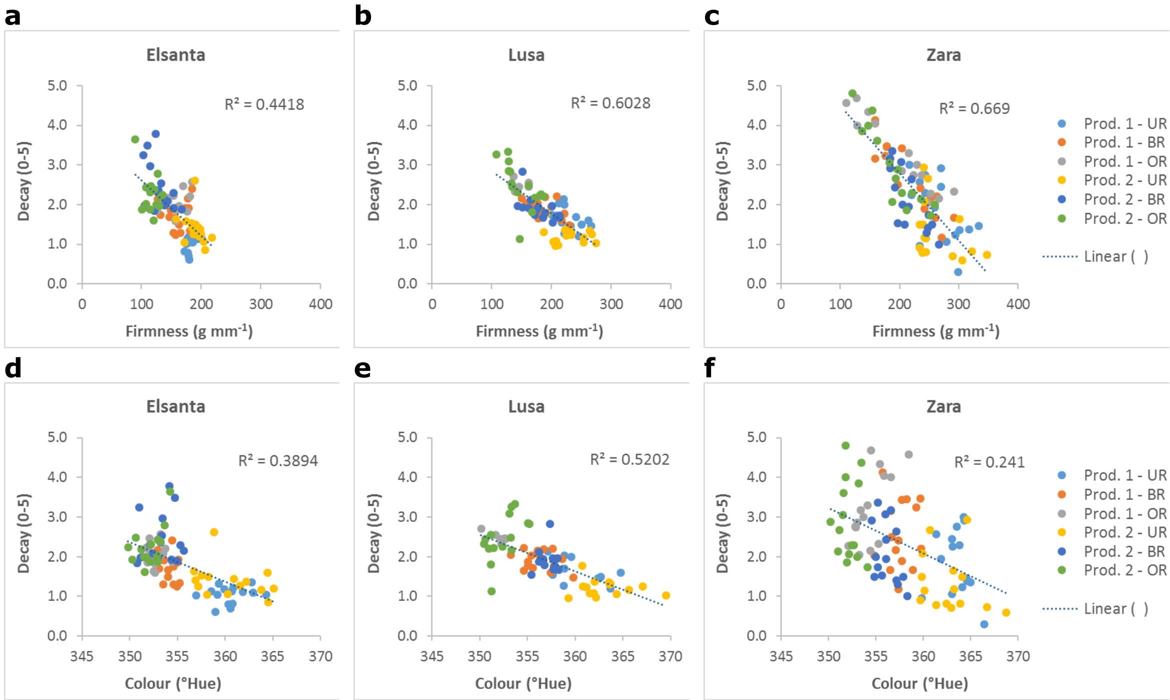

Figure 12: Relations between decay, firmness and colour of strawberries at different ripening stages in different production periods during storage. Shown are correlations between firmness and decay (a-c) and between colour and decay (d-f). Strawberry varieties used were Elsanta (a and d), Lusa (b and e) and Zara (c and f). Strawberries were harvested at two production periods: 1 and 2. Ripening levels were unripe (UR), best ripe (BR) and overripe (OR). Storage behaviour was assessed after 1, 6 and 9 days at 4 °C and grouped per ripeness level and production period. Data points represent means per punnet and the trend-line and R^2 were assessed over all data points.

Instead, we focussed on assessing whether strawberry firmness could be predicted using VIS-NIR. To this end, we processed the NIR data per strawberry and ran a PLS on all the measurement points to calibrate VIS-NIR models to predict the firmness per strawberry variety. For Elsanta, the calculated root mean square error of the calibrated model (RMSEC) was 39.97 g mm^{-1} (Figure 13a), which means that a VIS-NIR measurement can predict the firmness of an Elsanta strawberry with an error of about 40 g mm^{-1}. For Lusa, the RMSEC was similar as that of Elsanta, at 38.47 g mm^{-1}. For Zara, the RMSEC was 47.72 g mm^{-1}, which was likely due to the larger variety in strawberry size. Overall, these values were not as high, but could be increased by averaging the data per punnet prior to analysis (data not shown). However, within this analysis, the number of samples to also validate the model would be insufficient.

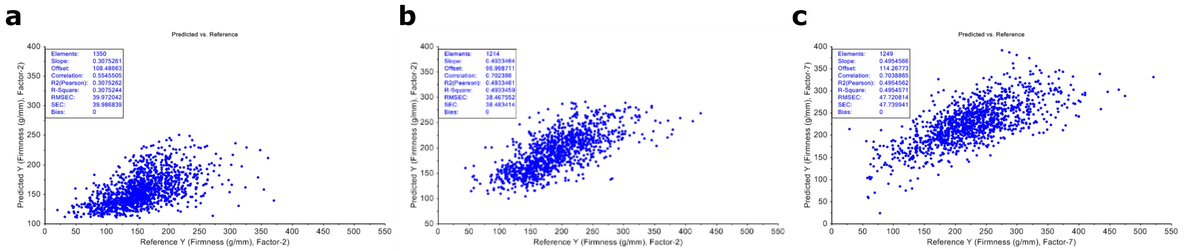

Figure 13: Calibrated NIR prediction models based on firmness. Strawberry varieties used were Elsanta (a), Lusa (b) and Zara (c). The number of elements used for the calculations were 1350, 1214 and 1249 for Elsanta, Lusa and Zara, respectively. Calibrated root mean square errors (RMSEC) for the 3 varieties were 39.97 g mm^{-1} for Elsanta, 38.47 g mm^{-1} for Lusa and 47.72 g mm^{-1} for Zara.

In order to generate and validate a model that could predict firmness values based on NIR spectra, we combined all the project datasets on strawberries from 2016-2018 that included both NIR and firmness measurements. Since the strawberry varieties Elsanta and Lusa had been used throughout the project, these were the most abundant and, therefore, used. The NIR and firmness data were averaged per punnet and a PLS was done in which a random 70% of the sample data was used to calibrate and the remaining 30% to independently validate NIR-firmness models for the two varieties.

For Elsanta, data from 125 punnets were used to calibrate the model and the remaining 54 punnets to validate it (Figure 14a). The predicted root mean square error (RMSEP) of the model was 16.02 g mm^{-1}. For Lusa, data from 235 and 100 punnets were used to calibrate and validate the model, respectively, resulting in a RMSEP of 18.64 g mm^{-1}. These RMSE values were quite a bit lower compared to those calculated based on only the 2018 data. Since the inclusion of different seasons generally causes more variation and with that a reduction in the robustness of the generated models and an increased RMSE, the largest contributor to this decrease was likely due to averaging the data per punnet. This is not unlikely due to the variation between harvested strawberries and the fact that strawberry quality is strongly influenced by the quality of the other strawberries within the same punnet.

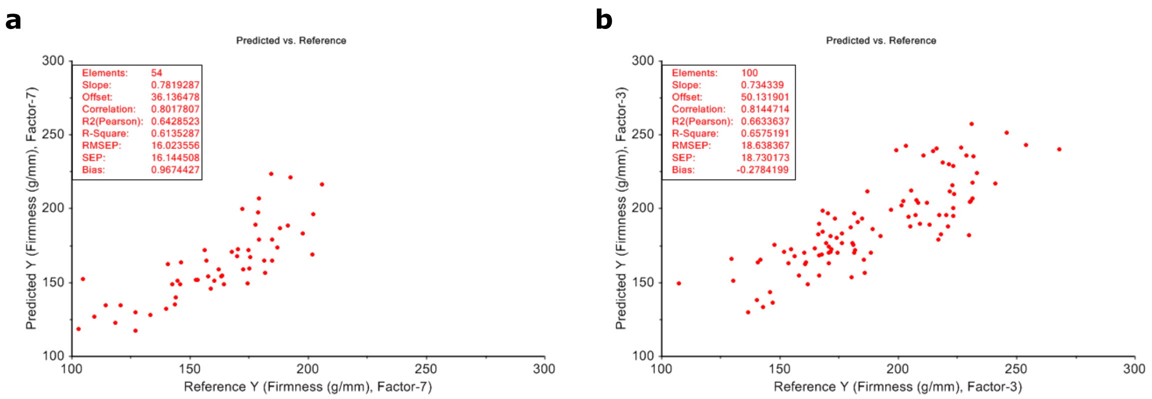

Figure 14: Independent Validation NIR prediction models based on firmness data from 2016-2018. Strawberry varieties used were Elsanta (a) and Lusa (b). All data were averaged from 15 strawberries per punnet, if available. Total numbers of punnets were 179 for Elsanta and 335 for Lusa. Predicted root mean square errors (RMSEP) for the 2 varieties are 16.02 g mm^{-1} for Elsanta and 18.64 g mm^{-1} for Lusa.

Overall the data confirmed the relationship between firmness and decay that was found in chapter 2.3 for both Elsanta and Lusa and also show that this relationship is present in Zara. Interestingly, the relationship between starting firmness, decay and ripening was linear, but differed from the linear progression during storage. Furthermore, colour and fruit decay also showed some correlation for Elsanta and Lusa, though not as strong as between firmness and decay. For Zara, the relationship between colour and decay was very weak, which was largely due to Zara showing a relatively strong decay during storage. Finally, we showed that NIR could be used to assess firmness with a certain margin of error. Whether application of VIS-NIR would be able to predict firmness and fruit decay has not been assessed here. However, these results, in combination with the observed linear relationships between storage time, firmness loss and fruit decay (Figures 8 and 12), VIS-NIR shows promise for predicting firmness and fruit decay. As such, it would certainly be interesting to study these relationships.

5 Conclusions

Within GreenCHAINge Fruit & Vegetables WP3, we first developed scales to visually assess strawberry quality. Besides these subjective visual methods, we also developed methods to objectively assess strawberry colour using our colour cabinet and strawberry firmness using the FirmTech FT7. Furthermore, we assessed the use of volatile and spectral analyses for strawberry quality analysis. These quality scales and objective assessment methods where then applied to assess the effects of season, ripening level and storage time on these quality parameters.

In general:

- Season had some effect on decreasing the strawberry diameter later in the season, but otherwise had little to no effect on strawberry quality.
- Ripeness level had a strong effect on all quality parameters, except for calyx decay.
- Storage of Elsanta and Lusa had strong effects on colour, firmness and fruit decay, but had little effect on diameter and calyx decay.

Interestingly, throughout the project, we have observed strong relations between firmness and fruit decay, the strength of which differed per cultivar; with less firm strawberries showing increased fruit decay. Strawberry volatile analysis using PTR-ToF-MS did not provide satisfactory results to assess strawberry quality.

- However, spectral analysis using VIS-NIR showed that firmness can be determined non-destructively.
- Combined with the relatively linear relations between storage time, firmness loss and fruit decay, VIS-NIR shows promise for predicting firmness and fruit decay.

Practical application for the findings in this report would likely take the shape of an automated system in which all punnets are scanned using VIS-NIR and by a model are predicted for firmness and fruit decay. Besides VIS-NIR, a given percentage of punnets that would also be subjected to in-depth quality control checking firmness and fruit decay, the data of which would then be fed into a 'living' dataset to maintain and update the quality prediction model.

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