

Assessing quality and reducing batch variety in Golden Honeydew Melons

GreenCHAINge Fruit & Vegetables WP2, BO-29.03-001-010

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Contents

Summary

The general objective in GreenCHAINge Fruit & Vegetables Work package 2 (GreenCHAINge) is to obtain high quality and uniform melons and papaya's on the shelf. This report focuses on the melons, being one of the exotic products for the breeding company East West Seed and the wholesaler Frankort & Koning. To obtain high quality and uniform melons on the shelf in supermarkets, it is essential to:

- Store melons at optimal conditions;
- Harvest melons at an optimal maturity stage.

Consumers have high demands, and will only re-purchase melons in case appearance, taste and smell are appreciated. Within GreenCHAINge, we developed scales to visually assess melon quality. For the peel we observed three reoccurring peel issues; brown freckles, brown spots and grey areas, for which we generated visual classifications. Besides these subjective visual methods, we also developed methods to objectively assess melon quality using colour imaging. To assess the smell of melons and quantify the production of volatiles, we stored melons in closed drums and measured the headspace using PTR-ToF-MS.

Depending on the demand in the market, melons are stored for up till several weeks at the wholesaler before arrival at the supermarket. This storage time is called the buffer period. In this project we investigated the effect of the buffer period and temperature on the quality of melons.

Besides storage conditions, also the moment of harvest is thought to affect quality. However, not only the moment of harvest, but also the flowering moment varies. Therefore we investigated the effect of maturity (period from flowering until harvest) on quality of melons after 2-3 weeks of transport oversees and subsequently after a buffer- and shelf life period.

The aim of this study is to measure the parameters that contribute to the quality of the melon variety Golden Honeydew. These quality parameters are used to investigate the effect of storage (buffer) conditions and/or maturity on melon quality. We conclude that melon quality decreases during a buffer period. However, when storing at lower temperatures like 4°C, colour increase and volatile production seem just delayed: As soon as conditions become favourable to induce ripening (20-22°C), colour and volatile production increase. However, peel disorders like brown freckles and brown spots increase after a buffer period at 4°C. Therefore, buffering at 7°C might still allow induction of ripening at 20-22°C, while keeping the peel disorders to an (acceptable?) minimum.

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 East West Seed and Frankort & Koning, who partly financed this project.

This report is confidential until October 2019 and intended only for East West Seed, Frankort & Koning and WFBR. From October 2019 onwards the information is "public".

1 Introduction

Melon (*Cucumis melo* L.) is consumed worldwide and appreciated for its unique aroma and taste. The content of vitamin C and other biologically active compounds like β-carotene and phenolic compounds have positive effects on human health (Lester & Hodges, 2008). Main production areas for melons are Central and South America. After harvest, melons are exported all over the world. To obtain optimal quality and appreciation of consumers it is important to harvest melons at the right moment in their development. The melon variety Golden Honeydew, with its smooth yellow/orange peel is popular for its sweet taste. Most melons, are non-climateric fruits and accumulate sucrose while attached to the plant, resulting in a sweet taste. However, Honeydew melons react to ethylene (Passam and Bird, 1978) which could imply that Golden Honeydew melons are climacteric. Also, with increased maturity, the production of volatile compounds increases, which contributes to the aroma for which melons are appreciated. Harvesting melons at fully mature stage is therefore most optimal for sweetness and the desired aroma. However, to increase shelf life, melons are harvested already at a partially mature stage. Increased shelf life is crucial to allow transport for two to three weeks in a reefer container oversea. Upon arrival in the harbour, melons are stored for up to three weeks, either at a wholesale company like Frankort & Koning, or for further transport to retail until consumption. The time period in which melons are stored between the moment of arrival at the harbour, until arrival at the supermarket varies depending on market demands and is called a "buffer period". Melon quality, and in particular the post-harvest quality of new varieties, is met by high demands from the European market, which requires a pristine condition of peel quality and high quality of flavour (Frankort & Koning, personal communication).

Current transport conditions, 7-10°C in a reefer container (Paull, 1990; Suslow et al., 1997) are dated. Nowadays both breeding for novel melon varieties as well as the quality of storage facilities have significantly progressed. Along with these progressions, questions have arisen on whether Golden Honeydew varieties could be stored at lower temperatures to delay ripening and microbial decay, while avoiding chilling injury. Chilling injury typically develops in chilling sensitive fruit upon returning to non-chilling temperatures after having resided at too low temperatures for too long time (Paull, 1990; Snowdon, 1990). Chilling injury symptoms include pitting and browning of the peel, increased surface decay, scald formation, change of pulp texture, pulp water infiltration and inability to ripen (Suslow et al., 1997; Ben-Amor et al., 1999; Sevillano et al., 2009).

The purpose of this research project on melons in GreenCHAINge is to improve the quality and reduce batch variety of Golden Honeydew melons that have been produced in Brazil and transported by ship to the Netherlands. The main research questions to obtain this goal are:

- What are the quality parameters that contribute to Golden Honeydew melon quality?
- How does extended storage (buffer period) at different temperatures affect Golden Honeydew melon quality?
- How can we reduce the large batch variety present in Golden Honeydew melons?

The main hypotheses are that optimised buffer temperature and optimal harvest stage can increase quality and uniformity of melons on the shelf. High quality melons will in turn increase consumer appreciation of the product and thereby increase overall melon consumption.

In this project, we determined which quality traits are important to evaluate transport and storage of Golden Honeydew melons, as well as how to quantify these quality traits using objective measuring techniques, if possible. Using the developed methods and classifications, we have investigated the effects of variation in storage conditions (both buffer period as well as temperature) and maturity on quality and uniformity of different Golden Honeydew melon cultivars.

The results show that the quality of Golden Honeydew melons after extended transport and/or storage largely depends on temperature and melon cultivar. At low temperature, particularly the quality of the peel will decrease, likely due to chilling injury. Furthermore, harvesting melons 1-2 days earlier or later will not affect the variation in quality within a batch. If anything, melons that have been harvested too early may be more susceptible to decreased peel quality.

2 Material and methods

Material and storage

For all chapters, golden honeydew melons (Cucumis melo L., Inodorus Group) were grown at Agrícola Famosa (Brazil), transported by ship to Rotterdam (the Netherlands), by road to Frankort & Koning (Venlo, the Netherlands) and subsequently to Wageningen Food and Biobased research (WFBR, Wageningen, the Netherlands).

- For chapter 3.1: Investigating melon quality in relation to different storage conditions; Golden Honeydew melons (cvs Goldex, Natal and Lual), class 8, were transported by ship for 2 weeks at 10 °C. After arrival at WFBR, the melons were numbered and stored at either 4 or 7°C and 90% RH for up to 21 days. After 2, 3 and 4 weeks of such storage, 5 melons of each cultivar were measured non-destructively and placed at 20°C. After 1.5 days, the melons were measured non-destructively and after 8 days the melons were measured both nondestructively and destructively to assess internal symptoms of cold damage.
- For chapters 3.2. and 3.3: Investigating melon quality in relation to colour and volatiles; Golden Honeydew melons (cvs Goldex and Natal), were grown and transported as above and stored at either 4, 7 or 10°C and 90% RH for three weeks, followed by one week at 22°C and 85% RH to simulate shelf life.
- For chapter 3.4: Investigating the effects of both harvest and flowering time; Flowers from Golden Honeydew melon plants (cv. Royal Ariana) were labelled 26 days after transplant (DAT), one day after spam bond removal. The spam bond is a thin white cloth used to cover and protect the melons after transplant into full soil and reduces the fertilization of the flowers by insects. Flowering started a few days prior to spam bond removal. Only full open flowers were labelled. To avoid border-effects, the labelled flowers were selected from three adjacent rows in the middle of a field. Over 100 flowers were labelled from each of the selected rows. Melons were harvested 60, 62 and 63 DAT, with 62 DAT coinciding with the commercial harvest. After harvest melons were stored at 12°C until transport, two days after the final harvest. Transport occurred at 12°C and 85% RH and after 3.5 weeks arrived at WFBR. Upon arrival at WFBR, the melons were scored for initial peel quality (brown freckles and spots) and stored at 12°C and 85% RH until the start of the analysis series. Exactly 4 weeks after harvest, melons were analysed, stored for 7 days at 12°C and 85% RH to simulate a one week buffer period and analysed once more. Subsequently, the melons were kept at 20°C and 85% RH to simulate ripening 'on the shelf', followed by a final analysis.

Volatile organic compound analysis

Volatile organic compound (VOC) production was determined by placing individual melons in highdensity polyethylene (HDPE) drums (Engels Logistiek B.V., Eindhoven, the Netherlands) with red rubber septa (Suba-Seal, Sigma-Aldrich) mounted in the lids. After four hours of headspace accumulation, headspace was sampled using a PTR-ToF-MS. The PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) had a drift voltage of 1000V at 60°C and 3.8 mbar, resulting in an E/N of 133 Td. Sampling flow rate was 60 mL/min and the mass range was 20–512 m/z. Samples were taken from the drums by direct injection into the PTR-ToF-MS drift tube through a heated (110°C) peek inlet connected to a syringe needle. Sampling was done for 60 seconds total; the first 5-10 seconds consisted of ambient air, followed by 35 seconds of sample headspace and 20 seconds of carbon-filtered air.

PTR-MS-ToF data was analysed using the program PTRwid (Holzinger, 2015), after which the data per sample were averaged over the sampling period (from second 15-35). These data were subsequently normalized against $H_3^{18}O^+$ (mass 21.03), accumulation time and melon weight.

Respiration

Amounts of O_2 consumed and CO_2 produced were measured after headspace accumulation of individual melons in 10 L high-density polyethylene drums (Engels Logistiek B.V., Eindhoven, the Netherlands) containing septa (Suba-Seal, Sigma-Aldrich) in the lids. Headspaces accumulation was done for 4.75 hours and O_2 and CO_2 percentages were measured using a CheckMate 3 CO_2/O_2 headspace gas analyser (Dansensor A/S, Ringsted, Denmark).

Weight

Melons were weighed using the balance MS6002TS, Mettler-Toledo GmbH, Giessen, Germany. connected to a laptop for automated logging of the data.

Visual quality

Visual quality was scored on a five-point scale: 5, undamaged; 4, some dents, speckles, scratches and/or lignification; 3, multiple speckles, some firm brown and/or spots; 2, one or two small soft brown spots; 1, large soft brown spots and/or black spots.

Peel quality

Melon peel quality was determined based on three types of disorders; brown freckles, brown spots and grey areas (including 'water spots'), see figure 1. The severity of brown freckles on the melon peel was assessed using a quality scale ranging from zero till four: 0 (total area of freckles on the melon leaf surface is less than 1 mm²), 1 (small areas of freckles totalling $< 2 \text{ cm}^2$), 2 (total area of freckles is 2 - 20 cm²); score 3 (large areas totalling 20 cm² up till 50% of the melon surface area) and score 4 (total area of freckles covers over 50% of the melon surface). For the disorder called "brown spots" a similar scoring system, this time based on the total area of brown spots on the leaf surface, was used. The disorder called "grey sheen" was assessed by the area of grey per total melon surface in a range from 0 (no grey surface), score 1 (0-12.5% grey surface); score 2 (12.5-25% grey surface), score 3 (25-50%) until score 4 (50-100% grey surface).

Figure 1: From left to right; freckles, brown spots and gray areas on melons.

Colour and lignification

Peel colour was determined by making pictures in a standardized light cabinet and using a colour rendition chart (Colour checker classic, X-rite Europe GmbH, Regensdorf, Switzerland) to analyse average colour values. Pictures were made of both sides of the melon and the average colour value was determined from the corrected images of both sides of the melon. 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 separate melon background and lignification from the background in order to assess their respective Hue colour values and relative surface. Among these colour values, 60° and 0° represent yellow and red, respectively.

Internal colour and hollow heart

From 3 cm thick slices, cut from the equatorial region of the melon, pictures were made in a standardized light cabinet using a colour rendition chart (Colour checker classic, X-rite Europe GmbH, Regensdorf, Switzerland) to standardize colour values. 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 separate fruit flesh, seed list and cavities from background, peel and damaged flesh in order to assess their respective Hue colour values and relative surface (Figure 2a-c). Among these colour values, 60° and 0° represent yellow and red, respectively.

Figure 2: Examples of internal image analysis. Shown are examples of melons that are firm (a), overripe (b) and show hollow heart (c). Colours in figures a and b indicate outer peel (dark yellow), inner peel (light yellow), healthy fruit flesh (orange) and damaged fruit flesh, seed lists and seeds (light brown). Colours in figure c indicate peel (light yellow), inner peel, seed lists and seeds (light brown) and hollow heart (black).

Firmness

- For chapter 3.1: Firmness of cultivars Goldex, Natal and Lual was assessed using an acoustic firmness sensor (Aweta). Melons were placed with the stele upwards on the AWETA pedestal and twisted 120 degrees between each of three measurements. From these three measurements, the median value was used as the firmness value.
- For chapter 3.4: Firmness of cultivar Royal Ariane was assessed using a Fruit Texture Analyzer (FTA, Güss Manufacturing Ltd, Strand, South Africa) using a 1 cm² probe surface, a trigger threshold of 100 g, a measurement speed of 10 mm $s⁻¹$ and a penetration distance of 8.9 mm. Firmness was determined on 3 cm thick slices cut from the equatorial region of the melon, halfway between peel and seed-list (Figure 3). The slices had been cut at the junctions of the seedlists into three parts, each part containing one seedlist, and the firmness value of the melon was calculated as the average of these three parts.

Figure 3: Internal analysis of Golden honeydew melons. Internal analysis consisted of: Cutting of the 3 cm thick slice prior to imaging (a), Imaging for image analysis (not shown), Dividing the slice into the 3 seed list-containing parts (b), Firmness analysis using an FTA (c) and Soluble solids content determination (d).

Soluble solids content

Total Soluble Solids Content (SSC) in °Brix was assessed using a refractometer (HI 96801, Hanna Instruments Inc, Woonsocket, RI, USA). The melon material used to measure the SSC was the same as that used for the firmness analyses. Juice was extracted from the material by lightly squeezing the fruit flesh over the refractometer.

3 Results

Before melons reach the supermarket, they are stored for up to three weeks at the wholesaler. Such storage, also called a "buffer period", is storage in addition to the regular transport chain (in this case from Brazil to The Netherlands). In this project we investigated whether storage of the Golden Honeydew melons cultivars Goldex, Natal and Lual for up to four weeks at reduced temperatures is feasible without quality losses such as those related to chilling injury.

3.1 Melon quality and variation in storage conditions

Weight

Storage of fruits is known to cause decrease in weight (and thereby value) due to water loss. In this experiment, size and weight of melons was measured after 0, 2 and 8 days at 20°C after 2, 3 or 4 weeks buffer time at 4 or 7°C. Melon weight varied between 1500 and 2000 g, irrespective of the buffer time (Figure 4). The weight at day 0 after buffer time varied between the different buffer times and storage temperatures, but this difference can be attributed to natural variation in melon weight. After two and eight days of simulating shelf life (storage at 20°C), melon weight decreased as expected.

Fiaure 4: Weight of melons during shelf life after varying buffer period lengths at different temperatures. Melon cultivars tested were Goldex (a), Natal (b) and Lual (c). Buffer period lengths were 2, 3 or 4 weeks and occurred at temperatures of 4 blue bars) or 7 °C (red bars). Shelf life quality parameters were measured after 0, 2 and 8 days at 20°C. Data represent means \pm 95% CI, n=5.

Firmness

Acoustic firmness of the melons was measured at 0, 2 and 8 days of shelf life. Including the buffer time of 2, 3 or 4 weeks of storage after arrival at WFBR, the measurement days translated to 14, 16 and 22; 21, 23 and 29 and 28, 30 and 36 days of total storage time prior to analysis.

Over 0, 2 and 8 days of shelf life, acoustic firmness for melon cultivar Goldex was similar irrespective of buffer period or duration of storage at either 4 or 7°C (Figure 5). Although increased variation was visible in the firmness of cultivar Natal and Lual, this variation was not significant and most likely caused by differences in peel surface structure. While the peel of Goldex was even and smooth, the peel of Natal an Lual was more uneven/rough. The roughness in the peel may have caused variations in the angle at which the pin of the acoustic firmness instrument hit the melon peel surface and subsequently the way the soundwaves travelled through the melon. Based on these results, we deemed acoustic firmness an unsuitable method to measure the firmness of melons, particularly when the peel is uneven.

Soluble Solids Content

In experiment I, SSC (°Brix) was determined after 8 days of shelf-life at 20°C post either 2, 3 or 4 weeks buffer period at 4 or 7°C. The SSC differed slightly between the different melon cultivars, but did not differ significantly between the buffer periods or buffer temperatures (Figure 6).

Figure 6: Soluble Sugar Contents (SSC in °Brix) of melons after storage and subsequent shelf-life at 20°C. Melon cultivars used were Goldex (a), Natal (b) and Lual (c). Melons were stored during a buffer period of 2, 3 or 4 weeks at either 4 (blue) or 7°C (red), followed by eight days of shelf life at 20 °C. Data were collected after 8 days at 20°C and represent means \pm 95% CI, n=5.

Respiration analysis

Respiration was assessed in melons of cv Goldex after 2 days of shelf life at 20 °C post a buffer period of 2 or 3 weeks at either 4 or 7°C. The O_2 consumption of Goldex melons with a buffer period of 3 weeks seems higher compared to melons with a buffer period of 2 weeks. The temperature of the buffer period did not cause a significant change in O_2 consumption. CO_2 production followed the same pattern as $O₂$ consumption, albeit less strong (Figure 7).

Figure 7: O₂ consumption and CO₂ production rates of melon cv. Goldex at day 2 of shelf life at 20°C after a buffer period of either 2 or 3 weeks at 4 (blue) or 7°C (red). Data represent means \pm 95% CI, $n=5$.

Volatile analysis

Volatile production of individual melons was assessed after a buffer period of 4 weeks at either 4 or 7°C (cold melons) and after 2 days of shelf life at 20°C succeeding the buffer period (warm melons). Volatile measurements were done using PTR-TOF-MS. Cold melons produced a single clear peak over the baseline of 10000 cps h^{-1} with a mass / charge ratio of 33, indicating the production of the volatile methanol (Figure 8a-b, Table 1). While this peak showed large variation between the individual melons, it was clear that the production of methanol was larger in the melons stored at 4°C compared to those stored at 7°C. Warm melons showed more and higher peaks than cold melons (Figure 8c-d). The respective peaks indicated not only methanol, but also included ethanol, acetaldehyde and a number of esters (Figure 8c-d, Table 1). Interestingly, melons that had been stored at 4°C seemed to show a higher production of volatiles than those stored at 7°C.

Figure 8: Volatile production of individual melons (cv. Goldex) either immediately after 4 weeks buffer period at 4°C or 7°C (cold melons) or after 2 days at 20 °C succeeding the 4 weeks buffer period (warm melons). Individual melons were numbered M1 to M5. Different volatiles are indicated by differences in their mass/charge ratio (m/z ratio).

Table 1: Tentative identification of volatiles produced by melon (cv. Goldex) after 4 weeks buffer period and after 2 days at 20°C succeeding the 4 weeks buffer period (warm melons). Abbreviations: n, unknown. (Fallik et al., 2001; Bai et al., 2003; Kourkoutas et al., 2006)

M/z ratio	Mol. formula	Tentative identification	References
33.034	CH4OH+	methanol	Bai et al 2003
43.017	C2H2OH+	acetate fragment	Bai et al 2003
45.033	$C2H4OH+$	acetaldehyde	Bai et al 2003
46.036	13CCH4OH+	acetaldehyde*	Bai et al 2003
47.048	$C2H6OH+$	ethanol	Bai et al 2003, Kourkoutas et al 2006
48.052	13CCH6OH+	ethanol*	Bai et al 2003, Kourkoutas et al 2006
51.044	n	n	Bai et al 2003
61.028	C2H4O2H+	acetic acid	Bai et al 2003
63.043	$C2H6O2H+$	n	Bai et al 2003
65.060	n	n	Bai et al 2003
73.064	$C4H8OH+$	n	Bai et al 2003
75.044	C3H6O2H+	methyl acetate	Bai et al 2003, Kourkoutas et al 2006

Visual quality

Visual quality of the melons was assessed at 0, 2 and 8 days of shelf life. Including the buffer time of 2, 3 or 4 weeks of storage after arrival at WFBR, the measurement days translated to 14, 16 and 22; 21, 23 and 29 and 28, 30 and 36 days of total storage time prior to analysis. Visual quality declined depending on cultivar and additional storage time (Figure 9). The visual quality score (VQS) of melon cultivar Goldex hardly declined during shelf life after 2 weeks of buffer time, while the VQS dropped down to one unit in case of shelf life after 3 or 4 weeks of buffer time. Similar to that of Goldex melons, the VQS of melon cultivar Natal hardly declined during shelf life after 2 weeks of buffer time. However, especially for cultivar Natal, visual quality dropped severely during shelf life after 3 or 4 weeks of buffer time. Lual melons already showed a decline in VQS of 1 unit during shelf life after 2 weeks of buffer time, but the decrease in quality was less than during the shelf life after 3 or 4 weeks of buffer time. No significant differences were observed when comparing the buffer temperatures of 4 and 7°C.

Figure 9: Visual quality score (VQS) of melons during shelf life after varying buffer period lengths at different temperatures. Melon cultivars tested were Goldex (a), Natal (b) and Lual (c). Buffer period lengths were 2 (diamonds), 3 (triangles) or 4 weeks (circles) and occurred at temperatures of 4 (blue) or 7 °C (red). Shelf life quality parameters were measured after 0, 2 and 8 days at 20°C, which translates as days 14, 16 and 22; 21, 23 and 29 and 28, 30 and 36 after arrival at WFBR for 2, 3 and 4 weeks of buffer period, respectively. Data represent means ± 95% CI, $n=5$

Peel quality

Peel quality is an important trait for melons. Melons with defects on the skin, also called peel disorders, are less attractive for purchase. Following the experiment described above, we determined the three main peel disorders; brown freckles, brown spots and grey peel areas; and developed quality scales for these disorders (see Material and Methods). To test these quality scales we set up a follow-up experiment in which we stored melons (cvs Natal and Goldex) for 21 days at either 4, 7 and 10°C, followed by a shelf-life period at 22°C from days 21 to 28.

In Goldex melons, brown freckling (brown spots < 1 mm) was barely present prior to buffering, but increased during the buffer period storage and at an increased rate during the subsequent shelf-life at 22°C (Figure 10a). While the melons stored at 10°C on average showed a minor increase of a few cm² (score 1) during storage and shelf-life, melons stored at lower temperatures showed a stronger increase, covering up to half the melon (score 3) when stored at 4°C. Brown spots, > 1mm and not composed of multiple brown freckles, increased very little and usually consisted of expanding spots that were already present upon measurement at day 0 (Figure 10b). New spots appeared rarely during either storage or shelf-life. Brown spots formation did not differ with regard to buffer storage temperature. Grey area in the peel were the most difficult to score, as they were not clearly visible

under all angles of light. They were not observed initially, but started showing after buffer storage and increased strongly during subsequent shelf life at 22 °C (Figure 10c). Melons stored at higher buffer storage temperatures appeared to show more grey area.

Natal melons exhibited more brown freckles prior to buffering compared to Goldex melons and the freckling increased at comparable rates during buffer storage and slightly lower rates during shelf-life (Figure 10d). Melons stored at 4°C showed the strongest freckle development, but in Natal the melons stored at 10°C exhibited freckle formation to similar extents as those stored at 4°C. Brown spot formation in Natal melons was similar to that in Goldex melons during storage, but increased slightly more rapidly during shelf-life. Brown spot formation was similar in all buffer storage temperatures (Figure 10e). Grey area formation was clearly visible in Natal melons at the end of the buffer storage period and increased to similar levels as in Goldex melons after the shelf-life period (Figure 10f). In Natal, grey area formation did not show a clear difference with respect to buffer storage temperature.

Figure 10: Peel disorders of melons during 3 weeks of storage at different temperatures and subsequent shelf-life at 22 °C. Melon cultivars used were Goldex (a, b and c) and Natal (d, e and f). Peel disorders scored were brown freckles (a and d), brown spots (b and e) and grey areas (c and f). Storage temperatures were 4°C (blue diamonds), 7°C (red squares) and 10°C (green triangles). Data represent means, n=15.

3.2 Melon quality and colour

Melon colour is not only important for consumer appeal, but the intensity of the colour also provides information about the ripening stage. As golden honeydew melons ripen, their peel colour intensifies from a whitish yellow to a deep yellow (cv. Goldex) or even orange (cv. Natal), depending on the cultivar. However, variations in colour of the peel of melons are very subtle and therefore difficult to assess by the human eye. Objective judgement (phenotyping) is feasible using camera's and image analysis. Within the GreenCHAINge project, one of the goals is to develop reliable, objective and high throughput phenotyping methods. Therefore, standardized images of fruits were made in a special developed standardized light cabinet. Subsequently the images were analysed for various colourrelated attributes. This analysis was composed of three different steps: first, an image of the product was generated using the standardized light cabinet. Second, the series of images was used in conjunction with a newly developed software called "Colour Learning" to classify the colours in the images. Third, the colour classification was used in another newly developed software called "Colour analysis" to quantify the size and colour information for each respective class in all the different images. Upon image analysis the colour value was indicated by a so called "Hue" value. The higher the hue value, the lighter the yellow colour (Figure 11). Using these new tools in the follow-up experiment described above, we analysed the peel of melon cultivars Goldex and Natal for changes in colour before and after 3 weeks of buffer period at either 4, 7 or 10°C, and after a subsequent week of storage at 22°C to simulate shelf life.

Figure 11: Hue value (a) and °Hue (b) as standardized values to quantify colour. Melons typically showed Hue values from 40 to 25, which translates into 56.5 to 35.3 °Hue, which represent a colour range from darker yellow to darker orange. In earlier experiments were used Hue values, but we started using °Hue in later experiments to facilitate comparison with existing literature. It is important to be aware that both Hue and °Hue are often used, depending on the targeted research field.

Goldex melons had a golden yellow Hue prior to the buffering period and during storage and shelf-life, the average colour deepened gradually, irrespective of temperature (Figure 12a). During shelf-life, the Hue decreased faster than during storage and did not differ significantly between the different buffer storage temperatures. Next we looked at the rate of colour change in the individual melons during the buffer storage period (Figure 12b). These results showed that the colour changed significantly faster with higher temperatures. Looking at the rate of colour change after storage, during 1 week at 22°C, the rates were much higher than those during storage (Figure 12c). However, they were significantly lower in the melons that had been stored at lower temperatures (Figure 12c).

Natal melons started at an dark yellow Hue prior to buffer storage and showed a similar progression of colour as the Goldex melons (Figure 12d). Also in Natal, the average colour did not differ between the different buffer storage temperatures, likely due to the large variation between the melons. The rate of colour progression during buffer storage of Natal showed was similar to Goldex, but showed more variation between the individual melons (Figure 12e). As a result, the significance in the change is strongly reduced. During shelf-life, Natal melons on average changed colour 40% slower than Goldex melons, but showed a similar trend with higher buffer storage temperatures showing a lower rate of colour change (Figure 12f). Also here, the variation between the Natal melons was markedly larger than that in Goldex melons, which nullified the significance of the observed trend.

A potential source for the increased variation in colour of the Natal melons is enhanced shadowformation by the roughness in its peel surface, which in Goldex is smooth. Narrowing the classes using the colour learning program to exclude some of the shading effects could reduce the variation somewhat, but would also create new problems in distinguishing ripe and shaded peel. Despite the variation, we see a clear trend in both cultivars; the increase in peel colour development is higher at higher storage temperatures and this relation is reversed when the melons are returned to room temperature conditions. *A key thing to realize from this experiment is that the continuous scale of a standardized light cabinet allows the visualisation of even subtle colour differences. The ability to measure these small differences is what has made the assessment of colour change rates possible.*

Figure 12: Colour progression and colour change rates of melons during buffer storage and subsequent shelf-life. Melon cultivars used were Goldex (a-c) and Natal (d-f). Colour progression (Hue) was shown over the complete storage duration (a and d), whereas colour change rates were calculated separately during buffer storage (b and e) and shelf-life (c and f). Melons were stored at 4 (blue), 7 (red) or 10°C (green) for 3 weeks (buffer period) followed by 1 week at 22°C (shelf life). Data represent means +/- 95% CI, n = 15. Statistical notations (ANOVA with Bonferroni post-tests) represent: $p < 0.1$ (+), 0.05 (*) and 0.01 (**), 0.001 (***).

3.3 Volatiles as biomarkers for melon quality

As described previously, the production of certain volatiles, in particular methanol, can differ depending on the storage temperature in melons of cv. Goldex. To investigate whether the same holds true for cv. Natal, we used a similar experimental setup to assess the volatile production during storage at different temperatures. Melons were stored for 3 weeks at either 4, 7 or 10°C and subsequently for one week at 22°C. Volatile measurements were taken prior to storage, on the final day of storage and after two days at 22°C. In this experiment, we focussed on the production of aldehydes, esters, ethanol and methanol. Aldehydes and esters are fruity odours that relate to fruit ripening (Pesis, 2005; White et al., 2016). Acetaldehyde and ethanol have been connected to various postharvest conditions, including ripening, stress and rot (Pesis, 2005). The production of methanol relates to cell wall degradation and fruit softening (Ortiz et al., 2010; White et al., 2016). Acetaldehyde production during storage was reduced at lower storage temperatures, but at shelf-life temperature this production was significantly higher in the melons that had been stored at lower storage temperatures (Figure 13a). Ester production during storage did not differ between storage temperatures, but at shelf-life temperature, ester production was significantly higher in the melons that had been stored at lower storage temperatures (Figure 13b). Ethanol production during storage did not differ between storage temperatures, but at shelf-life temperature, ethanol production was significantly higher in melons stored at 4°C (Figure 13c). Methanol production increased throughout the storage period to levels similar as at shelf-life temperature (Figure 13d). While prior to buffer storage (on day 0) the production of methanol did not differ significantly between the different storage temperatures, after storage and during shelf-life it was significantly higher in melons stored at 4°C.

Figure 13: Production of volatiles in Golden honeydew melon (cv. Natal), as determined by PTR-TOF-MS at different times during buffer storage and shelf-life. Volatiles shown here are acetaldehyde (a), esters (b), ethanol (c) and methanol (d). Buffer storage temperatures were 4 (blue), 7 (red) and 10°C (green) and shelf-life temperature was 22°C. Measurements were taken on days 0 and 21 (melons at storage temperature) and on day 23 (shelf-life temperature). Data represent means +/- 95% CI, n = 15 and statistical notations (ANOVA with Bonferroni post-tests) present significant differences (p < 0.05) between a: 4 and 10°C, b: 7 and 10°C, c: 4 and 7°C.

3.4 Melon quality related to maturity

In chapter 1, we observed that the melons showed large variation in all observed attributes, except weight, on which they had been selected prior to packing. In response to storage, these variations became larger as the storage time extended. In a commercial setting, such increase in variation will reduce the uniformity in ripeness at the retailer and consumer level, which is undesireable. An important part of this project is to reduce this variation.

Since this variation is already detectable shortly after storage and all melons have been subjected to the same processes after harvest, a plausible cause could be searched at the time of harvest. Maturity at harvest has been connected to resistance of fruit to post-harvest stresses and protocols. Harvesting fruit earlier can allow longer storage, although the fruit may ripen less well (Snowdon, 1990). By harvesting fruit later, at or after the climacteric ethylene peak, they may be more resistant to chilling injury (Snowdon, 1990). Some fruit, including muskmelon and papaya, exhibit increased batch uniformity and reduced susceptibility to chilling injury after having been subjected to an ethylene treatment to initiate the climacteric peak in the entire batch prior to cold storage (Snowdon, 1990; Ben - Amor et al., 1999). Besides harvest time, another source for the variation in melon maturity stages could be the differences in flowering and fertilization times.

In this chapter, we have tested how harvest and flowering time can contribute to a reduced batch variety and reduce chilling-related discorders, such as the brown freckles observed in chapter 3.1, in Golden Honeydew melons (cv Royal Ariana). To assess the influence of harvest and flowering times, we collaborated with the farm Agrícola Famosa, who managed to control the timing of harvest and the labelling of flowers during the experiments. In total, three experiments were conducted: First, we studied the influence of harvest. Second, we studied the influence of flowering time. Third, we studied the influence of harvest and flowering time together.

The first experiment showed that harvesting melons 2 days earlier or 2 days later did not reduce the batch variation. No significant differences between the harvest times were observed in either weight, peel quality, colour, firmness and SSC (data not shown). Furthermore, similar to earlier experiments, we observed large variation between the melons for all scored attributes, which suggests that the chosen harvest times did not reduce the batch variation.

The second experiment showed that 53% of the flowers that had been labelled 1 day after spam bond removal (DASBR) developed into harvestable melons, compared to 13 and 4% at 3 and 5 DASBR, respectively. Melons from flowers that had been labelled later showed reduced colour development and seemed to have a reduced weight, peel disorders, colour development and SSC. Rate of colour development and firmness loss did not differ significantly between the labelling times. However, due to a single harvest time, the melons from flowers that had been labelled later effectively had a younger age. Furthermore, the strongly reduced number of melons reaching harvest among the later labelled flowers increased the variation in the measured quality attributes.

The results from the first two experiments suggested that melons harvested at a more mature age were more likely to develop peel disorders. However, since these experiments had either a varied flowering time or fruit set efficiency and also had small variations in storage time between harvest and analysis, hard conclusions could not yet be drawn.

To assess whether melon maturity indeed relates to the observed post-harvest peel disorders and whether controlling the melon maturity reduces variation in quality within a melon batch, a third experiment was done using a slightly different setup (Figure 14). In this setup, flowers were labelled shortly after spam bond removal and melons were harvested at three different time-points. Harvested melons were stored at a single "safe" temperature of 12°C and were analysed at equal time intervals from harvest, i.e. after equal storage durations between harvest and analysis. The harvest times originally had been set at 4, 2 and 0 days prior to the commercial harvest date for optimal comparison with the earlier experiments. However, the commercial harvest date was unexpectedly moved to an earlier date. As such, we decided to use "days after transplant" as a reference unit.

Figure 14: Experimental setup to control melon maturity and harvest time followed by equal post-harvest treatments. In Brazil, melon plantlets were transplanted and grown under a spam bond. When flowering commenced, the spam bond was removed and over 300 flowers were labelled. Fruit set was 36% and after 60, 62 and 63 days after transplant (DAT), melons were harvested and placed at the transport temperature (12 °C) until all melons were harvested and transported to the Netherlands. Once there, starting 27 days after harvest, the melons were analysed over the course of one week of additional storage and one week of ripening at 20 °C, with equal times between harvest and each analysis time-point.

External melon physiology and peel quality

Upon arrival at WFBR, all melons were carefully randomized within each harvest day. Peel disorders, such as brown freckling and brown spots, were already visible at this time for each harvest day. After the randomization, melons were stored at 12°C until 27 days after their respective harvest time, which coincided with "day 1" of the analysis. On their first day of analysis, half of the melons were analysed both non-destructively and destructively. The other half was stored for yet another week at 12°C and one week at 20°C to simulate chain-time and shelf-life, respectively.

Non-destructive analyses were done on days 1, 8 and 15 and consisted of weight, peel quality scoring and imaging for colour analysis. Melon weight was similar for all harvest days and did not significantly change during the experiment (Figure 15a). Peel lignification was approximated using image analysis and suggested a mild difference between the harvest days (Figure 15b). However, since the difference is barely significant and unlikely to be caused during the final days on the field, this result likely is negligible. Peel colour showed a significant progression during the experiment (Figure 15c). Furthermore, the melons harvested at 62 DAT had a slightly lower hue than both 60 and 63 DAT. However, the underlying cause of this reduction is unknown. Peel colour changed at a constant rate in the melons harvested at 60 DAT, both during the additional week of storage at 12°C and during the ripening step at 20°C (Figure 15d). Melons harvested at 62 or 63 DAT showed a low colour change rate during the storage at 12°C, which at 20°C had increased to the level of the 60 DAT melons. However, since there appears to be a strong interaction for these data between harvest and analysis day, these results should be interpreted with extreme caution.

Figure 15: Physiological parameters of Golden Honeydew melons harvested at three different time-points on the various days of analysis after storage. Physiological parameters consisted of weight (a), peel surface lignification (b), peel colour (c) and peel colour change (d). Melons had been harvested 60, 62 and 63 days after transplant (DAT). During analysis melons were kept at 12 °C until day 8, after which they were placed at 20 °C. Data represent means \pm 95% CI, n > 12 with statistical notations (Two-way ANOVA with Tukey post-tests): ***, p < 0.001; **, p < 0.01; *, p < 0.05; ns, not significant.

Peel quality was scored based on the disorders brown freckles, brown spots and grey areas on the peel. As mentioned above, brown freckling was already present when the melons arrived at WFBR. During the experiment, brown freckling did not increase significantly (Figure 16a). However, melons harvested at 60 DAT showed significantly increased freckling compared to those harvested at 62 and 63 DAT. Formation of brown spots did not increase over time, but was reduced in the melons harvested at 63 DAT (Figure 16b). Grey areas on the peel were very hard to discern, particularly on day 1. Overall, grey area formation increased over time and started out to be slightly less abundant in melons harvested at 60 DAT (Figure 16c). However, as the experiment progressed, the three harvest days showed equal amounts of grey area formation.

Figure 16: Peel disorders of Golden Honeydew melons harvested at three different time-points on the various days of analysis after storage. Peel disorders consisted of brown freckles (a), brown spots (b) and grey areas (c). Melons had been harvested 60, 62 and 63 days after transplant (DAT). During analysis melons were kept at 12 °C until day 8, after which they were placed at 20 °C. Data represent means \pm 95% CI, $n > 12$ with statistical notations (Two-way ANOVA with Tukey post-tests): ***, p < 0.001; **, p < 0.01; $*$, $p < 0.05$; ns, not significant.

Internal melon physiology and flesh quality

To determine internal melon physiology and fruit flesh quality, destructive analyses were done on days 1 and 15. These analyses consisted of cutting out the centre slice, imaging for colour analysis, dividing the slice into three equal parts and measuring the firmness and SSC of the individual parts. Flesh colour was determined from the obtained images and while it showed some variation, the present interaction suggests to take caution interpreting the differences (Figure 17a). Flesh ripeness, as interpreted from the surface of flesh colour relative to the overall surface of the slice, showed some significance in variation related to the day of harvest (Figure 17b). However, also here interaction between harvest and analysis day calls for caution in the interpretation. Hollow heart, or 'Overgrowth', was interpreted as the surface of cavity relative to the overall surface of the slice. Hollow heart was barely present in this batch and showed no significant differences (Figure 17c). Flesh firmness on day 1 was 2.1, 1.8 and 1.8 kg cm⁻² for the melons harvested on 60, 62 and 63 DAT, respectively (Figure 17d). On day 15, these firmness values had decreased to 1.3, 0.8 and 0.8 kg cm⁻², respectively. The observed differences were significant both between 60 DAT and the later harvest times and the two measurement days. Soluble solids contents (SSC) were not significantly different between either harvest times or measurement days, averaging around 14.5 °Brix (Figure 4e).

Figure 17: Internal quality of Golden Honeydew melons harvested at three different time-points on the various days of analysis after storage. Internal quality parameters consisted of flesh colour (a), ripe flesh (b), hollow heart occurrence (c), flesh firmness (d) and soluble solids content (SSC) (e) and were measured on days 1 and 15. Melons had been harvested 60, 62 and 63 days after transplant (DAT). During analysis melons were kept at 12°C until day 8, after which they were placed at 20°C. Data represent means \pm 95% CI, $n > 12$ with statistical notations (Two-way ANOVA with Tukey post-tests): ***, p < 0.001; **, p < 0.01; $*$, $p < 0.05$; ns, not significant.

4 Discussion and conclusions

Melons are tropical fruits and only since a few decades available in most North-European supermarkets. Consumers have high demands, and will only re-purchase melons in case the appearance, taste and smell of the purchased product were appreciated. Depending on the demand in the market, melons are stored for up to several weeks at the wholesaler before arrival at the supermarket, even though this storage period (also called buffer time) might affect quality. In GreenCHAINge , we investigated the effects of different buffer times and buffer temperatures on the quality of melons. Beside these storage conditions, also the moment of harvest has been suspected to affect melon quality. We investigated the effects of different harvest moments and different flowering moments on the quality of melons.

4.1 Effects of storage of Yellow Honeydew Melon

During the GreenCHAINge project we observed that both weight and SSC (Brix) did not differ significantly between melons stored at different buffer periods and/or different buffer temperatures. Oxygen production of melons kept for 2 days at shelf life of 20°C was higher in the melons that had been stored for 3, rather than 2, weeks, possibly due to a slight increase in ripening. Regarding buffering at different temperatures, no significant difference in respiration was observed. Measuring firmness using an acoustic firmness sensor, proved not to be a good manner to determine firmness of melons, particularly when the peel is uneven. In later experiments, we developed a standardized method to determine firmness of the fruit flesh (Chapter 3.4).

Ripening is associated with increased colour and volatile production. Melons stored at 4°C show an increase in production of volatiles such as methanol, ethanol, acetaldehyde and various esters, both during storage and afterwards during shelf life at 20°C. Additionally, this increase in volatile production is accompanied by an increased rate of colour development, which during the shelf-life after storage is also higher in the melons stored at 4°C. Furthermore, the melons stored at 4°C experienced increased amounts of brown freckles. The increases in rates of colour development and volatile production suggest a ripening related process to be involved, likely related to ethylene. Processes related to ethylene and tropical fruits are chilling injury and ripening. Chilling injury symptoms include pitting, which is defined as brown sunken spots on the fruit peel surface (Lyons, 1973). Chilling injury sensitivity has been connected to the plant hormone ethylene and inhibition of ethylene production in the fruit has shown to increase tolerance to chilling (Ben - Amor et al., 1999). Ethylene also promotes ripening features such as peel colour development and the production of ester volatiles. As such, the symptoms observed in melons buffered at lower temperatures (4 and 7°C) are likely caused by chilling injury. Therefore, while buffering at reduced temperatures, such as 7°C, might reduce ripening processes in melons, they may also induce chilling injury symptoms, particularly when they warm up after the cold period. Tolerance to chilling injury may differ per cultivar and possibly pre-harvest conditions. We have shown here that chilling injury symptoms increase with lower temperatures, increased storage duration and differ between cultivars. Buffering at moderately cool temperatures is possible, but remains a risk, depending on cultivar, temperature, duration and customer acceptance, as it will most likely result in lower quality fruit.

4.2 Standardized colour measurements

Between the different GreenCHAINge work packages we have developed methods to objectively measure traits related to the quality of fruits and vegetables. Objective measurements allow standardized measurements independent of day, location or individuals. Also, quality measurements that are usually done by quality experts can be standardized using the same objective measurement methods all over the world. Furthermore, data derived from objective methods can detect much smaller differences in quality, allowing near-continuous classification or even continuous scales. Such improved scaling allows not only stronger statistics to detect smaller differences, but also to

investigate correlations to other traits, e.g. correlating the amount of peel defects to volatile production.

In the case of melons, we used image analysis of standardized pictures obtained in a newly developed "colour cabinet" to quantify several traits, such as peel colour, flesh colour, peel lignification, percentage of ripe flesh and hollow heart. While peel disorders would have been very interesting to include, insufficient camera resolution prevented an accurate separation of spots and freckles and were therefore scored visually. The data resulting from the objective image analysis allowed detection of much smaller changes in peel colour (Figure 12a), particular when considering the changes in colour for each individual melon (Figure 12b-c). This provided the insight that the rate of peel colour development depends largely on storage temperature (Figure 12b-c) and may be influenced by harvest date (Figure 15d), but seems independent from maturity (data not shown).

4.3 Volatile biomarkers for melon quality

Volatile analysis using PTR-TOF-MS shows promise in revealing differences in melon metabolism and potentially ripening, both during and after storage. Melons stored at low temperature (4°C) show a reduced volatile release compared to melons stored at 7 or 10°C, which is expected since volatile production is known to decrease upon storage at lower temperatures. However, storage at 4°C showed significantly more release of methanol compared to melons stored at 7 or 10°C. Since methanol is released as part of the pectin degradation pathway (Frenkel et al., 1998), which in turn is part of the cell wall degradation process, it is a candidate biomarker for storage issues related to cold damage, such as pitting, changes in pulp texture and pulp water infiltration. Indeed, at lower storage temperatures we observed more brown freckle formation, which coincided with the higher production of methanol. However, we did not see significant changes in pulp texture or pulp water infiltration. Upon return of melons to temperatures simulating shelf life, melons stored during the buffer period at lower temperatures (4°C) released more aroma volatiles (acetaldehyde, esters and ethanol) than melons stored at higher temperatures (7 and 10°C), coinciding with increased speeds in colour changes. The increased production of aroma volatiles accompanied by an accelerated change in peel colour suggests accelerated ripening in melons stored at 4°C during the buffer period. However, we have to consider that the production of volatiles may originate from the sites of the brown freckles. Besides the freckles producing the methanol as part of their own degradation, they might also act as pores allowing volatiles form inside the melon to disperse into the direct environment. Large variation in the data implies that the differences observed in the data would have to be further investigated. As it stands, methanol is a candidate volatile for the observed issues related to the brown freckles during storage, although further experiments would be required to model this relationship. Since other volatiles would require a warm-up, which is unwanted in a cold-change, they are of lesser interest.

4.4 Effect of melon maturity on quality and uniformity

Regarding batch uniformity, labelling flowers did not seem to affect the uniformity of the melon batches with regard to most measured attributes, with the exception of weight, firmness and brown freckling. In these cases, weight could not be considered as the melons underwent a selection after harvest and firmness and freckling showed a reduced variation with increased ripening and freckle formation. As such, we have to conclude that harvest time did not affect the batch uniformity of Royal Ariana melons.

Regarding melon quality, among the measured quality parameters, only the firmness, peel colour and the brown freckling showed clear differences between the harvest dates. Melons harvested at 60 DAT were more firm, had more freckles and seemed lighter in colour compared to melons that were harvested later. While in previous experiments a clear difference in colour had been visible, this was less obvious in this experiment. What is more, the melons harvested at 62 DAT were darker than both the 60 and 63 DAT melons, with is inconsistent with earlier observations. The higher firmness in the 60 DAT melons, as well as the lighter colour, would suggest that these melons were less ripe. The increased brown freckling on the 60 DAT melons is reminiscent of pitting and as such could be an indicator of cold damage (Lyons, 1973). Less ripe fruits may not have had their ethylene peak and are likely to experience this surge during storage, which makes them more sensitive to cold. Increasing the maturity of the melon prior to cooling could avoid the coincidence of the ethylene surge with the cold storage period and thereby increase tolerance to chilling injury (Suslow et al., 1997). Alternatively, application of 1-MCP could reduce the sensitivity to ethylene and increase tolerance to chilling injury (Pech et al., 2008). However, such implementation and the potential requirement for an ethylene treatment after the cold storage period would still need to be investigated.

4.5 General conclusions

Within GreenCHAINge , we developed scales to visually assess melon quality. Regarding peel quality we observed three recurring peel issues; brown freckles, brown spots and grey areas, for which we generated visual classifications. Besides these subjective visual methods, we also developed methods to objectively assess melon quality using our colour cabinet. To assess and quantify the production of volatiles, we measured the volatile production using PTR-ToF-MS. Using the developed methods and classifications, we have investigated the effects of variation in storage conditions (both buffer period as well as temperature) and maturity on quality and uniformity of different Golden Honeydew melon cultivars.

Buffering melons is possible but will likely result in reduced melon quality, depending on both temperature and melon cultivar. When buffering at lower temperatures (4°C) particularly the quality of the peel will decrease, likely due to chilling injury. Furthermore, depending on the cultivar, the melons may already be ripe when arriving in the Netherlands and further storage may result in overripening, particularly leading to the loss of pulp structure.

Harvesting melons 1-2 days earlier or later will not affect the variation in quality within the batch. However, while earlier harvested melons are more firm and lighter in colour after transport, they seem more susceptible to brown freckling. Flowering time may still influence the batch variety, but since later flowers generally lead to smaller melons, these will be filtered out based on size prior to transport.

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