Determining water loss characteristics in cucumber cultivars

Breeding for post-harvest quality Work Package 1, year 1

Ernst Woltering, Manon Mensink, Mariska Nijenhuis-de Vries, Najim El Harchioui, Esther Hogeveen-van Echtelt

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Institute: Wageningen Food & Biobased Research, Wageningen Plant Research

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1 Introduction

Water loss is a major determinant of postharvest shelf life. Horticultural products contain 80-95% water and a few percent of water loss will have an immediate effect on product quality. It is a major cause of postharvest losses. Some of the current trends for more sustainable fresh chains may have a negative impact on a product's capacity to retain water, or may require an improved control over water loss. Examples of these trends are cultivation techniques using less energy like next generation cultivation, alternative transport methods like transport by sea, and reduction of plastic packaging. Improved cultivar selection is proposed to be key in maintaining quality of peppers and cucumbers in sustainable chains. New methods are needed for high-throughput screening of post-harvest traits related to water loss in these products.

Therefore, a new public-private partnership project was started in 2020 entitled: Breeding for Post-Harvest Quality of Flowers and Vegetables. In this research project a consortium of breeding companies and research institutes Wageningen Food & Biobased Research and Wageningen Plant Research aim to develop knowledge and methods to develop germplasm better suited to the new (future) demands.

The objectives of this project are (i) to investigate the physiological, biochemical and genetic basis of processes involved in resistance to water loss, (ii) to apply this knowledge by developing high throughput and objective screening methods for crops like chrysanthemum, cucumber and pepper and (iii) to validate these methods in company's germplasm.

The project is subsidized by the Foundation TKI Horticulture and Starting Materials, commissioned by the Ministry of Agriculture, Nature and Food Quality. The breeding companies (Figure 1) fund the project with both cash and in-kind contributions. They are divided into 2 groups, based on the interest for either flowers or vegetables. There is also joint communication in the project to learn from each other. Researchers from Wageningen Food and Biobased Research together with Wageningen Plant Research lead the execution of the research in the project.

Company	**Interest**
Ball Helix –	Flowers + Vegetables
Nunhems Netherlands B.V. (BASF)	Vegetables
Dekker Breeding B.V.	Flowers
Deliflor Chrysanten B.V.	Flowers
Dümmen Orange The Netherlands, B.V.	Flowers
Enza Zaden Research & Development B.V.	Vegetables
Van Zanten Research B.V.	Flowers

Figure 1 Breeding partners in the Consortium

This report is written after the first year of research for the vegetable part in the project. It describes the work of Work Package 1, focussed on the question what is determining water loss in **cucumbers**. Why are certain fruits/cultivars losing more or less water? Can we find characteristics/traits involved in water loss.

Two experiments have been performed, one explorative experiment to determine the methodology and one experiment in which 12 cultivars are screened on water loss characteristics and fruit and quality characteristics. In addition to the experiments on cucumber, this work package also includes similar type of experiments on peppers. The latter are described in a separate report entitled "Determining water loss characteristics in pepper cultivars, WUR report 2171" .

The results of Work Package 2, which is focused on genetic diversity for water loss traits in broader populations is also described in a separate report. In this report, first 2 greenhouse experiments with peppers have been performed by researchers from Wageningen Plant Research, in which 2* 100 different cultivars peppers have been screened on post-harvest water loss and other characteristics with the aim to find genotypes with proposed better water loss characteristics. The results of these experiments are described in report: "Pepper diversity trial, WUR report nr 2173".

2 Material and Methods

Materials and methods will be described in the following parts:

- Starting material
- Weight loss and transpiration
- Visual assessment
- Colour & shape analysis for possible correlation with shape and yellowing during shelf life
- Skin prints of the stomata of some cucumbers

2.1 Starting material & conditions

2.1.1 Starting material for explorative experiment to develop methodology weighing cucumbers

On May 11th 2020 cucumbers were kindly provided by BASF:

- Cultivar A regular picked 30 pieces
- Cultivar A younger picked 30 pieces
- Cultivar B 30 pieces
- Cultivar C 6 pieces
- Cultivar D 5 pieces

All cultivars were placed at trays at (18 °C 70% RV).

2.1.2 Starting material for determining weight loss curves

Seeds from twelve cultivars supplied by Enza and BASF have been grown under code (K01-K12) at BASF under similar growing conditions. Cucumbers were harvested early morning, put into boxes and within 2 hours transported to Wageningen and placed in the conditioned shelf life room (18 °C, 70% RV, 24 hr light, ~10 µmol.m-2.s-1 PAR on the level of the shelves, air circulation ~ 0.1 m.s-1 at a constant level). Harvest dates:

- Harvest 1 (H1): 08-06-2020
- Harvest 2 (H2): 15-06-2020
- Harvest 3 (H3): 22-06-2020

Ten cucumbers per cultivar from 2 harvest dates have been used for a shelf life test, determining weight loss and various quality parameters. Five cucumbers per cultivar were placed per tray. All trays were placed randomly in the climate room. The third harvest was used for taking stomatal prints from each cultivar.

The age of every cucumber is indicated by the colour of a little ring around the stem. The colour correlates to the day of fruits set (Figure 2). Fruits from harvest 2 were older than fruit from harvest 1 with on average the same weight. An impression of the different cultivars is given in Figure 3.

Figure 2 Rings used to indicate the age of the cucumbers

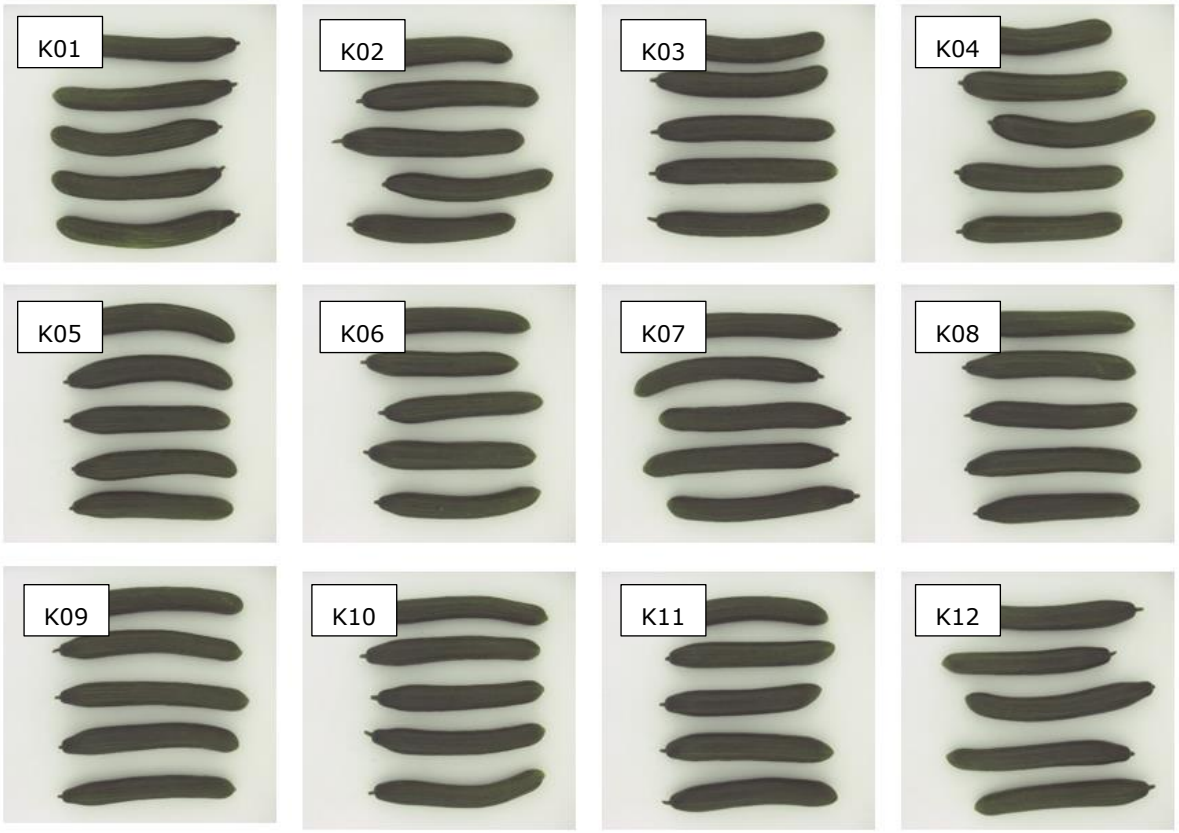


Figure 3 Impression of the long European type (LET) cucumber cultivars used in the experiment

2.2 Weight loss

2.2.1 Weight loss during explorative experiment

On May 12th 2020 the cucumbers were numbered, weighed on a two digit scale, Mettler Toledo (Tiel, NL) type MS6002TSDR (serial nr. B523026995, PHT0029)[1] and placed on trays. From cultivar A, fruits were obtained from a regular harvest and an early harvest (younger fruit); from cultivar B only fruits from a regular harvest were obtained. The stem of half of the cucumbers (= 15 pieces) was greased with vacuum grease. These cucumbers were weighed again. The grease application was done to investigate the amount of water loss via the stem versus the fruit itself. All trays were randomly placed in the climate room.
The first day the measurements were after 1.5 hours, 3.5 hours and 6 hours. The next three days the cucumbers were weighed twice a day. The second week measurements were done once a day (on week days only). The third week measurements were done on Monday and Friday.

2.2.2 Weight loss curves of 12 cultivars

During 14 days shelf life each individual fruit is weighed on the two digit scale, mentioned before. The first day every hour, the following days twice a day during weekdays.
Per time interval the transpiration (gram water loss *hour $^{-1}$ * start weight $^{-1}$) is calculated and plotted in a graph. This resulted in water loss curves. For data analysis, to be able to compare curves and determine coefficients, curve fitting is done in two different ways which will be explained below and will be evaluated on its fit for use in the Results section.

[1] Calibration certificate nl0048-018-061819-ACC-RVA, issued 18th June 2019, guideline EURAMET cg-18 v4.0 (11/2015), method WIKA/02

2.2.3 Curve fitting 1: Genstat model

For data analysis, to be able to compare curves, the linear-by-linear (rectangular hyperbola) model in Genstat (Figure 4 and Annex 2) was used because of its good match with the data points of the weight curves. The formula for this model is:

$$y = \mathrm{A} + \frac{B}{(1 + \mathrm{D} * \mathrm{x})}$$

Where x is time and y = the weight expressed as % of the starting weight (%W).

All the cucumbers follow a similar like water loss curve and show a good fit/observed relationship. The reaction time constant (D) and end value (A) are estimated.

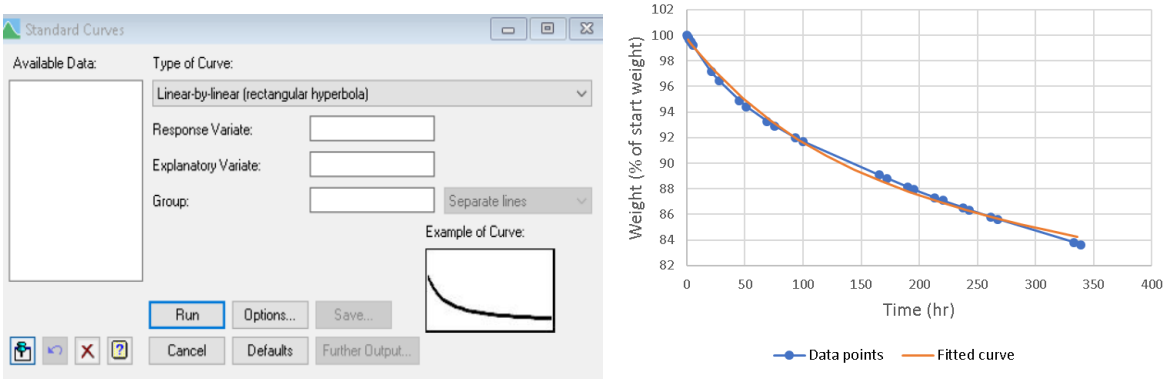


Figure 4 ***Screen shot of Genstat's linear-by-linear (rectangular hyperbola) standard curve function (left). Example of the weight measurement as % of the starting weight and the fitted curve (right). Data shown of fruit no. 1 of cultivar K01 from harvest 1.***

This method provides 3 coefficients per cucumber which describe the curve, A, B and D. Using these 3 parameters, the transpiration curve formula can be derived:

$y = -B * D/(1 + Dx)^2$

Whereby Y = transpiration and x = time.

2.2.4 Curve fitting 2: Kinetic model

The second approach was taken by fitting a first order kinetics model (Pers. comm. Tijskens and Schouten (2021)).

A mathematical model was developed using the complete data set. From this, the best parameters were estimated for each cultivar and harvest. The formula for this model is:

$$W = (W_0 - W_{min}) * e^{-k*time} + W_{min}$$

In which W= water content in %, W_o = 100%, W_{min} = final weight end value (in %), k = reaction (time) constant. An example of the model fitting is shown in Figure 5.

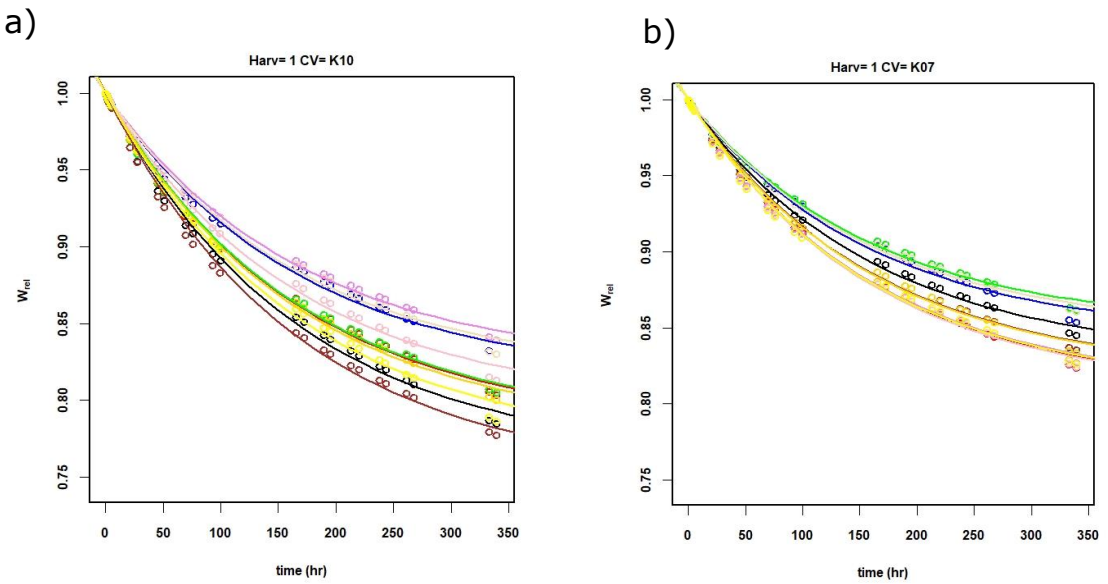


Figure 5 ***Examples of water loss kinetics of individual fruit of two cultivars. a): %W in fruit from K10 harvest 1, b) %W in fruit from cv K7 harvest 1, (provided by Pol Tijskens, 2021, WPR). Measurement data points and best fit of the kinetic model are shown.***

2.3 Visual assessment

2.3.1 Visual assessment during explorative experiment

In this explorative experiment visual assessment was not performed..

2.3.2 Visual assessment of 12 cultivars

Visual assessment was performed to test possible correlation of quality parameters with weight loss. Visual assessment was done by two persons on day 4, 7 and 14 on the following features:

- Overall visual quality: 1-10 with 6 being acceptable.
- Manual firmness: 1-10 with 6 being acceptable.
- Dehydration: yes/no
- Blisters: yes/no
- Yellowing: yes/no

Firmness was also manually determined at the start of the experiment and after 1 day.
In Figure 6 examples of dehydration, blistering and yellowing are shown.

Figure 6 ***From left to right example of cucumbers showing dehydration, blistering and yellowing.***

2.3.3 Colour & shape analysis

The colour and shape were measured using the Smart Colour Inspector, running Colour Cabinet Software (WFBR, Wageningen, V1.6, 23-01-2019) (see Figure 7).

The Smart Colour Inspector is designed by WFBR and built by IPSS Engineering (both Wageningen, The Netherlands). It is mounted with LED arrays (4038 K) on five sides and is equipped with an RGB camera (MAKO G-192C POE, Allied Vision Technologies GmbH, Stadtroda, D) that takes images from above according to standard settings.

For each measurement series, the system is calibrated with a white background (Forex® PVC Plate White 6mm) and a 24-plane color chart (Color checker classic, X-rite Europe GmbH, Regensdorf, S), see Figure 8. Based on this calibration, the RGB images are standardized to official L*a*b* (D50) values of Macbeth ColorChecker[2].

The images are used to determine the shape and the L* value of the L* a* b* colour space of the fruits. The software for these image analysis is developed by the Computer Vision group of WFBR, using National Instruments (NI), and consist of two parts. For teaching segmentation Colour Learning Software is used (WFBR, Wageningen V1.09, 22-01-2019). For image analysis Colour analysis Software is used (WFBR, Wageningen, V3.14, 06-12-2018).

From the analysis software two parameters[3] are derived for the shape:

- Surface area (2D)
- Max feret diameter = the largest diameter of the 2D object in the image; this resembles the length of the cucumber. To determine the length of the cucumber the stem is cut of by a line in the picture (see Figure 9).

For estimating surface and volume of the fruit we assume the fruit is a cylinder:

- Height (h): max feret diameter
- Radius (r): ½*equivalent rectangle short side (derived by Surface area 2D and Max feret diameter)
- Surface area = $2\pi rh+2\pi r2$
- Volume = $\pi r2h$

To reveal the differences between the cultivars on length or width, anovas were performed in Genstat with Tukey as posthoc test (p = 0.05). As variable length or width was taken, as factors cultivar, serie and cultivar*serie.
Since the cucumbers apeared to shrink the amount of shrinkage is calculated.

[2] Pascale D (2006) RGB coordinates of the Macbeth color checker, BabelColor Company, Montreal, Quebec, Canada.(2006, Jun.)

[3] https://zone.ni.com/reference/en-XX/help/372916T-01/nivisionconcepts/particle_measurements/.

Figure 7 ***Smart Colour Inspector to make (color calibrated) pictures of the cucumbers for determining shape and colour of the cucumbers***

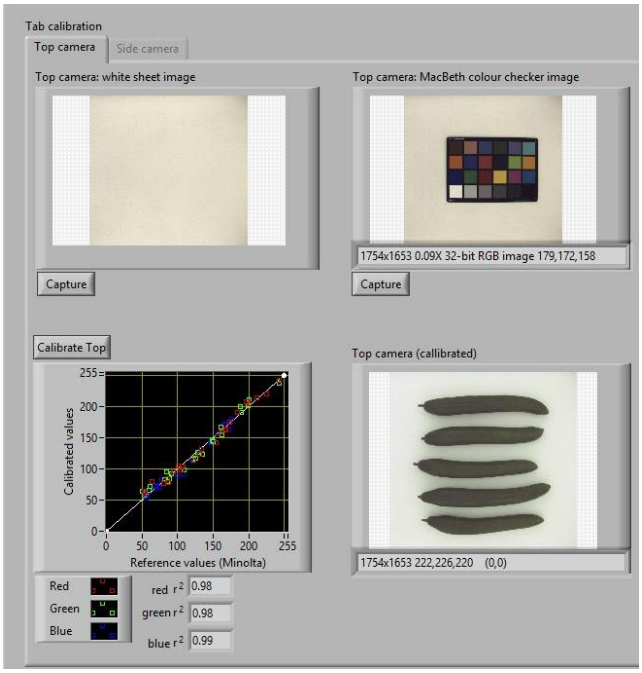


Figure 8 ***Smart colour Inspector: top: calibration with white background and colour card; bottom: calibration line based on colour card and example of picture of cucumber***

Figure 9 ***Analysis of 5 individual cucumbers with Smart Colour Inspector. Stems are cut off with a line in order to exclude it from the length of the cucumber.***

2.3.4 Skin prints of the stomata of some cucumbers

To investigate the amount, form and shape of the stomata and the cuticula skin imprints. This was done with cucumbers of harvest 3 about one week after harvest. Nail polish was used to make the imprint in the mid-section and at the stem end of 1 fruit per cultivar (see Figure 10). The imprints were studied by microscopy. The stomata density and stage, trichomes and the blisters were studied.

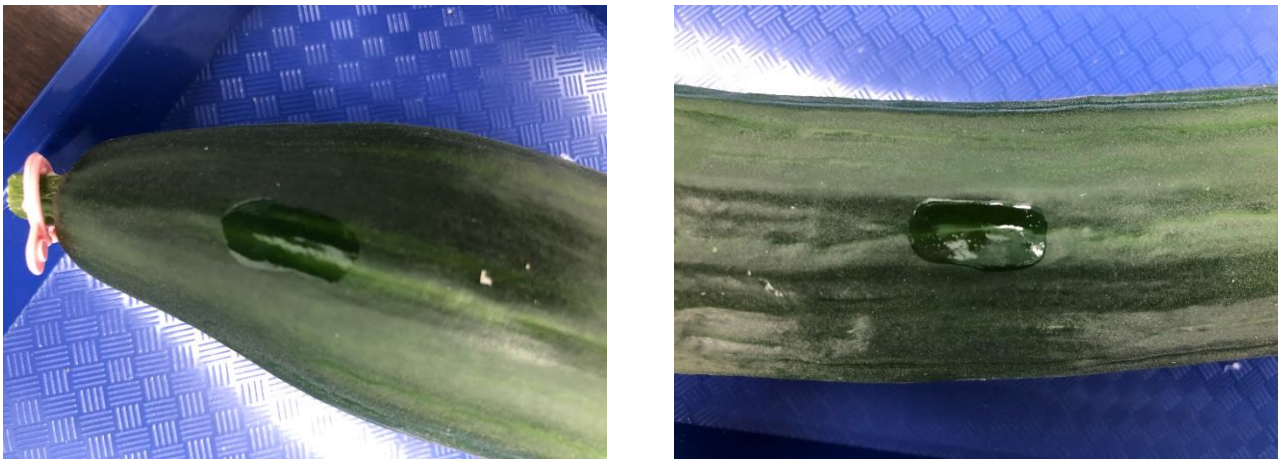


Figure 10 ***Imprint with nail polish of the peel in the stem end (left) and the mid-section (right)***

3 Results

3.1 Explorative experiment to develop methodology weighing cucumbers

A preliminary experiment was done to answer following questions:

- What is the required frequency of water loss measurements?
- What is the required time period to measure water loss?
- What is the contribution of the stem end to the total water loss of the fruit?
- Is the age of the fruit influencing the water loss?

Cucumbers from two cultivars were provided, coded A and B. Fruits from cultivar A were picked both at the regular harvest stage (indicated by R from regular) and at a slightly younger stage which would normally be harvested the next round (indicated by Y from young). The stem end was either left untreated (-), or water loss was blocked by treatment with silicon grease (+). Water loss was determined over time and was expressed in % of start weight (%WL).

%WL and calculated transpiration rate of treated and non-treated fruit is shown in Figure 11, Figure 12 and Figure 13. Contribution of the stem end to the total %WL was minor; no significant differences were observed in %WL between treated and non-treated fruit. Therefore, in further experiments the stem end was left untreated. %WL was slightly higher in younger compared to regular harvested fruit. However, it should be taken into account that surface/volume ratios are different. Smaller fruit have a higher surface/volume ratio and, if all other conditions are similar, are expected to show faster weight loss. However, differences in skin features should be taken into account.

In further experiments, fruit of approximately the same commercial weight (i.e. around 450 g) were used and, although fruit growing time was registered, no corrections for age were applied.

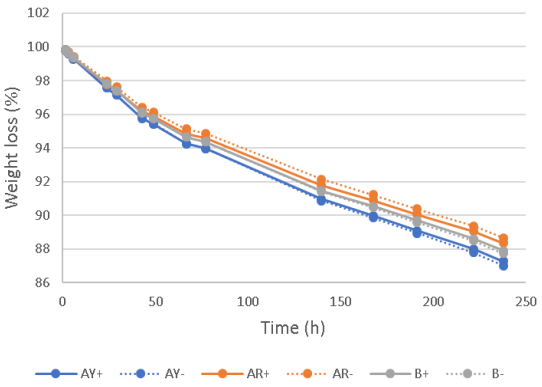


Figure 11 ***Water loss (%) curves of cucumbers at 18°C and 70% RH and continuous light. Data are shown from cultivar A and B, with (+) and without (-) sealing of the stem. Fruits of cultivar A were harvested in a regular stage (R) and young stage (Y).***

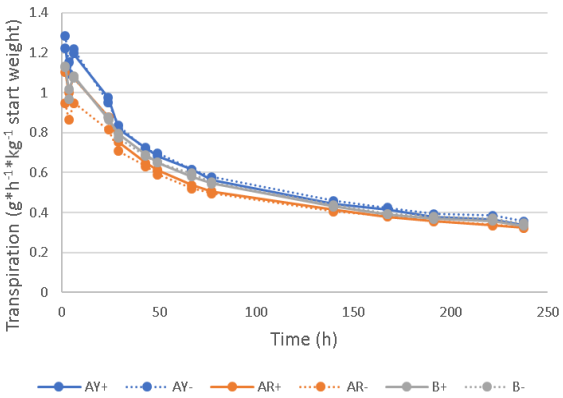


Figure 12 ***Transpiration (g*h^{-1}*kg^{-1} start weight) curves of cucumbers at 18°C and 70% RH and continuous light. Data are shown from cultivar A and B, with (+) and without (-) sealing of the stem. Fruits of cultivar A were harvested in a regular stage (R) and young stage (Y).***

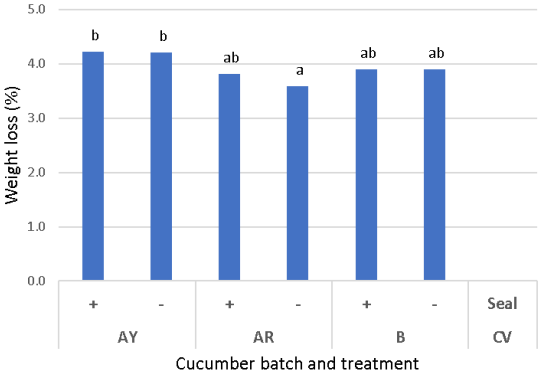


Figure 13 ***Water loss (%) of cucumbers after 48 hr of dehydration at 18°C and 70% RH and continuous light. Data are shown from cultivar A and B, with (+) and without (-) sealing of the stem. Fruits of cultivar A were harvested in a regular stage (R) and young stage (Y). N=10, bars showing a different letter are significant different (Tukey post hoc test, α 0.05).***

3.2 Features starting material

At the start of the experiment, fruit were weighed, 2D surface of each fruit was determined with computer image analyses. From this image the total fruit surface (cm^2) and the fruit volume (cm^3) were estimated. These measurements were repeated at different times during dehydration. In addition, regular visual inspection of quality features was performed.

3.2.1 Fruit weight, area and volume

Fruit from the second harvest had on average the same weight as fruit from the first harvest, however, fruit from harvest 2 were on average 2 days older (Figure 14). There were differences in fresh weight (FW) at the start between the cultivars and sometimes also between harvests (e.g. K4, K7, K12). Only in the case of K12, this was statistically significant. In general, fruit weight differences were within about 10% from the average.

Fruit calculated area and volume are shown in Figure 15. As expected, there was a good correlation between fruit weight and fruit estimated volume (Figure 16) indicating that the volume estimation from 2D images is to a certain extent reliable and that there are no big differences in density of the fruit flesh between cultivars. Calculated average fruit density was 0.92 g/cm^3 which is close to values mentioned in literature.

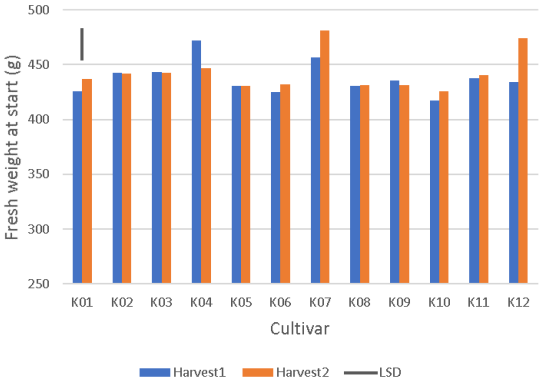


Figure 14 ***Average fresh weight (g) at start of 12 cucumber cultivars from 2 harvests. N=10, Least Significant Difference (LSD) value is indicated in the figure. Result of the post hoc test to show significant differences can be found in Annex 2,***

Table 1.

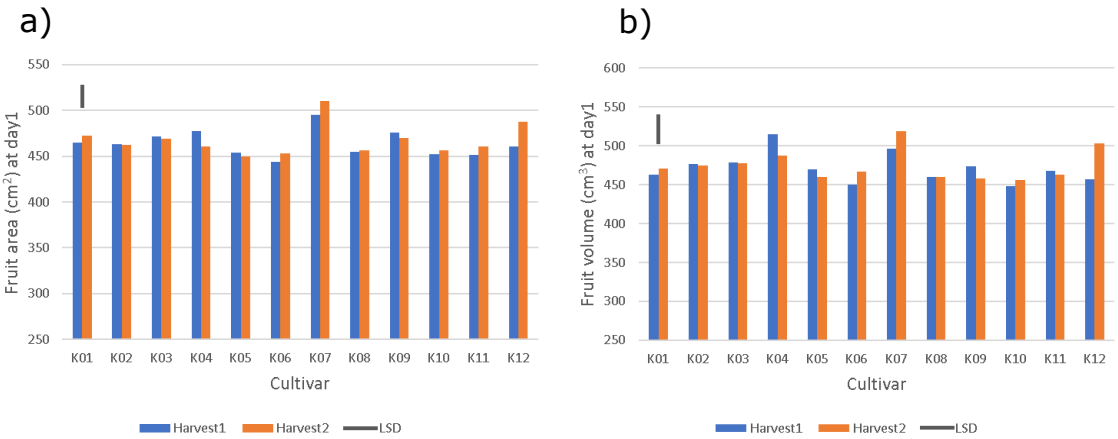


Figure 15 ***Fruit properties at day 1 of 12 cucumber cultivars from 2 harvests. a) Average calculated surface area (cm²), b) Average calculated fruit volume (cm3). Gray bar indicates least significant difference (LSD). Result of the post hoc test to show significant differences can be found in Annex 2, Table 2 and Table 3.***

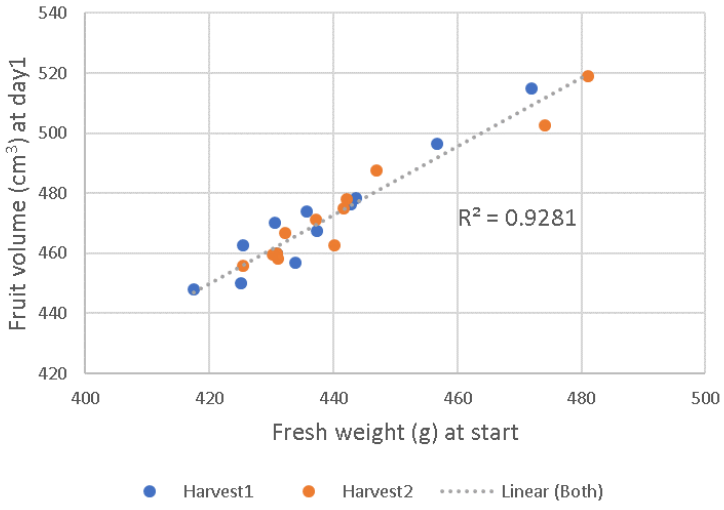


Figure 16 ***Relation between fruit weight (g) and calculated fruit volume (cm³). Data points represent the average value of 10 individuals of 12 cucumber cultivars from 2 harvests.***

Fruit length and width at day 1 were calculated from the 2D images (Figure 17). This shows clear differences between the cultivars. In general, the longer fruit were also thinner (Figure 18), leading to approximately similar weights of fruit. On average, the length/width (L/W) ratio was about 7. K4 showed the lowest and K7 the highest L/W ratio.

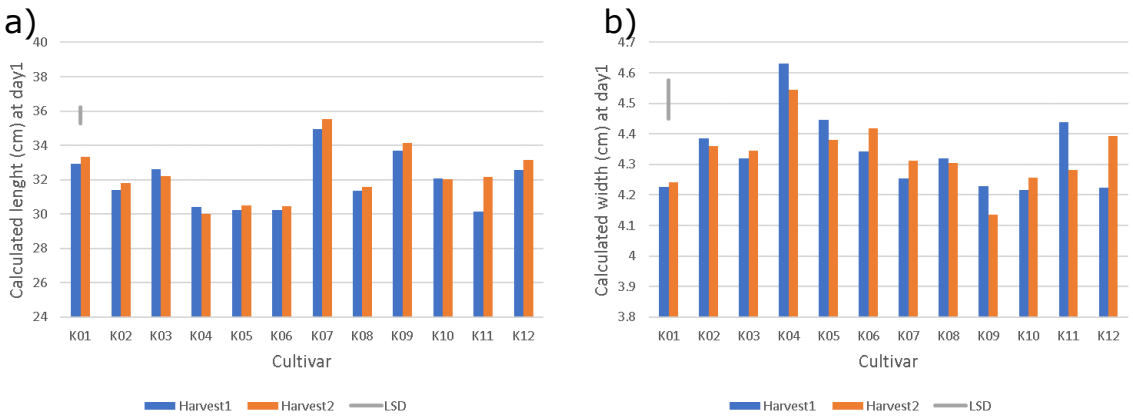


Figure 17 ***Fruit properties at day 1 of 12 cucumber cultivars from 2 harvests. a) Calculated length (cm) and b) calculated width (cm). Bar indicates least significant difference (LSD). Result of the post hoc test to show significant differences can be found in Annex 2 Table 4, Table 5.***

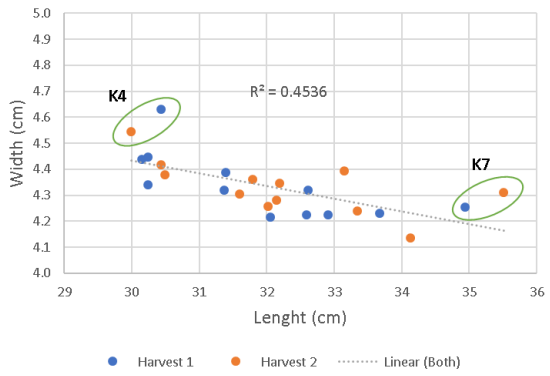


Figure 18 ***Correlation between length (cm) and width (cm).***

3.2.2 Area/volume ratio

There were differences between cultivars in both "area/volume" and "area/FW" ratios. Lowest area/volume ratio was 0.93; highest was 1.02, amounting to a maximum difference of about 10% in this population (Figure 19a). A similar difference was seen in the area/FW ratios (Figure 19b). Both ratios showed a reasonable correlation with each other, indicating that both these values can be used to compare cultivars. All tested fruit were regular commercial fruit and of more or less similar size, so big differences in area/volume were not expected. When screening larger populations of diverse fruit, this may become a more relevant factor. The lower the area/volume ratio, the less %WL is expected, if peel resistances are similar. Cultivar K4 shows a relatively low value and would therefore be expected to perform better.

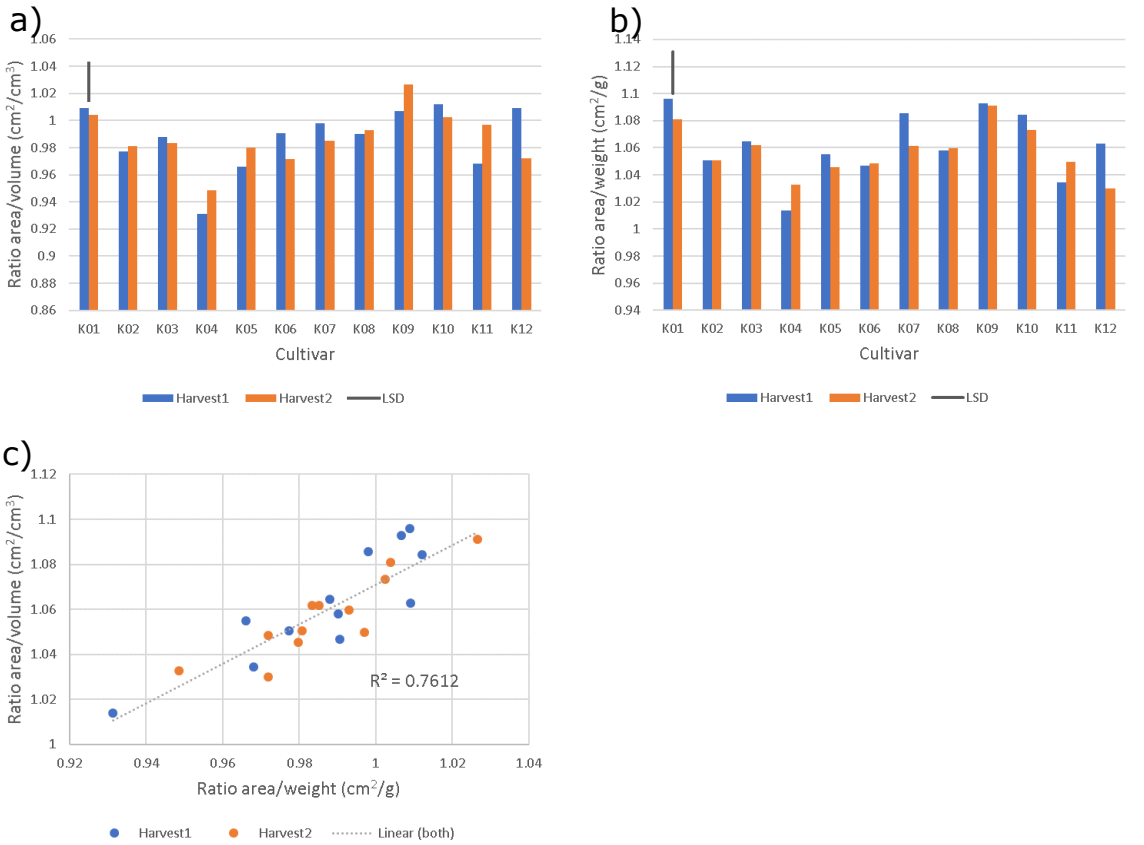


Figure 19 ***Fruit properties at day 1 of 12 cucumber cultivars from 2 harvests. a) Initial surface area to volume ratio, b) initial surface area to fresh weight ratio., c) Correlation between "surface area/volume" and "area/FW"-ratios. Surface area and volume both calculated from 2D images. Grey bar indicates least significant difference (LSD). Data points in C represent the average value of 10 individual fruit. Result of the post hoc test to show significant differences can be found in Annex 2, Table 6 and Table 7.***

3.2.3 Fruit firmness

Fruit firmness was measured manually on a scale of 1-10. At start, slight differences in firmness a were apparent between cultivars, however differences between harvest were more pronounced.

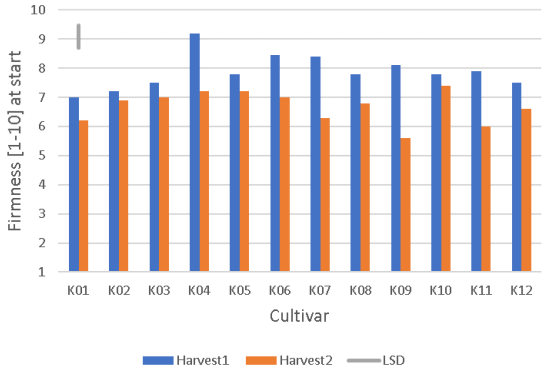


Figure 20 ***Manually assessed firmness at start [1-10; 1= very soft, 10 is very firm] of 12 cucumber cultivars from 2 harvests. Grey bar indicates least significant difference (LSD). Result of the post hoc test to show significant differences can be found in Annex 2, Table 8.***

Cultivar K4 shows the highest firmness on average (Figure 20) at start. This cultivar K4 was the cultivar with a relatively low ratio area/volume and area/weight.

3.3 Water loss behaviour during dehydration

3.3.1 Analysis of water loss curves via 2 models

Water loss of cucumbers over time, under the conditions described in 2.1 and expressed as % of starting weight, showed a curve that seems to indicate a non-linear weight loss over time (Figure 21a).

In case water loss is presented as transpiration in $g.kg^{-1}.hr^{-1}$ (Figure 21b) it is clearly visible that the transpiration has reduced by about 50% within 2 days and by about 70% after 10 days of shelf life.

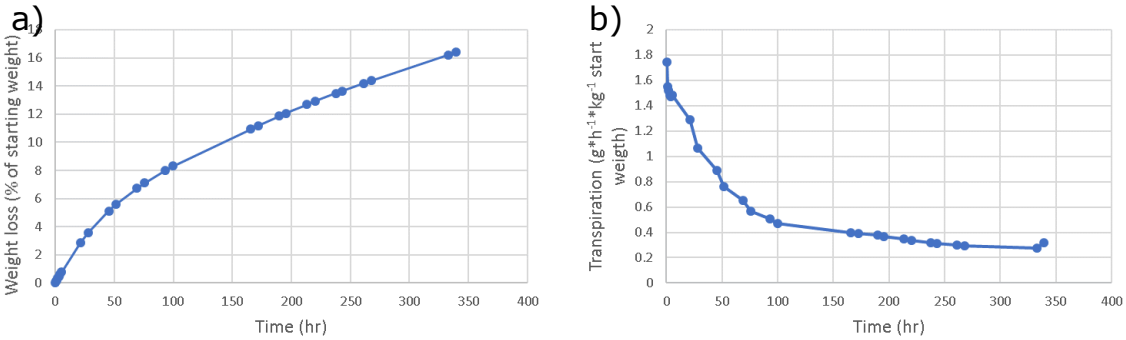


Figure 21 ***Example of the water loss curves: a) the development of the weight loss (%) over time, b) calculated transpiration ($g.h^{-1}.kg^{-1}$) over time. Data shown of fruit no. 1 of cultivar K01 from harvest 1.***

To determine the coefficients of the water loss curve, two models were tested. In both methodologies only 2 parameters are estimated: a reaction constant and an end value. The reaction (time) constant indicates the rate of %WL between the start (100%) and the end level (asymptote of the curve).The end level indicates the amount of water in the fruit that was available for loss under the experimental conditions. The dynamics of the curves show that transpiration-continuously declines over time, indicating a regulatory mechanism.

Genstat model

The Genstat (GS) model was applied to the %W curve of each individual fruit and from these values averages were calculated per cultivar and harvest. The reaction (time) constant (D) and end value (A) are estimated (as described in 2.2.3). Comparing these estimated values of the different fruit batches it shows little variation in rate constant between cultivars and harvests (Figure 22a.) but some more variation in end value (Figure 22b). Consequently there was no correlation between values D and A (Figure 22c). Concerning the end value, it shows that only 25 – 30% of the water in the fruit is actually available for transpiration.

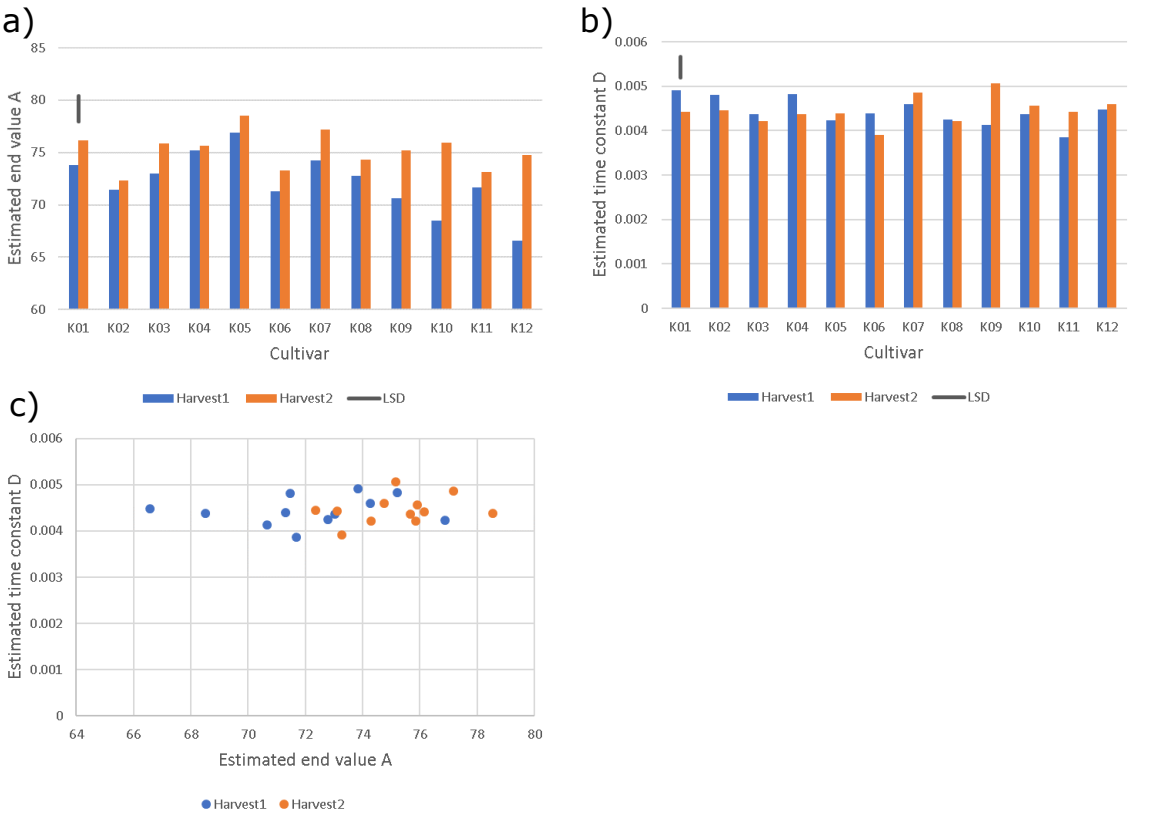


Figure 22 ***Parameters of the Genstat model: a) Estimated end value A, b) Estimated time constant D, c) correlation between A and D values. For the ANOVA-table and post hoc test of A and D see Annex 2, Table 9 and Table 10. Grey bar indicates least significant difference (LSD).***

Kinetic model:
The second approach was taken by fitting a first order kinetics model as described in paragraph 2.2.4. In this case a mathematical model was developed using the complete data set. From this the best parameters were estimated for each cultivar and harvest.

The k value (time constant) showed only slight differences, indicating that water loss dynamics (between start and end value) are very similar between cultivars and harvests (Figure 23a). A high K value would mean that the fruit goes more fast from 100% water to the end level than when a low K is measured. The end value Wmin, as calculated with the kinetic model showed slight differences between cultivars (Figure 23b); in general, bigger differences were observed between harvests in some cultivars than between cultivars within one harvest. A high end value would mean that the fruit is capable to "hold" its water very well; a low end value means that the fruit loses its water more easily. A low K and high end value would probably be the preferred situation. There was no correlation between K and end values (Figure 23c).

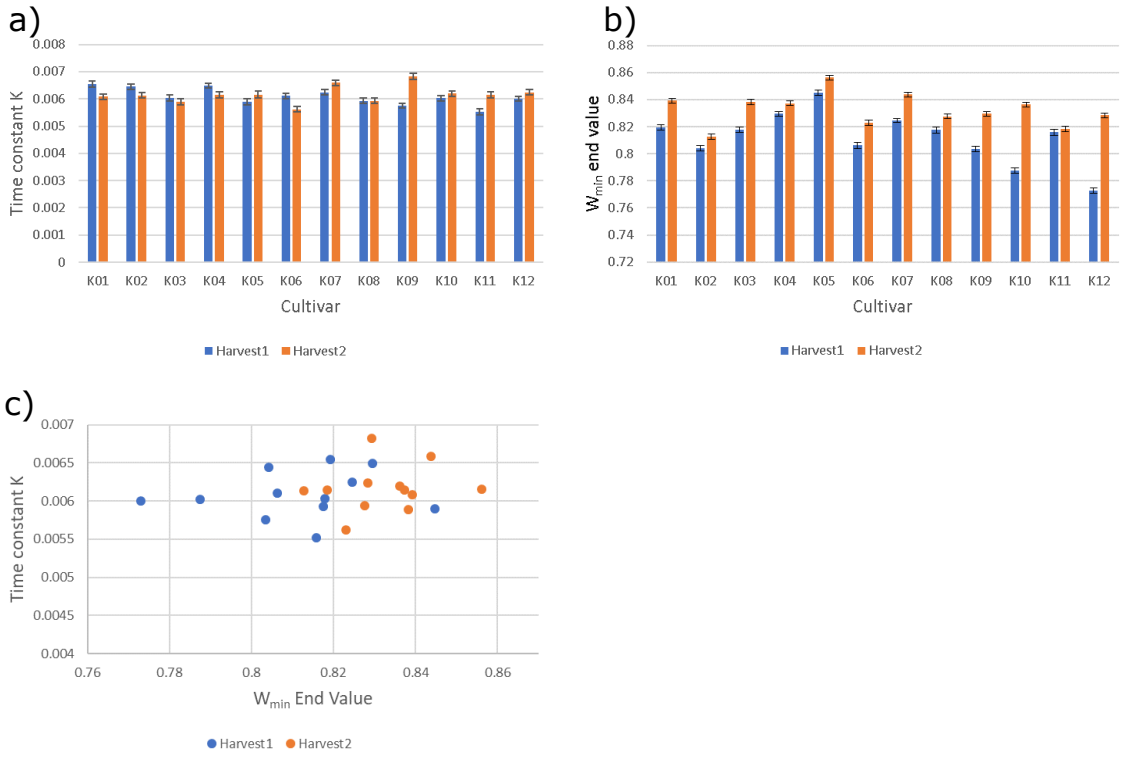


Figure 23 ***Parameters of the kinetic model. a) Estimated end value W_{min}, b) Estimated time constant K, Error bars indicate standard deviation. c) Correlation between K and W_{min}.***

When comparing both models, good correlations between both the end values and the time constants can be observed (Figure 24). This shows that both models can be used to compare the water loss behaviour of the fruit.

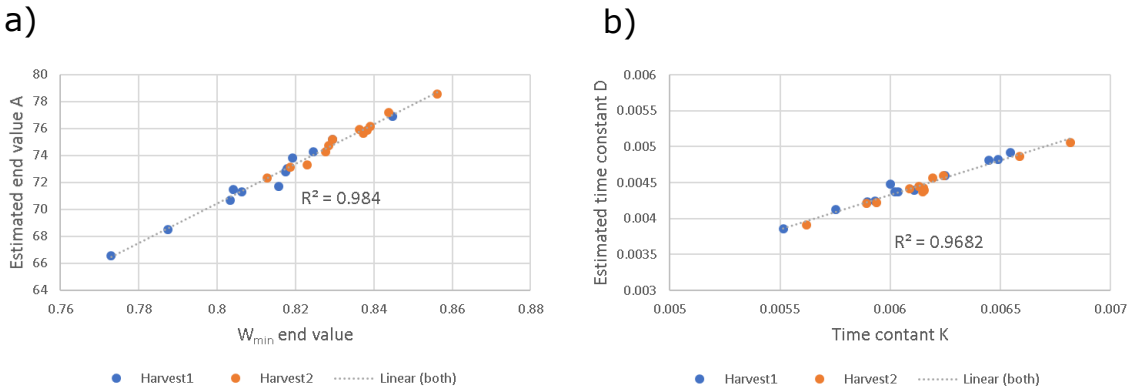


Figure 24 ***Comparing the GS and kinetic models. A) Correlation between the end values (W_{min} vs A), b) correlation between the time constants (K vs D).***

As the reaction constants (K and D) were in general very similar (not statistically different) for the whole data set, only the end values are of interest when considering differences between cultivars or harvests. Since the reaction constant is very similar in all cucumber batches, the %WL (or % of start weight) of the batches can be compared at any moment during the dehydration course (Figure 25). The calculated end values show high correlation with %WL (% from starting weight) values measured e.g. after 7 days (Figure 26).

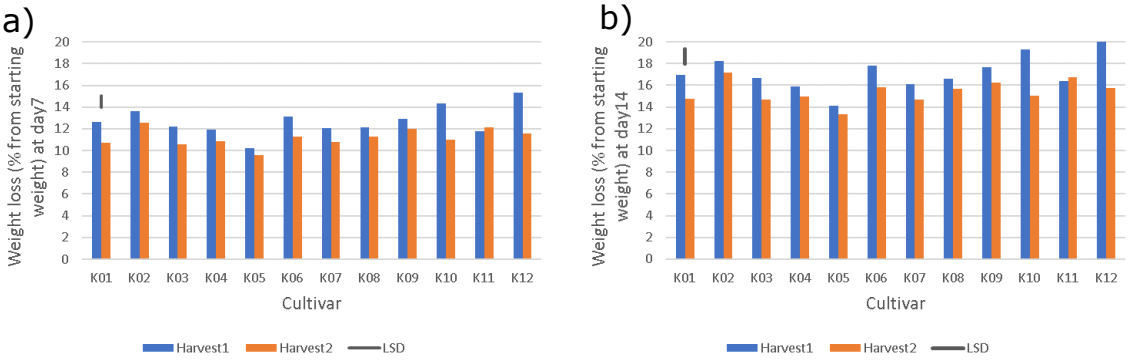


Figure 25 ***Average weight loss (% from starting weight) during shelf life. a) Weight loss at day 7, b) weight loss at day 14. Grey bar indicates least significant difference (LSD). For the ANOVA-table and post hoc test of A and D see Annex 2, Table 11 and Table 12.***

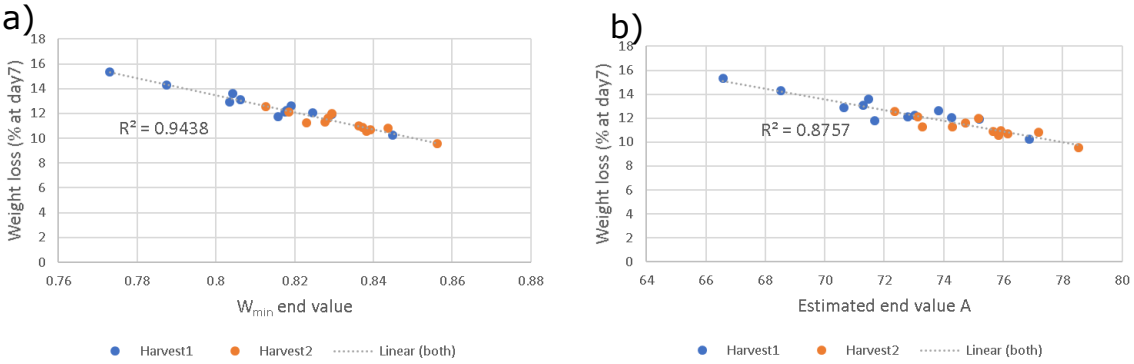


Figure 26 ***Correlations between %WL after 7 days and calculated end values of two models. a) %WL d7 and W_{min}, b) %WL d7 and A value.***

According to the differences in water loss (end value, or %WL at any time during the dehydration experiment)), the following can be concluded:

- In harvest 1 the cultivars 10 and 12 lost most water, cultivars 04 and 05 lost the least water;
- In harvest 2, cv 02 lost most water; cv 05 lost the least water.
- In harvest 2, end values were in general higher and showed less variation than in harvest 1.
- The lack of consistency in the ratings is due to the variability between the harvests.
- For cultivars 2, 6 and 11, the response was similar in the 2 harvests, the same is true for cv 5. For these selected cultivars it can be concluded that the performance of cv 5 is better (less water loss) than the performance of cultivars 2, 6 and 11.

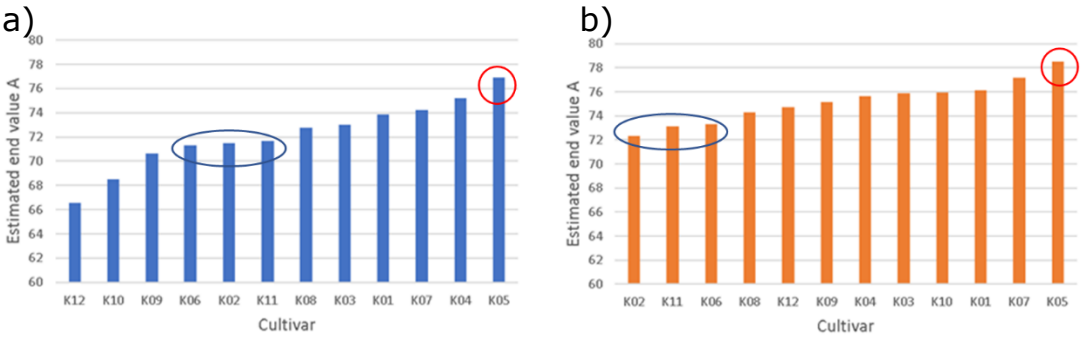


Figure 27 ***Rating of cultivars and harvests according to estimated end value A . a) fruit from harvest 1, b) fruit from harvest 2. For the ANOVA-table and post hoc test of A see Annex 2, Table 9.***

3.3.2 Water loss per surface area

Transpiration of fruit per surace area (g water loss per surface area) was calculated as an average over 7 or 14 days (total water loss in g was expressed per estimated surface area of the fruit at day 1). Again this shows minor differences between cultivars and harvests. This could indicate that the peel properties of these fruit in fact are very similar. There was a good correlation between transpiration and the end level of the weight loss curves estimated by both the kinetic and GS models. However, in this case the same weighing data have been used, and there was not a direct measurement of local transpiration. To determine if this relation is pivotal one should measure local transpiration of the peel and correlate it to WL% of a fruit.

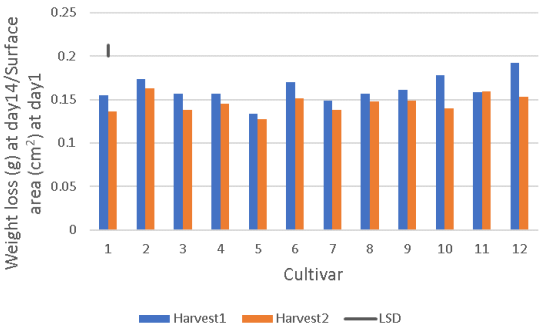


Figure 28 ***Average water loss per surace area (g/cm²) of different cultivars and harvests over 14 days. Grey bar indicates least significant difference (LSD) For the ANOVA-table and post hoc test of A see Annex 2, Table 13.***

3.3.3 Conclusions and discussion water loss curves

%WL is determined by the specific transpiration (g water loss per area unit per h) relative to the starting amount of water (weight). %WL is therefore dependent on the surface area/volume (or weight) ratio. At a given transpiration, a fruit with a higher surface area/volume ratio will show more %WL decline than a fruit of similar weight with a lower surface area/volume ratio. This means that %WL may partly be explained by the shape of the fruit (long and thin vs short and thick). Looking at the starting features, it was seen that K04 had a low area/volume ratio and was therefore expected to perform better in %WL. Indeed this cultivar has relatively low % WL compared to other cultivars, and had a high end value A. Looking at the specific weight loss/ area, K04 was an average scoring cultivar. The other cultivars did not greatly differ in this ratio, so differences in WL% are supposed to be connected to difference in transpiration rates.

The specific transpiration rate (g water loss/ per area unit /h) is dependent on the properties of the skin (resistance to water flow). Skin resistance is dependent on the chemical composition of cuticle and wax layer and on the existence of openings (stomata, cracks, lenticels). The status of the water in the fruit may also affect transpiration. If the water is "bound" in the tissue matrix, e.g. because of high osmotic potential, transpiration will be restricted. All these factors may contribute to the explanation of the %WL behavior and the possible differences between genotypes.

The shape of the %WL curve shows that the rate of %WL is high at the start and slowly comes to a low level of water loss, which eventually will virtually stop. Possible explanations for this behavior are discussed below:

- As the curves develop to an expected 'end value' at around 75% of the fruit fresh weight, it indicates that only about 25% of the water in the fruit would actually be available for transpiration (under the conditions in our shelf life room). If the available water is also to a certain extent "bound" in the tissue, the resistance to transpiration may increase over time as more water is lost. Currently we have no method to determine the water status in a nondestructive manner. On the other hand we have not looked at the development of weight loss after 14 days, so we don't know if 'end value' is really an end value.

- From the water loss over time, the transpiration (in g/kg FW) at each time point can be calculated. From this we can observe that transpiration (in g/kg FW) shows a decline of more than 50% over the first 2 days and by over 70% during 14 days. The reason for this is not immediately clear. Transpiration (expressed per FW) is, among others, determined by the fruit surface area. During the time frame of the experiment the fruit do show some shrinkage, resulting in a drop of surface area by 5-6% in 14 days. This means that the declining surface area can in itself not explain the huge drop in transpiration.
- The fruit shrinking may apart from lowering the surface area also have effects on the structure or compactness of the tissue and, especially the compactness of cuticle and wax layer. This may restrict transpiration. Currently we have no method available to quantify these effects.
- The cucumber fruit has stomata, but they seem to be mostly closed (Figure 29). It is not clear if they are still functional (so that they can open and close) and if they contribute to water loss. It may be that the more complete closure of the stomata over time restricts the transpiration. This is an aspect that we will further investigate.
- The resistance of the cuticle and wax layer is dependent on the chemical composition of these layers. It is possible that chemical composition changes during the dehydration.

Although we cannot yet fully explain the kinetics of the %WL curves, it is clear that the kinetics are pretty similar for all fruit, meaning that differences between these cultivars or harvests are not explained by different %WL kinetics (e.g. different stomata behaviour or different chemical changes in cuticle).

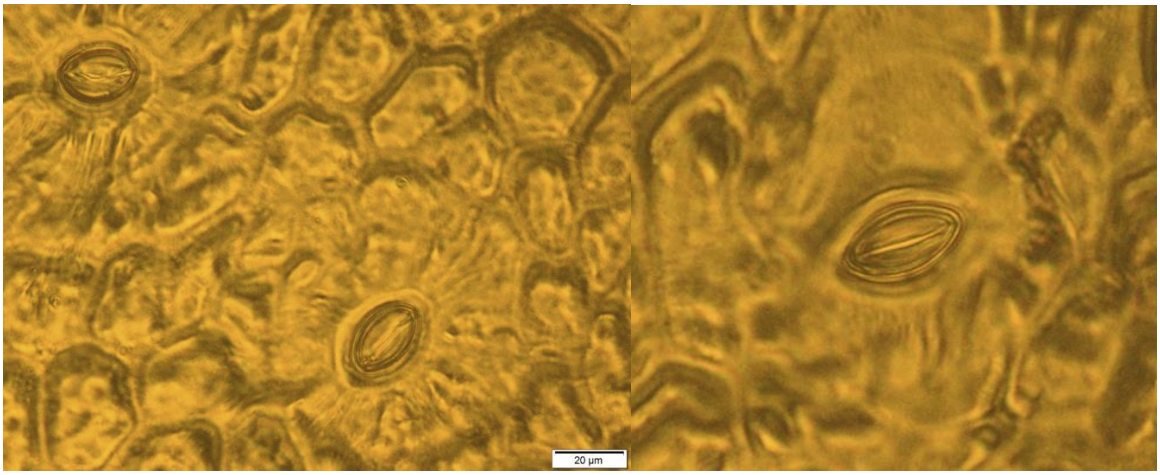


Figure 29 ***Example of stomata in cucumber (peel imprints), fruit were tested about 1 week after harvest. Stomata are fully closed.***

3.3.4 Quality features and water loss

3.3.4.1 Firmness

During the dehydration fruit showed changes in firmness, shrinking and yellowing. Surprisingly, fruit firmness first decreased, but later increased again. This trend was initially observed, but not sufficiently quantified, in all cultivars from the first harvest. Therefore it was decided to do more frequent firmness evaluations in the first period of shelf life for the fruits from the second harvest (Figure 30).

It was observed that firmness declined during the first 2-3 days, but firmness was generally back at the starting value at day 4. During the remainder of the shelf life period firmness slowly decreased again. The reason for this behaviour is not clear. It should be consideerd that the fruit lost most of their water during the first couple of days. A correlation of firmness and percentage waterloss was not apparent.

Apparently there is a mechanism that restores the firmness values following initial decrease. Partly the shrinking of the fruit can be responsible for this. The manual/organoleptic measurement of firmness in fact extimates "stiffness" of the whole fruit which may not be equivalent to tissue firmness measured with e.g. a penetrometer. On the whole the behaviour of all cultivars was very similar.

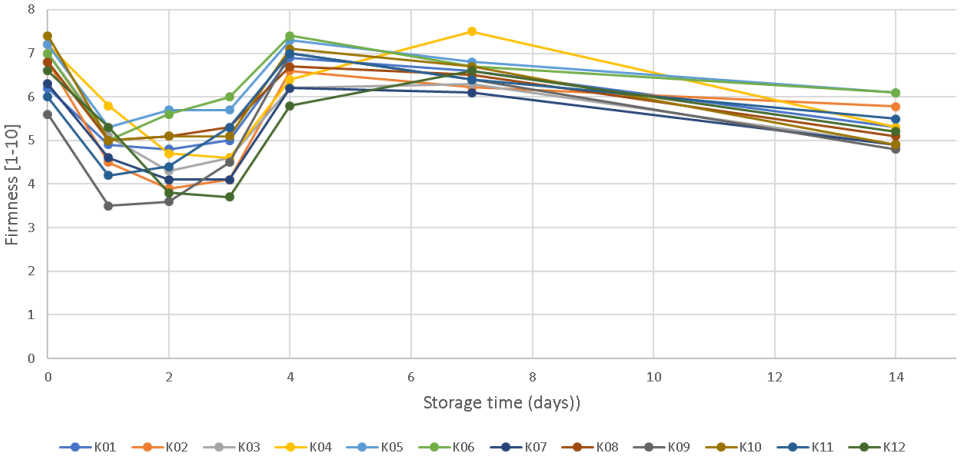


Figure 30 Manually assessed change in firmness over time in 12 cultivars of harvest 2. Data points represent the average value of 10 individual fruit.

3.3.4.2 Shrinking

Shrinking led to a decrease in both the estimated fruit surface area and volume. For both area and volume, an average decrease of about 10-12 % was observed during the dehydration experiment with no clear differences between cultivars and harvests (data not shown).

There was quite a strong correlation between shrinkage% and weight loss% after 1 week, but much lower after 2 weeks (R^2 of 0.70 after 1 week, 0.28 after 2 weeks) (Figure 31).

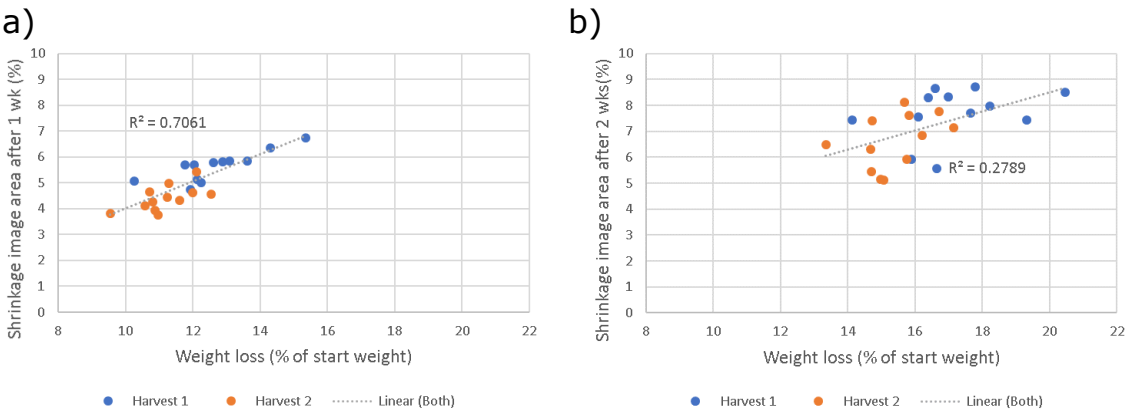


Figure 31 Correlation between % shrinkage of the image area and the weight loss (as % of start weight) a) after 1 week, b) after 2 weeks.

3.3.4.3 Colour

At day 1, 7 and 14, color changes were determined based on the 2D-images. All fruit tend to become more dark in the first week (lower L-value,

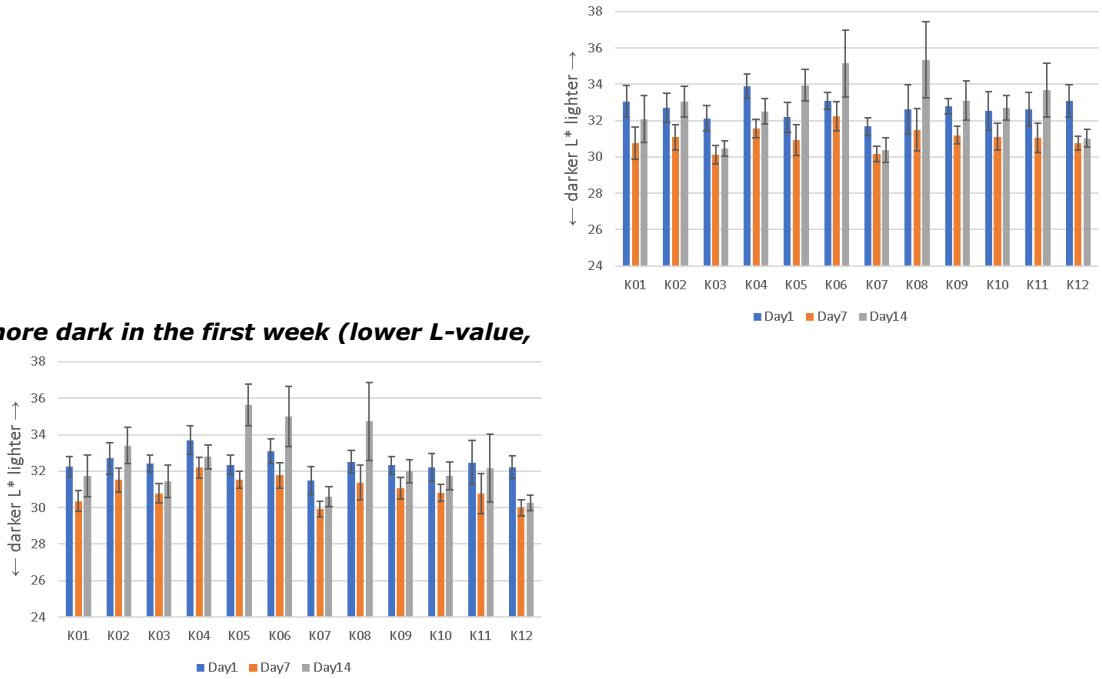


Figure 32). Later fruit became more yellow. In our experiment, fruit were exposed to continuous light. Yellowing was mostly present of the lighted side of the fruit. The reason for the initial darkening is not known. It may be related to the earlier mentioned shrinking of the fruit and its possible effect on concentrating the chloroplasts. Another reason may be the continued synthesis of chlorophyll from available substrates.

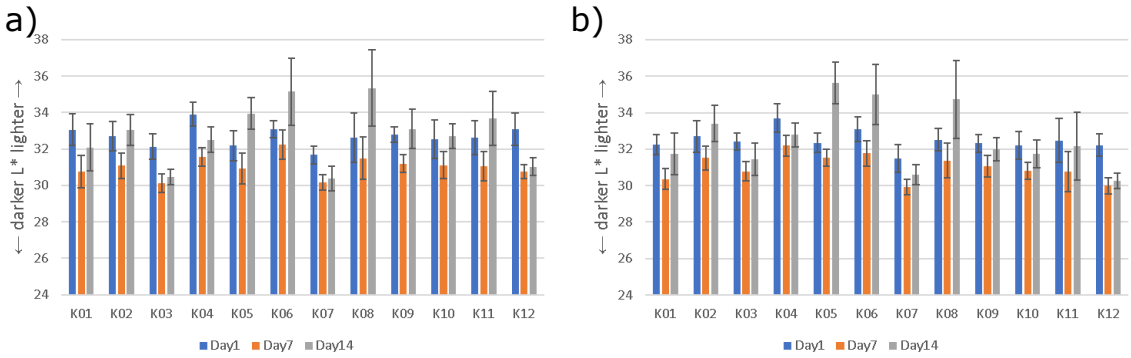
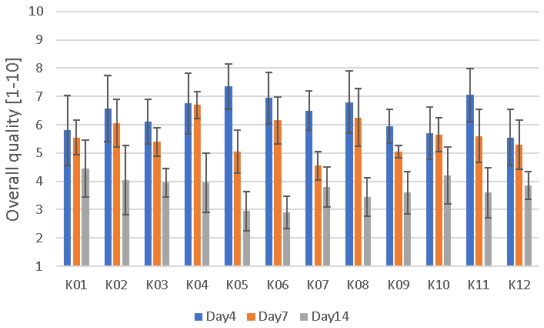


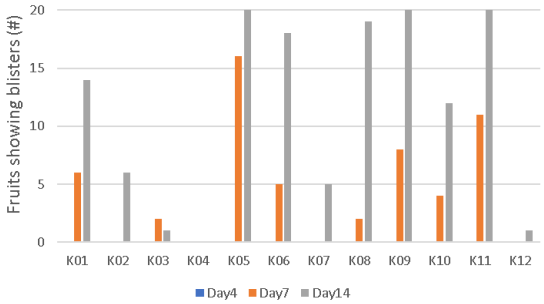
Figure 32 Change in color (L-value) of fruit of 2 harvests. Color was measured on the lighted side of the fruit. a) fruit from harvest 1, b) fruit from harvest 2

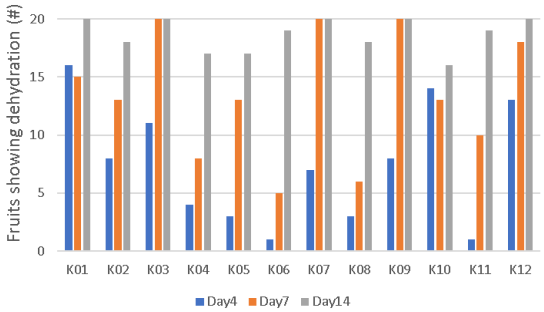
3.3.4.4 Visual quality inspection

Visual inspections of the fruit were done at the start and after 7 and 14 days. The visual quality score is affected by the signs of dehydration (wrinkling), but in addition also by yellowing and development of blisters. In addition to visual quality score the incidence of fruit showing clear dehydration symptoms (wrinkling), clear yellowing and blistering was scored. Overall visual quality decreases over time in all cultivars. Cultivars K5 and K6 received high scores at day 4, but relatively low scores on



day 14 (





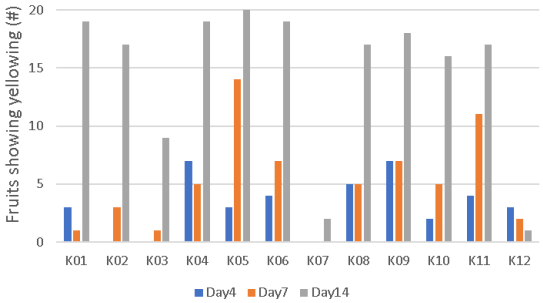
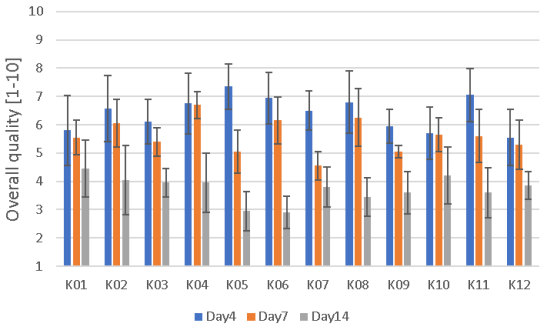
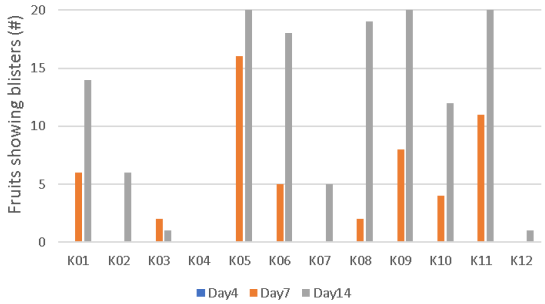
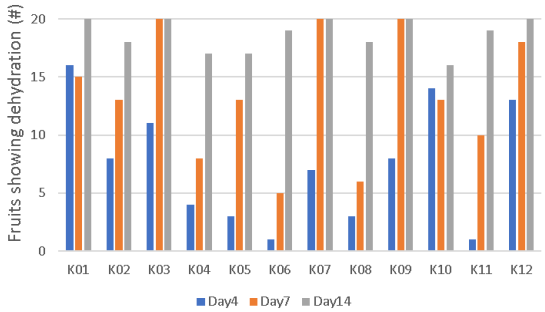


Figure 33a, see Annex 3 for harvest 1 and harvest 2 in separate figures). All cultivars increasingly showed dehydration symptoms, most cultivars showed clear yellowing (except K7 and K12), about



half of the cultivars showed blisters (





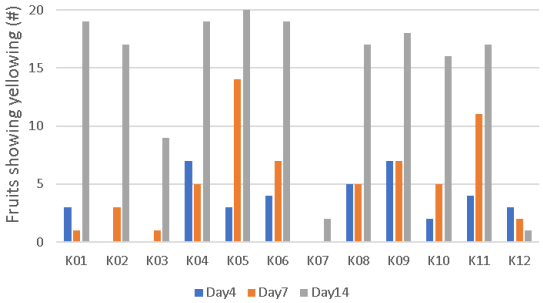


Figure 33 b, c, d).

A weak correlation could be found between the overall quality score at day 4 and the WL% after 7 days of the different cultivars (0.36 R^2, Figure 34)Figure 34 Correlation between weight loss (% from start weight) after 7 days and average overall quality scores at day 4 per cultivar over 2 harvests, with cultivars K5 and K12 as best and worse performing cultivars. The observations later in time did not correlate better. Also looking specifically at the evaluation of the number of cucumbers showing first dehydration or yellowing symptoms, was not improving the correlation.

a) b)

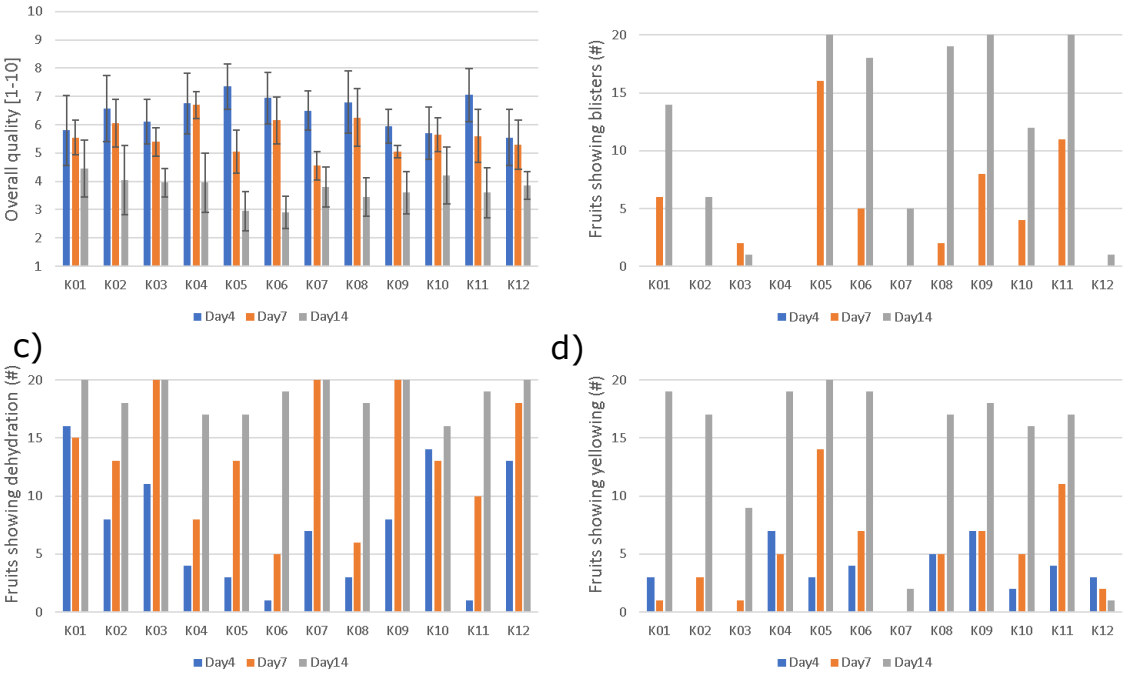


Figure 33 ***Quality features of 12 cultivars from 2 harvests together, assessed at day 4, 7 and 14 of the shelf life. a) average overall visual quality, bars indicate standard deviation, b) number of fruit showing blisters, c) number of fruit showing dehydration symptoms, d) number of fruit showing clear yellowing. N=20.***

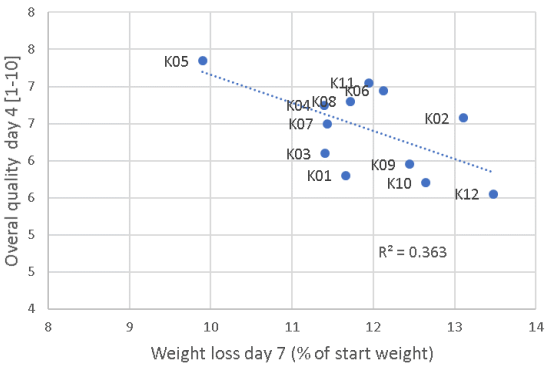


Figure 34 ***Correlation between weight loss (% from start weight) after 7 days and average overall quality scores at day 4 per cultivar over 2 harvests.***

4 Conclusions and discussion

%WL of cucumber fruit shows an exponential decline over time. This means that during dehydration the transpiration rate (g weight loss/FWstart/hr) continuously decreases. After 2 days, the transpiration rate is about 50% of the value at the start, after 14 days it is about 30% of the start value. There is no good explanation yet for this behaviour. As cucumber has ample stomata, it may be hypothesized that the slow closure of the stomata may be responsible for this behavior. This has to be verified. As another possibility, the decreasing "availability" of free water may also lie on the bases of the decreasing transpiration rate. As the %WL behaviour was very similar in the tested cultivars and harvests, it means that the possible stomatal closure (or in-availability of water) has similar dynamics in the different cultivars. Any differences in water loss between genotypes or harvests is not related to the kinetics of transpiration decline.

The %WL curves reach an estimated end value at around 75% of water. This means that under the test conditions only about 25% of water can be lost. This estimated end value is different for the different cultivars and harvest and can be used to rate the cultivars. A high end value could mean that the cultivars does not easily loose water which can be seen as a beneficial feature. End values differed between cultivars and harvests. Variation %WL was between 13 and 20% during the dehydration period, but high and low weight loss values were not consistently associated with cultivars indicating that the environmental conditions during cultivation affect these values.

As the %WL kinetics is similar for the different cultivars, a measure of %WL can be done e.g. after 1 day as a measure to compare cultivars. The %WL is mainly explained by the transpiration per area (g/cm^2/time). In addition, the ratio between surface area and volume (or weight) can be taken into account. This ratio did not differ much in the commercial cultivars that were tested, except for cultivar K4. Most differences between %WL of cultivars in this experiment can therefore be related to the transpiration (g/cm^2/time). The transpiration is thought to be mainly determined by the possible existence of functional stomata and by the properties of the cuticle and wax layer. Blocking the cut stem end with water insoluble grease was found to have little or no effect on transpiration.

During the dehydration experiment, fruit show up to 10-12% shrinkage. The % of shrinkage after 1 week showed a correlation with R^2 0.70 with the WL%. So the shrinkage is presumably related to the loss of water. During dehydration fruit firmness first decreases and later increases again. Fruit color first becomes more dark (green) and later more light again. These observations could be related. Currently we have no good explanation for this behaviour but both phenomena may also be related to the shrinkage of the fruit and could therefore also be related to the water loss.

The overall quality of the fruit decreases because of visual shriveling, yellowing and appearance of blisters. A weak correlation was seen between overall quality observation and WL% at day 4, with the best performing K5, and the worst K12.

In Figure 35 we show a simplified visualisation of some of the processes in cucumber and how they can be related to water loss and each other in cucumber. Water loss through transpiration decreases the water availability. This suppresses the transpiration. This explains the steadily decreasing transpiration. Water loss initially causes loss of firmness, but also it causes shrinking of the fruit. The shrinking may be the cause of the increase in firmness after an initial decease. Shrinking of the fruit may also cause the initial darkening of the fruit. Later, aging may cause yellowing of the fruit and water loss that is not compensated by shrinking will cause severe firmness loss.

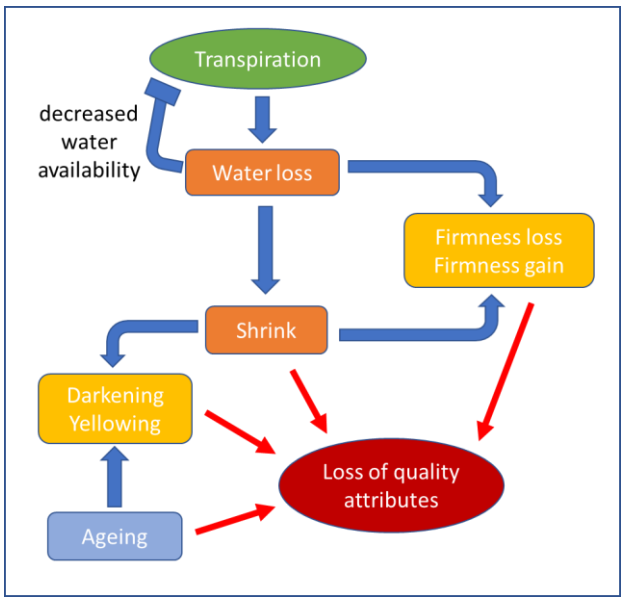


Figure 35 ***Visualisation of processes involved in water loss cucumber fruit.***

Annex 1. Climate conditions cucumber experiments

Temperature (°C) and relative humidity (%) during cucumber dehydration experiments

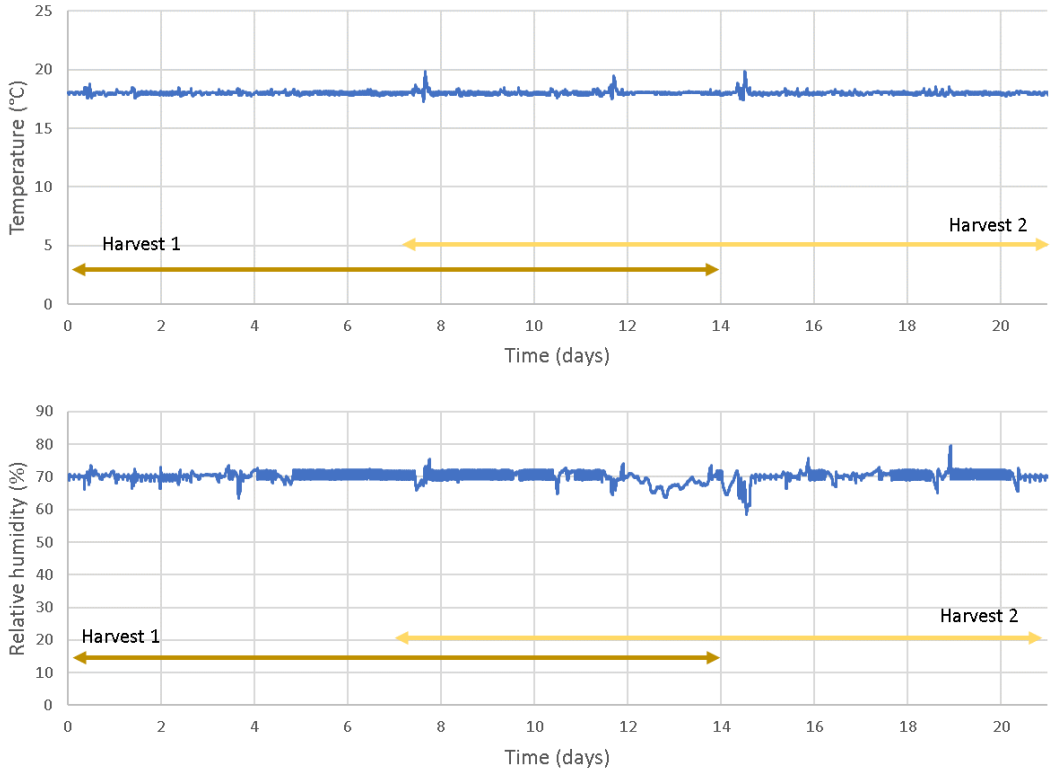


Figure 36 Temperature and relative humidity during dehydration tests

Annex 2. Statistical analysis

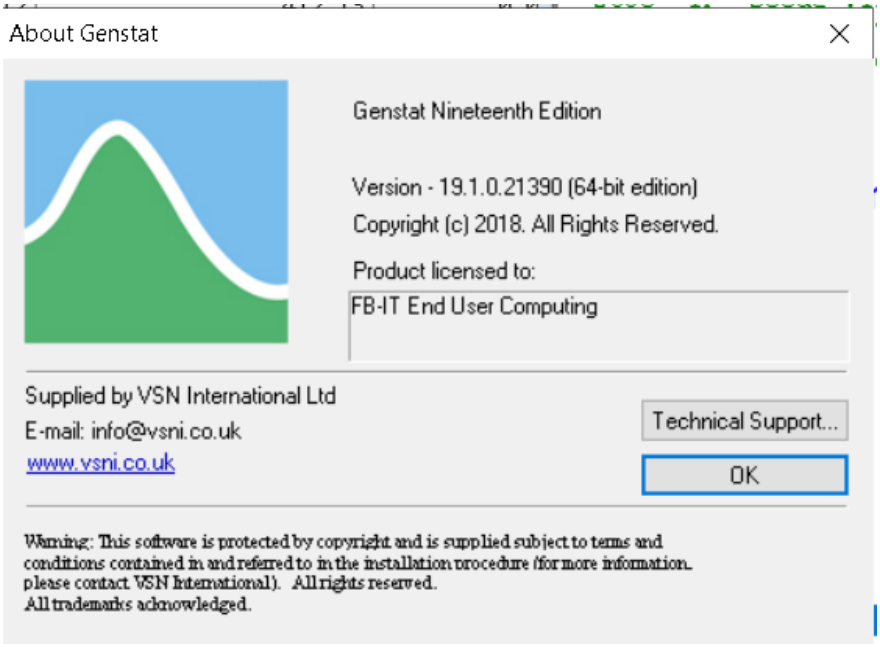


Figure 37 Information on used GenStat software

Table 1 ANOVA table, l.s.d. and Tukey post hoc test for the fresh weight (g) at start

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	43769.5	3979	4.32	<.001
Harvest	1	1586	1586	1.72	0.191
CV.Harvest	11	14006.8	1273.3	1.38	0.182
Residual	216	198741.3	920.1		
Total	239	258103.5			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	216	216	216
l.s.d.	18.91	7.72	26.74

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
431.3	442.2	442.9	459.5	430.4	428.7	468.9	430.9	433.4	421.4	438.8	454.0
ab	abc	abc	bc	ab	ab	c	ab	ab	a	abc	bc

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
425.5	442.8	443.6	471.9	430.6	425.1	456.7	430.9	435.8	417.4	437.4	433.9
ab	abc	abc	bc	ab	ab	abc	ab	abc	a	abc	abc
437.2	441.6	442.2	447.0	430.3	432.2	481.1	431.0	431.1	425.4	440.1	474.1
abc	abc	abc	abc	ab	abc	c	ab	ab	ab	abc	bc

Table 2 ANOVA table, l.s.d. and Tukey post hoc test for the fruit area (cm2) at day 1

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	46996.2	4272.4	7.68	<.001
Harvest	1	793.5	793.5	1.43	0.234
CV.Harvest	11	6876.3	625.1	1.12	0.344
Residual	216	120224.7	556.6		
Total	239	174890.7			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	216	216	216
l.s.d.	14.7	6	20.8

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
468.8	462.8	470.6	468.9	451.6	448.5	502.7	455.7	472.8	454.5	456.3	474.1
ab	ab	ab	ab	ab	a	c	ab	ab	ab	ab	b

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
465.2	463.4	472.0	477.1	453.7	443.8	495.2	455.1	475.6	452.5	451.6	460.5
abc	abc	abcd	abcd	ab	a	cd	ab	abcd	ab	ab	abc
472.4	462.2	469.2	460.8	449.6	453.1	510.2	456.4	470.1	456.6	461.1	487.8
abcd	abc	abc	abc	ab	ab	d	abc	abc	abc	abc	bcd

Table 3 ANOVA table, l.s.d. and Tukey post hoc test for the calculated fruit volume (cm3) at day 1

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	62664	5697	3.99	<.001
Harvest	1	692	692	0.48	0.487
CV.Harvest	11	19997	1818	1.27	0.242
Residual	214	305819	1429		
Total	237	388646			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	23.56	9.62	33.32

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
466.9	475.6	478.2	501.3	464.9	458.5	507.7	460.0	466.1	451.8	465.1	479.9
ab	abc	abc	bc	ab	a	c	a	ab	a	ab	abc

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
462.7	476.4	478.5	515.0	470.2	450.2	496.5	460.0	473.8	447.8	467.4	457.0
abc	abc	abc	bc	abc	a	abc	abc	abc	a	abc	abc
471.1	474.9	478.0	487.6	459.6	466.8	519.0	459.9	458.3	455.7	462.7	502.8
abc	abc	abc	abc	abc	abc	c	abc	abc	ab	abc	abc

Table 4 ***ANOVA table, l.s.d. and Tukey post hoc test for calculated fruit length (cm) at day 1***

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	518.307	47.119	43.27	<.001
Harvest	1	7.321	7.321	6.72	0.01
CV.Harvest	11	21.249	1.932	1.77	0.06
Residual	214	233.027	1.089		
Total	237	776.521			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	0.6504	0.2655	0.9199

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
33.13	31.59	32.4	30.21	30.37	30.34	35.22	31.48	33.9	32.04	31.15	32.87
de	bc	cd	a	a	a	f	bc	e	bcd	ab	de

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
32.91	31.4	32.61	30.43	30.25	30.24	34.94	31.37	33.67	32.06	30.15	32.59
efghi	abcde	efghi	abc	ab	ab	jk	abcde	hij	cdefgh	ab	efghi
33.34	31.79	32.19	29.99	30.49	30.44	35.51	31.59	34.13	32.01	32.15	33.15
ghij	bcdefg	defgh	a	abcd	abc	k	abcdef	ijk	cdefgh	cdefgh	fghi

Table 5 ***ANOVA table, l.s.d. and Tukey post hoc test for calculated fruit width (cm) at day 1***

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	2.42439	0.2204	10.67	<.001
Harvest	1	0.00176	0.00176	0.09	0.771
CV.Harvest	11	0.43484	0.03953	1.91	0.039
Residual	214	4.41919	0.02065		
Total	237	7.27941			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	0.08957	0.03657	0.12667

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
4.233	4.373	4.333	4.587	4.413	4.379	4.283	4.312	4.182	4.237	4.36	4.308
ab	bc	bc	d	c	bc	abc	abc	a	ab	bc	abc

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
4.226	4.387	4.319	4.631	4.447	4.341	4.254	4.32	4.229	4.217	4.439	4.223
ab	bc	abc	d	bcd	abc	ab	abc	ab	ab	bcd	ab
4.241	4.36	4.346	4.544	4.38	4.417	4.311	4.304	4.135	4.257	4.281	4.393
ab	abc	abc	cd	bc	bcd	abc	ab	a	ab	ab	bc

Table 6 ANOVA table, l.s.d. and Tukey post hoc test for ratio area/volume

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	0.085002	0.007727	8.2	<.001
Harvest	1	1.6E-06	1.6E-06	0	0.967
CV.Harvest	11	0.018937	0.001722	1.83	0.051
Residual	214	0.201574	0.000942		
Total	237	0.305463			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	0.01913	0.00781	0.02705

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
1.0064	0.9791	0.9857	0.94	0.9729	0.9812	0.9916	0.9916	1.0167	1.0073	0.9826	0.9905
cd	bc	bcd	a	b	bc	bcd	bcd	d	cd	bc	bcd

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
1.0089	0.9773	0.988	0.9313	0.9661	0.9905	0.9981	0.9902	1.0067	1.0121	0.968	1.0091
cd	abcd	bcd	a	abc	bcd	bcd	bcd	cd	cd	abc	cd
1.0039	0.9808	0.9834	0.9486	0.9797	0.9718	0.9851	0.993	1.0266	1.0025	0.9971	0.9719
cd	abcd	bcd	ab	abcd	abc	bcd	bcd	d	cd	bcd	abc

Table 7 ANOVA table, l.s.d. and Tukey post hoc test for ratio area/weight

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	0.09115	0.008286	6.68	<.001
Harvest	1	0.00144	0.00144	1.16	0.282
CV.Harvest	11	0.012082	0.001098	0.89	0.555
Residual	214	0.265299	0.00124		
Total	237	0.369726			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	0.02195	0.00896	0.03104

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
1.088	1.051	1.063	1.023	1.05	1.048	1.074	1.059	1.092	1.079	1.042	1.046
c	ab	bc	a	ab	ab	bc	abc	c	bc	ab	ab

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
1.096	1.05	1.064	1.014	1.055	1.047	1.086	1.058	1.093	1.084	1.034	1.063
d	abcd	abcd	a	abcd	abcd	bcd	abcd	d	bcd	abc	abcd
1.081	1.051	1.062	1.033	1.045	1.048	1.062	1.06	1.091	1.073	1.05	1.03
bcd	abcd	abcd	ab	abcd	abcd	abcd	abcd	cd	bcd	abcd	ab

Table 8 ANOVA table, l.s.d. and Tukey post hoc test for manually assessed firmness at start [1-10]

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	41.2656	3.7514	4.84	<.001
Harvest	1	87.068	87.068	112.24	<.001
CV.Harvest	11	31.2981	2.8453	3.67	<.001
Residual	214	166.0111	0.7758		
Total	237	324.1387			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	0.549	0.2241	0.7764

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
6.6	7.044	7.25	8.2	7.5	7.722	7.35	7.3	6.85	7.6	6.95	7.05
a	ab	ab	c	abc	bc	abc	abc	ab	bc	ab	ab

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
7	7.2	7.5	9.2	7.8	8.444	8.4	7.8	8.1	7.8	7.9	7.5
abcdef	bcdef	cdef	g	defg	fg	fg	defg	efg	defg	defg	cdef
6.2	6.889	7	7.2	7.2	7	6.3	6.8	5.6	7.4	6	6.6
abc	abcde	abcdef	bcdef	bcdef	abcdef	abc	abcde	a	bcdef	ab	abcd

Table 9 ANOVA table, l.s.d. and Tukey post hoc test for end value A from the Genstat model

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	887.271	80.661	11.14	<.001
Harvest	1	541.794	541.794	74.83	<.001
CV.Harvest	11	336.976	30.634	4.23	<.001
Residual	214	1549.472	7.241		
Total	237	3307.967			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	1.677	0.685	2.372

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
74.99	71.91	74.44	75.43	77.71	72.29	75.72	73.54	72.91	72.22	72.4	70.66
cde	ab	bcd	de	e	abc	de	bcd	abcd	abc	abc	a

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
73.84	71.47	73.02	75.2	76.89	71.3	74.26	72.79	70.66	68.51	71.68	66.58
cdefgh	bcde	cdefgh	defghi	hi	bcd	cdefghi	bcdefgh	abc	ab	bcdef	a
76.15	72.35	75.85	75.66	78.53	73.28	77.19	74.29	75.17	75.92	73.12	74.74
ghi	bcdefg	efghi	defghi	i	cdefgh	hi	cdefghi	defghi	fghi	cdefgh	cdefghi

Table 10 ANOVA table, l.s.d. and Tukey post hoc test for the time constant D from the Genstat model

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	9.62E-06	8.75E-07	3.18	<.001
Harvest	1	2.82E-08	2.82E-08	0.1	0.749
CV.Harvest	11	1.09E-05	9.91E-07	3.6	<.001
Residual	214	5.88E-05	2.75E-07		
Total	237	7.94E-05			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	0.000327	0.000133	0.000462

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	K02	K03	K04	K05	K06	K07	K08	K09	K10	K11	K12
0.004666	0.004628	0.004293	0.004596	0.004312	0.004154	0.00473	0.004232	0.004593	0.004468	0.004141	0.004536
ab	ab	ab	ab	ab	a	b	ab	ab	ab	a	ab

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	K02	K03	K04	K05	K06	K07	K08	K09	K10	K11	K12
0.004916	0.004806	0.004372	0.004823	0.004235	0.004396	0.004595	0.004243	0.004124	0.004375	0.003859	0.004474
bc	bc	abc	bc	abc	abc	abc	abc	ab	abc	a	abc
0.004416	0.00445	0.004214	0.00437	0.004388	0.003913	0.004865	0.004221	0.005062	0.004562	0.004423	0.004597
abc	abc	abc	abc	abc	a	bc	abc	c	abc	abc	abc

Table 11 ANOVA table, l.s.d. and Tukey post hoc test for weight loss (% of starting weight) at day7

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	191.833	17.439	11.75	<.001
Harvest	1	134.496	134.496	90.62	<.001
CV.Harvest	11	70.172	6.379	4.3	<.001
Residual	214	317.607	1.484		
Total	237	712.395			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	0.759	0.31	1.074

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	K02	K03	K04	K05	K06	K07	K08	K09	K10	K11	K12
11.66	13.08	11.41	11.4	9.9	12.17	11.43	11.72	12.44	12.64	11.94	13.47
b	cd	b	b	a	bc	b	b	bcd	bcd	bc	d

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	K02	K03	K04	K05	K06	K07	K08	K09	K10	K11	K12
12.61	13.62	12.24	11.92	10.25	13.09	12.05	12.14	12.9	14.32	11.77	15.35
defgh	ghi	bcdefg	bcdefg	ab	fgh	bcdefg	bcdefg	efgh	hi	bcdefg	i
10.71	12.54	10.57	10.88	9.55	11.25	10.81	11.29	11.99	10.97	12.12	11.6
abcd	cdefgh	abc	abcd	a	abcdef	abcd	abcdef	bcdefg	abcde	bcdefg	bcdef

Table 12 ANOVA table, l.s.d. and Tukey post hoc test for weight loss (% of starting weight) at day14

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	308.395	28.036	11.98	<.001
Harvest	1	189.385	189.385	80.9	<.001
CV.Harvest	11	113.07	10.279	4.39	<.001
Residual	214	500.98	2.341		
Total	237	1108.838			

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	0.954	0.389	1.349

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
15.85	17.68	15.68	15.43	13.73	16.81	15.39	16.13	16.92	17.19	16.55	18.1
bc	de	bc	b	a	bcde	b	bcd	bcde	cde	bcde	e

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
16.98	18.22	16.64	15.9	14.12	17.79	16.1	16.59	17.64	19.31	16.39	20.45
cdef	efg	bcde	bcde	ab	def	bcde	bcde	def	fg	bcde	g
14.72	17.14	14.71	14.96	13.34	15.83	14.69	15.68	16.21	15.07	16.72	15.74
abc	cdef	abc	abc	a	abcde	abc	abcd	bcde	abc	cde	abcde

Table 13 ANOVA table, l.s.d. and Tukey post hoc test for the ratio of weight loss (g) at day14 and the surface area measured at day1

ANOVA table

	d.f.	s.s.	m.s.	v.r.	F pr.
CV	11	0.028554	0.002596	12.85	<.001
Harvest	1	0.015273	0.015273	75.59	<.001
CV.Harvest	11	0.007843	0.000713	3.53	<.001
Residual	214	-2	0.043242	0.000202	
Total	237	-2	0.094557		

Least significant differences of means (5% level)

	CV	Harvest	CV. Harvest
rep.	20	120	10
d.f.	214	214	214
l.s.d.	0.008861	0.003617	0.012531

Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
0.1457	0.1684	0.1474	0.1509	0.1307	0.1605	0.1434	0.1524	0.155	0.1592	0.159	0.1726
bc	de	bc	bc	a	cde	ab	bc	bcd	cde	cde	e

Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)

K01	**K02**	**K03**	**K04**	**K05**	**K06**	**K07**	**K08**	**K09**	**K10**	**K11**	**K12**
0.1553	0.1736	0.1564	0.1567	0.1339	0.17	0.1484	0.1569	0.1615	0.1782	0.1583	0.1921
bcdefgh	ghi	bcdefgh	bcdefgh	ab	fghi	abcdef	bcdefgh	defgh	hi	cdefgh	i
0.1362	0.1632	0.1385	0.1451	0.1276	0.1511	0.1384	0.1479	0.1486	0.1403	0.1596	0.1532
abc	efgh	abcd	abcde	a	bcdefg	abcd	abcdef	abcdef	abcde	defgh	bcdefg

Annex 3. Overall quality in both harvests

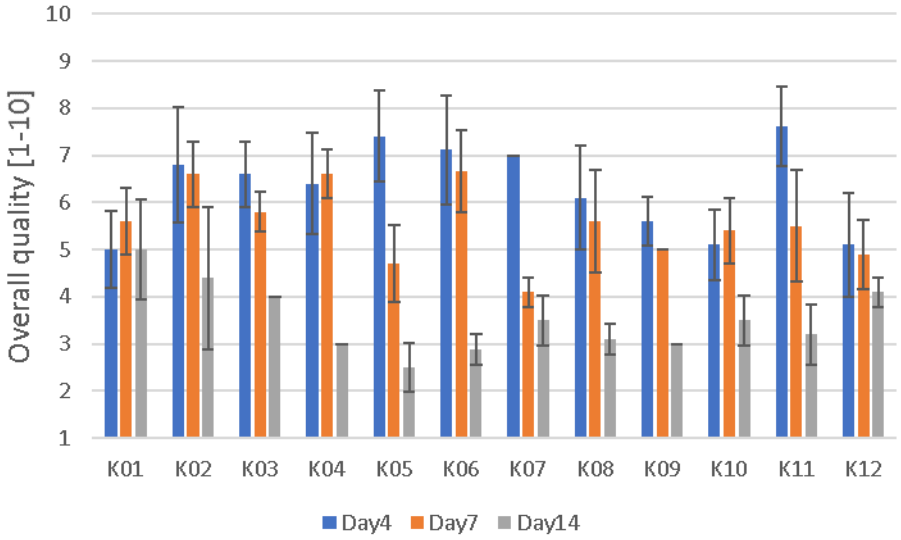


Figure 38 ***Average overall quality of 12 cultivars from harvest1, assessed at day 4, 7 and 14 of the shelf life, bars indicate standard deviation***

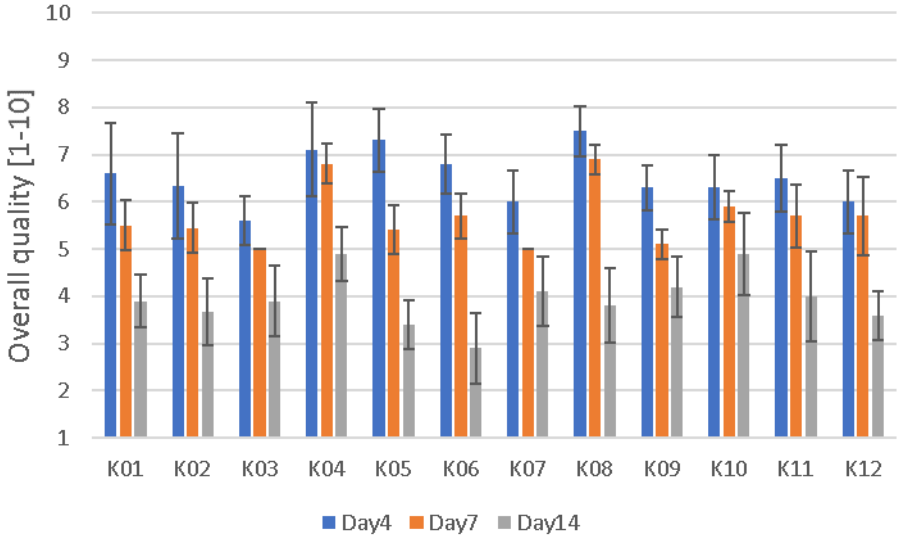


Figure 39 ***Average overall quality of 12 cultivars from harvest1, assessed at day 4, 7 and 14 of the shelf life, bars indicate standard deviation***

To explore the potential of nature to improve the quality of life

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