



Determining water loss characteristics in pepper cultivars

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

Ernst Woltering, Manon Mensink, Mariska Nijenhuis-de Vries, Najim El Harchioui,
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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. Therefore water loss 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 the 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 **peppers**. 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 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 experiments in pepper, this work package includes a similar type of experiments on cucumbers. The latter are described in a separate report entitled "Determining water loss characteristics in cucumber cultivars, WUR report 2172".

The results of Work Package 2, which is focused on genetic diversity for water loss traits in broader populations are 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 identify genotypes with proposed better water loss characteristics. "Pepper diversity trial, WUR report nr 2173".

2 Material & Methods

Several experiments were performed in 2020.

1. Explorative experiment to develop methodology for water loss characteristics peppers
2. Dehydration experiment in which water loss in time of 12 cultivars from 2 harvests was determined. This with the aim to correlate water loss in time to certain fruit characteristics and identify possible features / traits which could be a possible cause of water loss.

The setup of these experiments is described in this chapter.

2.1 Products

2.1.1 Explorative experiment

ENZA kindly provided WUR with different cultivars to develop the weighing strategy (see Table 1 and Figure 2).

Table 1 *Pepper products provided by ENZA for methodology development*

WUR code	ENZA code	Variety
Pepper A	R15958	Block
Pepper B	R16218	Mini-conical
Pepper C	R16524	Conical
Pepper D	R15986	Block
Pepper E	R16220	Mini-conical
Pepper F	R16490	Block



Figure 2 *Peppers provided by ENZA for methodology development*

2.1.2 Experiment with 12 cultivars determining water loss and other features

Each partner selected 4 cultivars, which were all grown at Enza in Enkhuizen, The Netherlands. Fruits were harvested and transported at the same day to Wageningen. From these 12 cultivars, 9 were blocky type peppers, 1 was mini-block and 2 were conical peppers (see Table 2). In Figure 3 pictures of the peppers are shown.

Table 2 *Pepper products provided by partners with WUR code*

WUR code	Variety
P01	Block
P02	Block
P03	Block
P04	Block
P07	Block
P08	Block
P09	Mini-Block
P10	Block
P13	Block
P15	Conical
P16	Block
P17	Conical

Two series of peppers were delivered:

- Serie 1 harvest date 5th October 2020
- Serie 2 harvest date 26th October 2020



Figure 3 *The 12 cultivars for water loss characterization in 2020 and their WUR codes*

2.2 Method Explorative experiment

From each of the six cultivars a number of peppers were placed on six trays. The total of 36 trays were placed randomly in the climate room (see Figure 4). In Table 3 the amount of peppers per tray are given.



Figure 4 *Peppers placed randomly in the climate room during method development for weighing*

Table 3 *The amount of peppers on the trays during method development for weighing*

WUR code	Variety	Tray 1	Tray 2	Tray 3	Tray 4	Tray 5	Tray 6	Total peppers
Pepper A	Block	4	3	3	3	3	4	20
Pepper B	Mini-conical	5	5	5	5	5	5	30
Pepper C	Conical	5	5	5	5	5	5	30
Pepper D	Block	3	2	3	3	2	3	16
Pepper E	Mini-conical	5	5	5	5	5	5	30
Pepper F	Block	5	5	5	5	5	5	30

To check the water loss through the calyx and stem, from half of the peppers the calyx and stem end were treated with water insoluble (vacuum) grease. This to prevent water loss via the calyx and stem end.

The trays were weighed per tray on a two digit scale, Mettler Toledo (Tiel, NL) type MS6002TSDR (serial nr. B523026995, PHT0029)¹. The first week all trays were weighed twice per working day. The second week the trays were weighed 1-2 times a day. After two weeks the peppers were cut in half to determine the percentage calyx compared to rest of the fruit (see Figure 5)

¹ Calibration certificate nI0048-018-061819-ACC-RVA, issued 18th June 2019, guideline EURAMET cg-18 v4.0 (11/2015), method WIKA/02



Figure 5 *Measurement % calyx*

2.3 Method Dehydration experiment with 12 cultivars

2.3.1 Determining weight loss curves

In this experiment the peppers were weighed individually in order to be able to connect weight loss to other traits like firmness, appearance, etc. Because of the low weight loss, weighing was performed on a three digit scale Mettler Toledo (Tiel, NL) type MS403TS/00 (serial nr. B623579527, PHT0032)² with a closed chamber to prevent air movement from disturbing the measurement (see Figure 6).

For determining weight loss, ten fruits were placed on two trays. The trays were placed randomly in the climate room. The first day each individual fruit was weighed five times, the following four days the fruits were weighed two times a day. Another weighing was performed on day 8, 9 and 15. The series was repeated with fresh peppers 3 weeks later.



Figure 6 *Weighing individual peppers on three digit scale with chamber*

Besides weighing, also quality evaluation was performed on day 1, 4, 7, 9, 11 and 14 days.

The fruits were individually evaluated on:

- Firmness manual impression score 1 (bad)-9 (very good), 6 = acceptable
- Overall visual impression/marketability score 1 (bad)-9 (very good), 6 = acceptable. The overall impression score could not be higher than the firmness score. Firmness score was leading, when more defects were detected the score was lowered.
- Pitting (skin): yes/no
- Internal rot, visible on the outside: yes/no
- Wrinkles (skin): yes/no
- Mould on pedicel (skin): yes/no.

² Calibration certificate nI0048-028-061919-ACC-RVA, issued 19th June 2019, guideline EURAMET cg-18 v4.0 (11/2015), method WKA/02



Figure 7 *Examples of defects of peppers; pitting(left) and shrivelling (right)*

For surface estimation and amount of water in pericarp at start:

- On the first day, a 2D image was taken and analysed per individual fruit
- At the end of the weighing experiment, the pericarp was separated from the rest of the fruit by removing the placenta, the calyx and the white parts of the capsaicin glands (see Figure 8). The pericarp was weighed on the earlier described 3 digit scale and dried at 80°C for 4 days to determine the dry weight. In this way the remaining water content can be calculated. Because also the water loss is determined, the amount of water present in the pericarp at start can be calculated. In this approximation we assumed that most of the fruit water loss is from the pericarp (and not from the internal tissues).

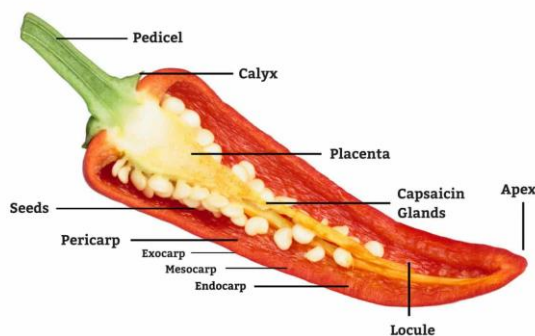


Figure 8 *Pepper anatomy (<https://www.pepperscale.com/pepper-anatomy/>)*

2.3.2 Determining features of the starting material

To determine features of the starting material, per cultivar 5 different fruits were selected and the following was measured:

- 2D-image with colour cabinet
- Fresh weight per fruit whole fruit
- Fresh weight pericarp
- 4 discs of known area were punched from the pericarp, between the glands. From these discs the following was measured:
 - o Thickness
 - o Fresh weight
 - o Dry weight

With these data various parameters could be calculated. The methods area described in more detail below.

2.3.3 Specific fruit area

With the disc's fresh and dry weight (4 days at 80°C) measurements and the disc area, the specific fruit area (area per gram product) can be calculated.

Specific fruit area:

- $SFA_{fw} = Area_{discs} [cm^2] / FW_{discs} [g]$
- $SFA_{dw} = Area_{discs} [cm^2] / DW_{discs} [g]$

2.3.4 Estimating surface area

Two methods were explored to determine the pericarp surface area of the whole fruit:

- a) Estimation surface area via 2D imaging
- b) Estimation surface area via disc method

a. Estimation surface area via 2D imaging

Pictures (2D-imaging) were taken with the Smart Colour Inspector running with Colour Cabinet Software (WFBR, Wageningen V1.6, 23-01-2019). 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, Regensburg, S), see. Based on this calibration, the RGB images are standardized to official $L^*a^*b^*$ (D50) values of Macbeth ColorChecker³.

The images are used to determine the surface of the pericarp. 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).

Per cultivar and series five peppers were 2D-imaged. Also a ruler was imaged so that afterwards the amount of pixels could be calculated to cm (1 cm = 37 pixels). Before estimating the area of the pericarp, the stem was excluded by a line in the image (see Figure 9). The area was then estimated by converting number of pixels to cm^2 .

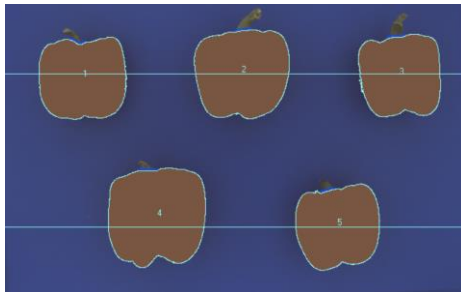


Figure 9 *Example of 2D-image of peppers. The stem is cut off and the pericarp is analysed*

b. Estimation surface area via disc method using SFA

The idea is to take discs with a fixed area and determine the fresh and dry weight. Thereafter the total weight (fresh and dry) of the pericarp is measured. The area/weight ratio of the discs is representative for the whole pericarp; in this way a reliable measure of surface area can be obtained.

To determine the surface area of the starting material the same peppers were used as for the estimation 2D-imaging. In this case the SFA_{fw} was used to determine the area:

- $Area (cm^2) = SFA_{fw} [cm^2/g] * FW_{pericarp} [g]$

³ Pascale D (2006) RGB coordinates of the Macbeth color checker, BabelColor Company, Montreal, Quebec, Canada.(2006, Jun.)

The total dry matter content is supposed not to change during dehydration experiments, therefore, the area estimation based on the ratio of area to dry weight of discs can be used to back calculate the original area of the peppers in the dehydration experiment. After the experiment, the pericarp total dry weight is determined. Fruit area can be back calculated using SFAdw.

- $\text{Area (cm}^2\text{)} = \text{SFA dw}_{\text{discs}} [\text{cm}^2/\text{g}] * \text{DW}_{\text{pericarp}} [\text{g}]$

2.3.5 Estimation of pericarp/whole fruit weight ratio and pericarp thickness

Fresh weight contribution of pericarp to whole fruit was determined by weighing the whole fruit and following dissecting weighing of the pericarp. Pericarp thickness was measured with a digital calliper in 4 pericarp discs (Figure 10).



Figure 10 *Preparing discs (left), measuring thickness (right).*

2.3.6 Weight loss curves

For pepper the water loss appeared to be merely linear in the time measured. Therefore, a linear regression was performed in excel per cultivar to determine the water loss per hour.

$$\text{Water loss} = a * \text{time} + b$$

a = water loss per hour = transpiration

To reveal the differences in transpiration between cultivars, an Anova was performed. Cultivar was taken as factor and weight loss as variable. If significant differences occurred, the Tukey post-hoc test ($p = 0.05$) showed the differences between the products. When comparing the two series (replicates) cultivar and replicate were taken as factors with their interaction and weight loss as variable.

2.3.7 Climate conditions

The dehydration tests were performed in a climate room with regulated temperature and relative humidity. Setpoints were set to 18°C and 70% RH.

The light in the climate room was continuously on, providing about 10 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ PAR on the level of the shelves. Air circulation was at a constant level of $\sim 0.1 \text{ m.s}^{-1}$.

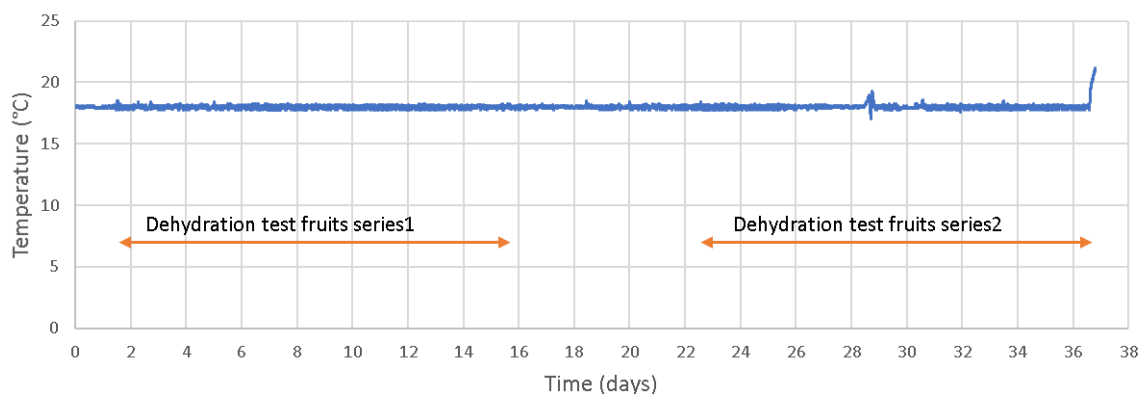


Figure 11 *Temperature (°C) during dehydration testing of peppers from series1 and series2*

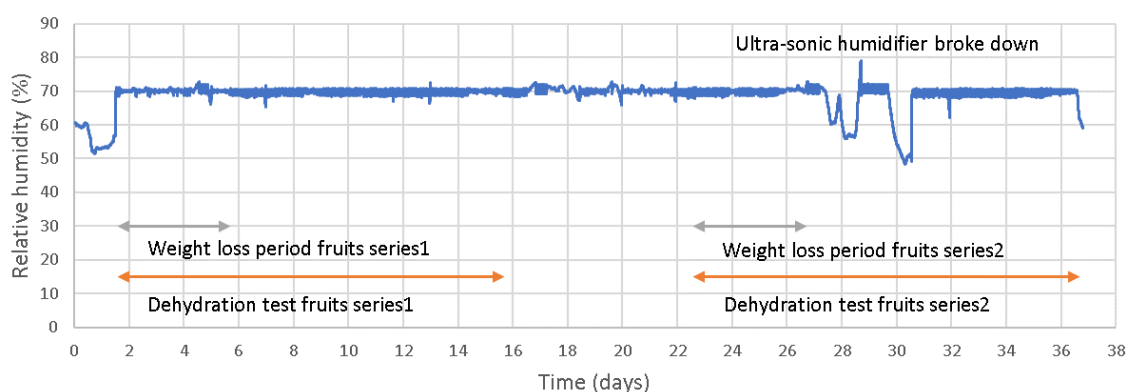


Figure 12 *Relative humidity (%) during dehydration testing of peppers from series1 and series2. Period of weight loss measurements is indicated (grey lines). After the weight loss period of series2, the ultra-sonic humidifier broke down and finally had to be replaced.*

In Table 4 the average temperature and relative humidity during the dehydration testing is shown.

Table 4 *Average temperature (°C) and relative humidity (%) during the dehydration testing of peppers, harvested in 2 series.*

	Temperature (°C)		Relative humidity (%)			
			Dehydration test		Weight loss period	
	Average	Std	Average	Std	Average	Std
Series1	18.0	0.1	70.1	0.6	70.1	0.7
Series2	18.0	0.2	68.4	4.8	70.1	0.5

3 Results

3.1 Methodology development

In the first explorative experiment and in the experiment with the 12 cultivars we explored various methods to measure and analyse sub-traits related to water loss. In this section we will describe the main results and learnings with respect to the method development.

3.1.1 Contribution of calyx to water loss

In the explorative experiment average weight loss was followed during 14 days from 6 cultivars. From half of the peppers the calyx was left untreated and from the other half of the peppers the calyx and stem were treated with vacuum grease to block water loss by these parts.

Judging from the weight loss curves (Annex 1) it can be concluded that weight loss (in % of start weight) was mostly linear over time and that %WL was not or only marginally affected by the grease treatment of the calyx. From this, we conclude that the calyx does not have a significant contribution to the fruit weight loss. Any differences in e.g. the ratio calyx to whole fruit between cultivars and individual fruit are not expected to affect the weight loss behaviour. Therefore in the following dehydration experiment it was decided to leave the calyx untreated.

3.1.2 Determination of fruit area

In the dehydration experiment, 2 methods to determine fruit area have been used and have been related to each other. One method to determine fruit area was using the SFAdw: DW (dry weight) of pericarp discs with known area was measured and the DW of the total pericarp was determined. The second was using 2D computer image analyses. The method using SFA is considered an accurate estimation; 2D-image analysis is much faster and non-destructive but less precise in relation to whole fruit surface.

In Figure 13, it can be seen that 2D-images estimated a much lower area than the SFA method. This may be due to the fact that only one 2D image of the fruit was taken. There was, however, a reasonable good correlation between the data from both methods. 2D imaging may be a suitable method to non-destructively estimate the fruit area. By using an appropriate correction factor (specific for fruit type, blocky or conical or spherical) it may be improved.

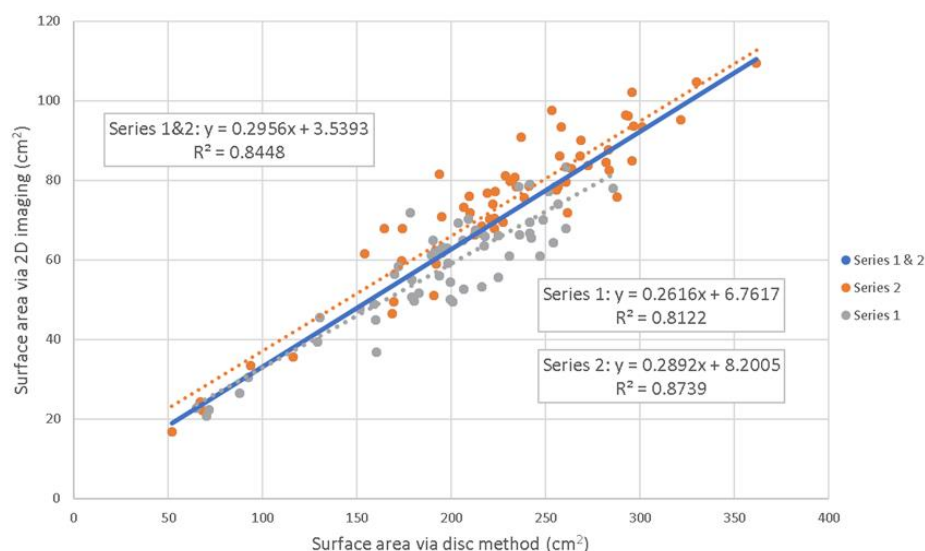


Figure 13 Correlation between calculated fruit surface areas using method based on SFAdw (disc method) and based on 2D-image analysis, in 10 peppers from all 12 cultivars from 2 harvests.

3.2 Results dehydration experiment 12 cultivars

3.2.1 Initial product features

There were big differences in fruit weight between cultivars, and differences in fruit weight between first and second harvests (Figure 14). In general blocky fruit of the second harvest were heavier compared to the blocky fruit from the first harvest.

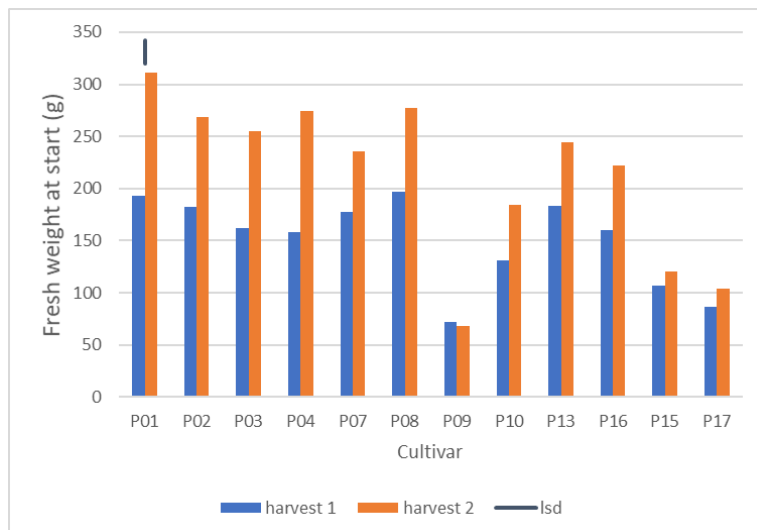


Figure 14 Fresh weight at start of fruit from 2 harvests. N=10, Lsd value is given in left top corner of figure. Result of the post hoc test to show significant differences can be found in Annex 2, Table 8.

Pericarp %DM was around 8 in the red blocky and the conical fruit; it was between 6 and 7 in the yellow blocky fruit (Figure 15A). The pericarp of blocky fruit is thicker (6-9 mm) than that of conical fruit (4-5 mm) (Figure 15). Specific fruit area SFA (based on DW Figure 15C) and SFA (based on FW Figure 15) showed some variation between cultivars. SFA was higher for the conical fruit compared to the blocky fruit. This also shows that the conical fruit have a thinner pericarp than the blocky fruit. For the yellow blocky fruit, SFAfw is relatively low, SFAdw is relatively high. This reflects the observation that the %DM of these fruit is relatively low.

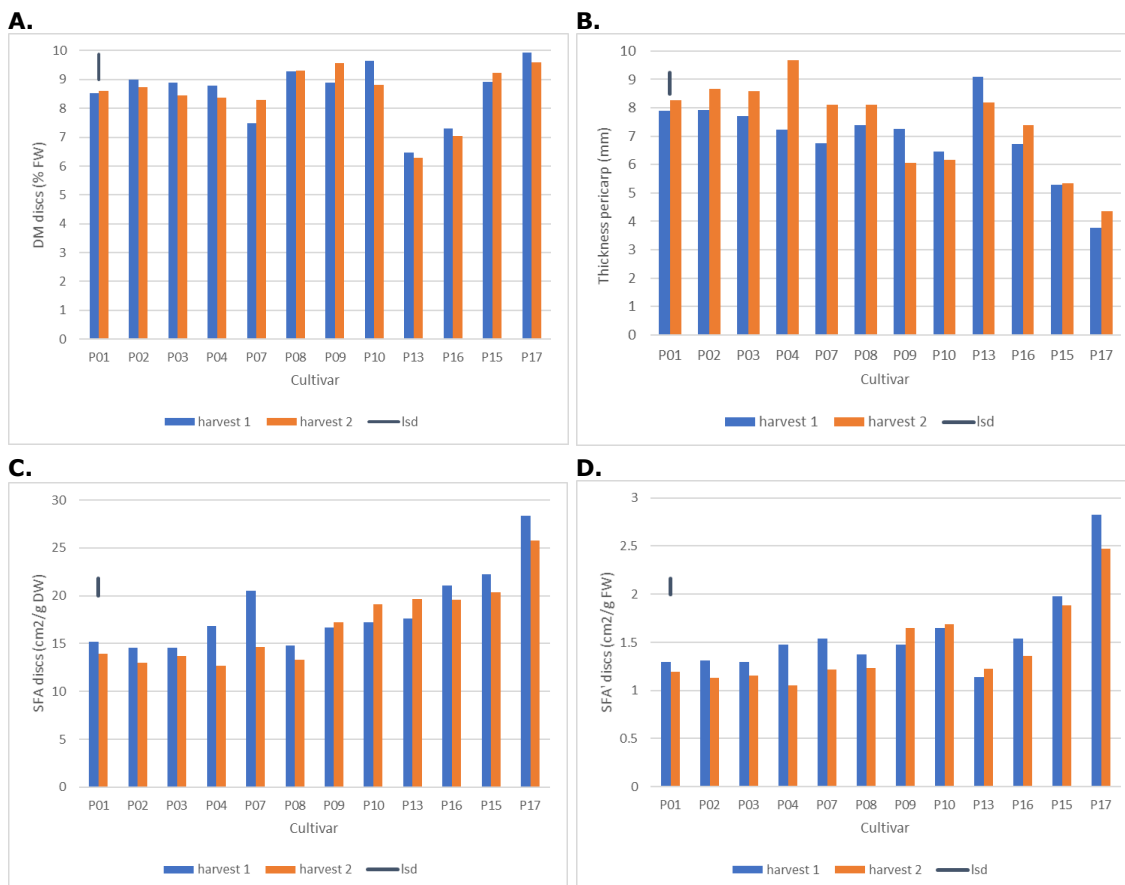


Figure 15 *Pericarp features of different cultivars of 2 harvests. A) Dry matter %, B) thickness in mm, C) Specific fruit area based on dry weight (SFAdw), D) SFA based on fresh weight (SFAfw). N=5, Lsd value is given in figure. Result of the post hoc test to show significant differences can be found in Annex 2, Table 9, Table 10, Table 11 and Table 12.*

Average pericarp surface area was estimated from the area/DW ratio (SFAdw) determined in pericarp discs. Area showed little variation among the tested cultivars. Only the red blocky fruit P09 differed from the rest, however this fruit also had a low fresh weight. The ratio weight pericarp to whole fruit was between 85 and 92%. As described in 3.1.1, we found that the calyx (and most likely also the internal tissues!) contribute very little to the total weight loss. The pericarp %WL will be the parameter that presumably is responsible for pericarp quality loss. Therefore it is relevant to correct %WL data for these differences in pericarp/whole fruit ratio.

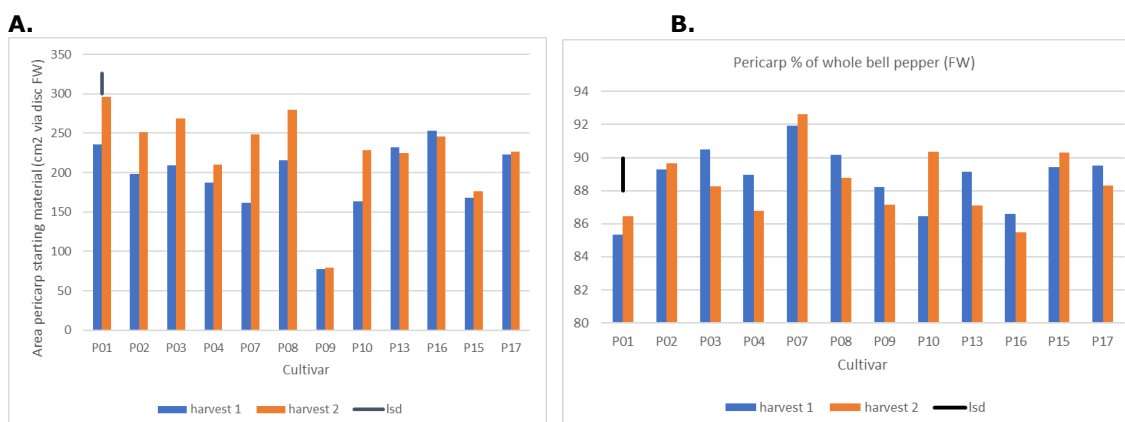


Figure 16 *Pericarp features of pepper cultivars of 2 harvests. A) Pericarp area estimated through discs (SFAfw), B) Estimated ratio between pericarp and remaining tissues. N= 5, Lsd value is given in figure. Result of the post hoc test to show significant differences can be found in Annex 2 Table 13 and Table 14.*

There was a good correlation between pericarp thickness (measured directly in mm) and SFAfw (area per weight in cm^2/g). This is illustrated in Figure 17. It means that both parameters are a good indication of pericarp thickness or pericarp mass. There is a reasonable correlation between either SFAfw or pericarp thickness on the one hand and pericarp %DM on the other hand (Figure 18). It shows that a thinner pericarp may have a higher %DM. In this relation, the yellow blocky differ from the other cultivars in the sense that the same relation holds true, but the yellow fruit in general have lower DM%.

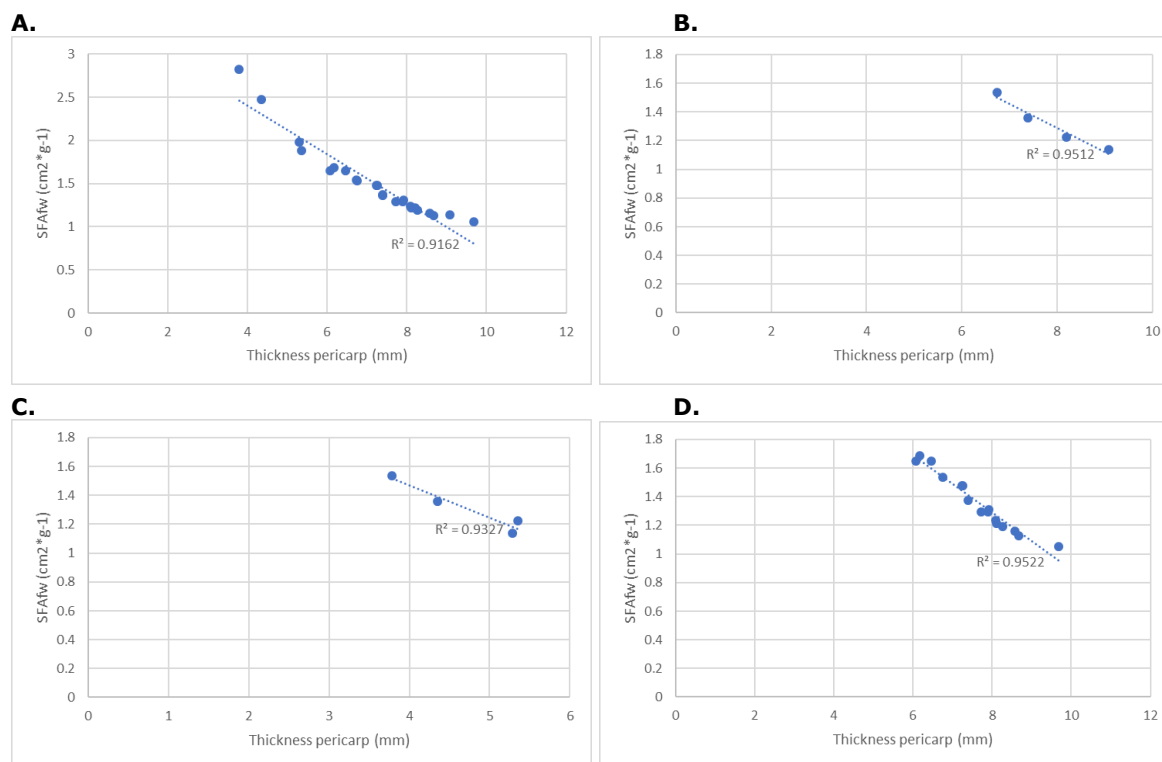


Figure 17 *Correlation between SFAfw and pericarp thickness. A) all fruit, B) yellow blocky, C) conicals, D) red blocky. Data points are averages per serie.*

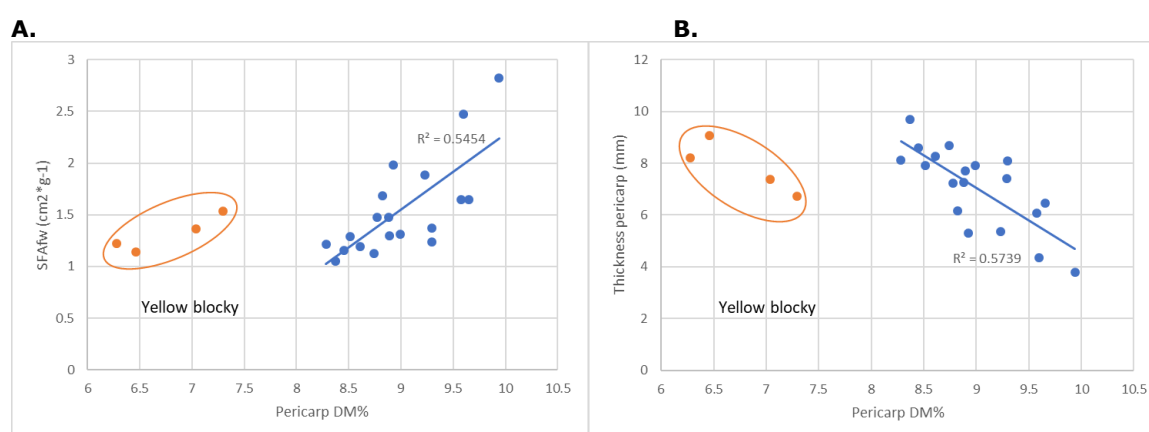


Figure 18 *Correlations between pericarp %DM and other features. A) %DM% and SFAfw, B) %DM and pericarp thickness. Data points are averages per serie.*

3.2.2 Weight loss

During the first day, all fruit were weighed 5 times, in the following days fruit were weighed 2 times per day. In all the samples, weight loss was strictly linear over time. Therefore either the slope of the water loss curve or the weight loss (in%) at any time can serve as a parameter to compare cultivars with respect to water loss behavior.

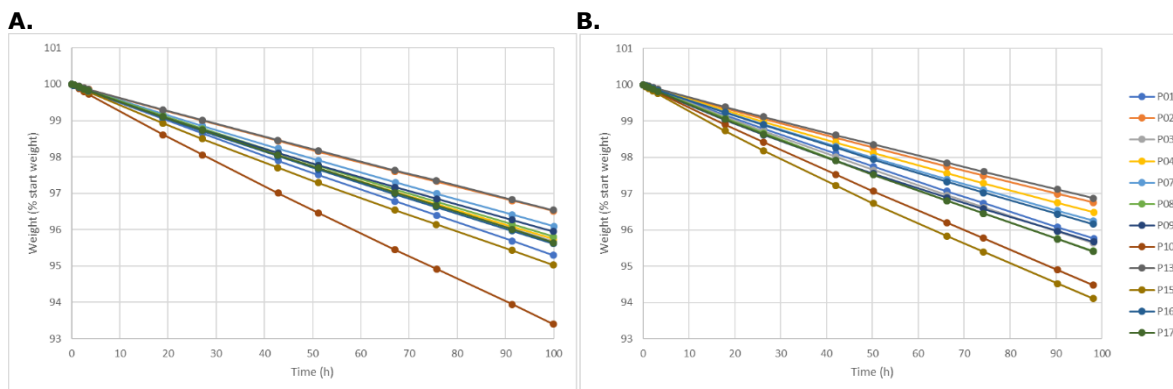


Figure 19 *Weight loss pattern of different cultivars (whole fruit, in %) during 2 harvests in the first 100 hours. A) first harvest, B) second harvest. Line shows the best linear fit through the measurement points .*

During the first 4 days of the experiment, weight loss accumulated to approximately 4-5%. Weight loss (in % of start weight of whole fruit) showed slight differences between cultivars and between harvests (Figure 20A). The lowest %WL was found in the yellow blocky fruit P13, the highest %WL was found in red blocky fruit P10 (almost double that of P13). Weight loss corrected for pericarp ratio shows a comparable picture as weight loss per whole fruit Figure 20B. We assume that the calculated %WL after correction is more likely presenting the “level of water stress” in the pericarp (and thus the expected quality loss in pericarp). Transpiration, defined as the loss of water (in g) per area unit, is strongly related to the difference in water vapor pressure between the tissue and the outside air and, in addition to the resistance of the peel (Figure 20C). Transpiration showed marked differences between cultivars, with a low value for P17 and highest values for P1, P8 and P10.

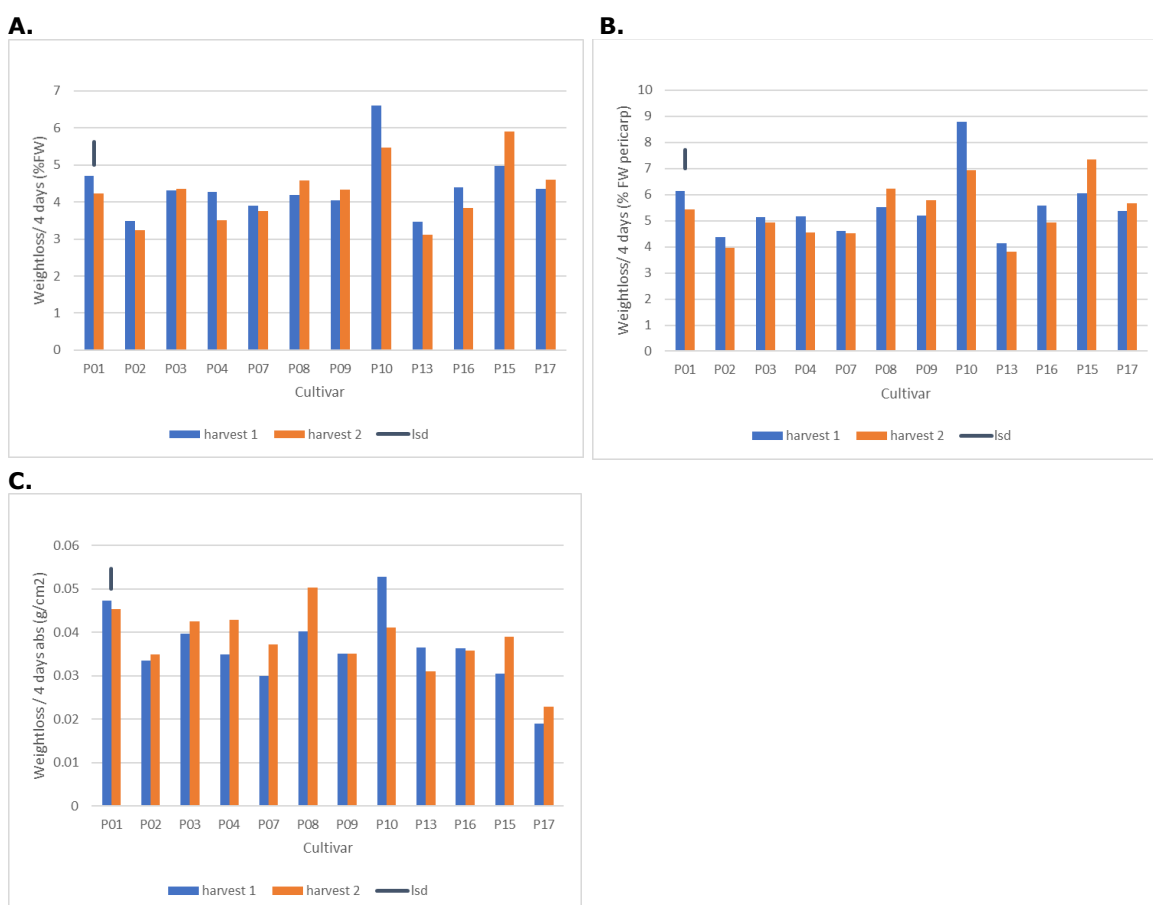


Figure 20 *Weight loss during desiccation of different cultivars of 2 harvests during 4 days. A) %WL of whole fruit at day 4. B) %WL corrected for ratio pericarp/whole fruit, C) Absolute weight loss in g/cm² pericarp/4 days (=Transpiration). Result of the post hoc test to show significant differences can be found in Annex 2 Table 15; Table 16 and Table 17.*

3.2.3 Correlations between initial water loss behaviour and initial parameters

Above data show the variation between cultivars in different initial parameters and in the behavior during desiccation under standardized conditions. An analysis has been done to link the initial parameters with the %WL.

In this analyses, the %WL after 4 days of desiccation was used as the parameter to be explained. Both the %WL of whole fruit and the corrected %WL of pericarp was used. The latter probably better reflects the level of water stress in the pericarp and thus the expected quality problems (shriveling, softening). The analyses were done for both harvests separate and for the harvests together; and for the separate groups: red blocky, yellow blocky, red + yellow blocky, conicals and all fruit together.

%WL fruit and %WL pericarp was correlated with the following initial parameters:

- FW of whole fruit
- FW of pericarp
- DM% of pericarp
- Pericarp thickness
- Specific fruit area based on dry weight (SFAdw in cm²/gDW)
- Specific fruit area based on fresh weight (SFAfw in cm²/gFW)
- Calculated area of whole fruit
- Transpiration (g water/cm² pericarp)

The results of the 2 harvests were very comparable (data not shown). Therefore the results are shown for the analyses where the 2 harvests were taken together (Table 5, Table 6).

Table 5 *Correlations between initial parameters and %WL of whole fruit. The analyses were done for red blocky, yellow blocky, red+yellow blocky, conicals and for all fruit together. Results from both harvests were taken together. Correlation coefficient R is given.*

	Red (blocky)	Yellow (blocky)	Red + yellow (blocky)	Conical	All	Data from measurement
Serie 1 + 2	%WL day 4	%WL day 4	%WL day 4	%WL day 4	%WL day 4	
FW Whole fruit (start g)	-0.27	-0.57	-0.29	0.11	-0.35	desiccation
FW Pericarp (g est.)	-0.27	-0.51	-0.29	0.18	-0.34	dessication
Thickness pericarp discs (mm)	-0.45	-0.56	-0.47	0.44	-0.42	disc initial material
SFA discs (cm ² /gDW)	0.32	0.44	0.10	-0.48	0.19	disc initial material
SFA' discs (cm ² /gFW)	0.48	0.62	0.50	-0.45	0.33	disc initial material
Area (cm ² via discs DW)	-0.12	-0.20	-0.14	-0.26	-0.17	dessication
Transpiration (g/cm ²)	0.74	0.67	0.75	0.87	0.53	dessication
DM pericarp (% FW)	0.37	0.67	0.42	-0.33	0.41	disc initial material
Sample size n	159	40	199	39	238	

Table 6 *Correlations between initial parameters and %WL of pericarp. The analyses were done for red blocky, yellow blocky, red+yellow blocky, conicals and for all fruit together. Results from both harvests were taken together. Correlation coefficient R is given.*

	Red (blocky)	Yellow (blocky)	Red + yellow (blocky)	Conical	All	Data from measurement
Serie 1 + 2	%WL day 4	%WL day 4	%WL day 4	%WL day 4	%WL day 4	
FW Whole fruit (start g)	-0.26	-0.45	-0.28	0.20	-0.31	dessication
FW Pericarp (g est.)	-0.33	-0.62	-0.36	0.19	-0.37	dessication
Thickness pericarp discs (mm)	-0.48	-0.62	-0.50	0.45	-0.41	disc initial material
SFA discs (cm ² /gDW)	0.30	0.50	0.08	-0.50	0.15	disc initial material
SFA' discs (cm ² /gFW)	0.51	0.68	0.53	-0.47	0.31	disc initial material
Area (cm ² via discs DW)	-0.19	-0.31	-0.22	-0.26	-0.23	dessication
Transpiration (g/cm ²)	0.79	0.82	0.81	0.90	0.61	dessication
DM pericarp (% FW)	0.49	0.72	0.48	-0.33	0.45	disc initial material
Sample size n	159	40	199	39	238	

In red and yellow blocky fruit there are clear **positive** correlations between %WL on the one hand and transpiration and SFAfw (cm²/gFW) on the other hand. The %WL was **negatively** correlated with pericarp thickness (note that SFAfw and pericarp thickness are negatively correlated).

In conical fruit %WL was **positively** correlated with transpiration and pericarp thickness, but **negatively** with SFAfw. This seems to indicate that in the blocky fruit high %WL is caused by a **higher transpiration and lower pericarp thickness**; in the conicals high **%WL is caused by high transpiration and higher pericarp thickness**. These results seem to be contradictory. It may be caused by the limited number of samples available in the conicals.

In the blocky fruit, a weak positive correlation was seen between pericarp DM% and %WL, in conicals this was the other way around. Again, this may be caused by the limited number of samples available in the conicals.

In general correlations were a little bit better when initial parameters were correlated with %WL of only the pericarp than with %WL of the whole fruit. Correlation became worse when more different types of fruit (red blocky, yellow blocky, conical) were clustered together.

No correlations were found between %WL and fruit FW, pericarp FW, fruit area and SFA_{dw}.

Below, the correlations between %WL (of pericarp) and the individual parameters are graphically presented (Figure 21). Because of the seemingly different behavior of conical compared to blocky, correlations are shown separate for blocky fruit and conicals.

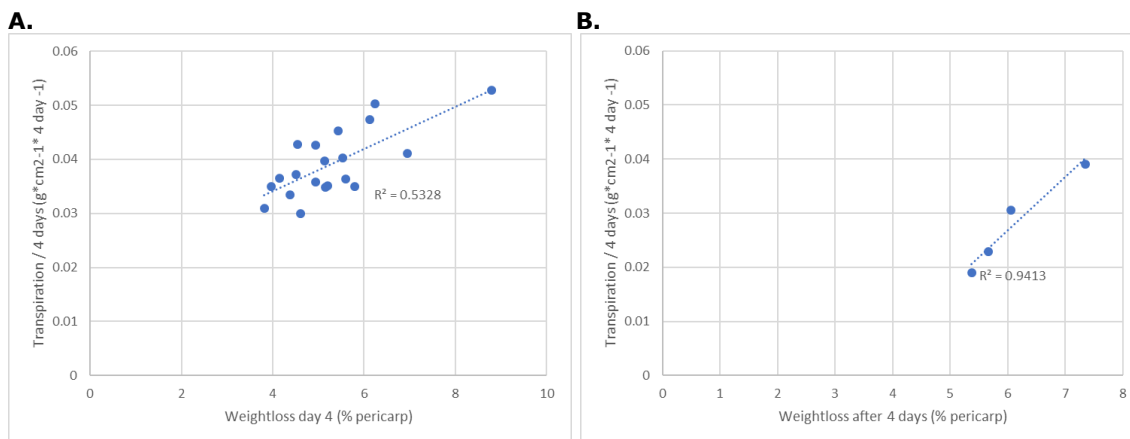


Figure 21 *Correlation between pericarp %WL and Transpiration. A) red and yellow blocky fruit, B) conicals.*

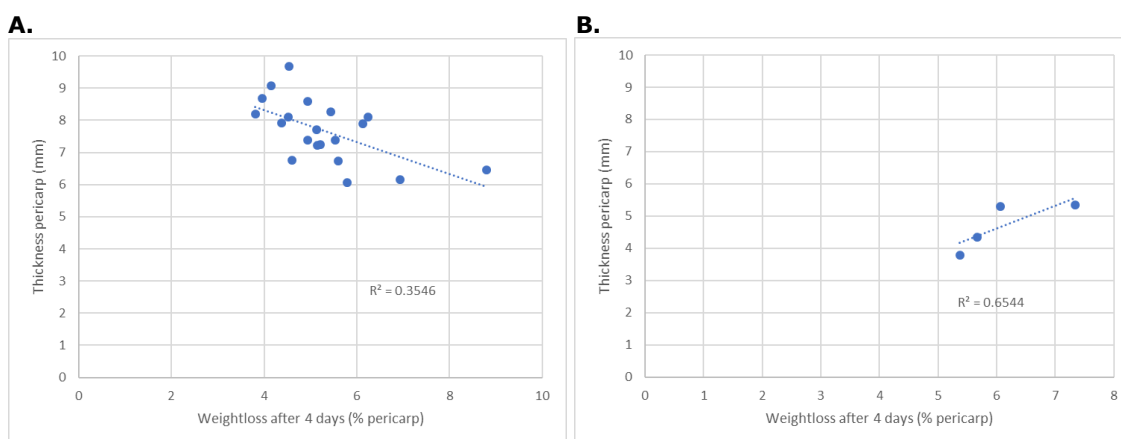


Figure 22 *Correlation between pericarp %WL and pericarp thickness. A) red and yellow blocky fruit, B) conicals.*

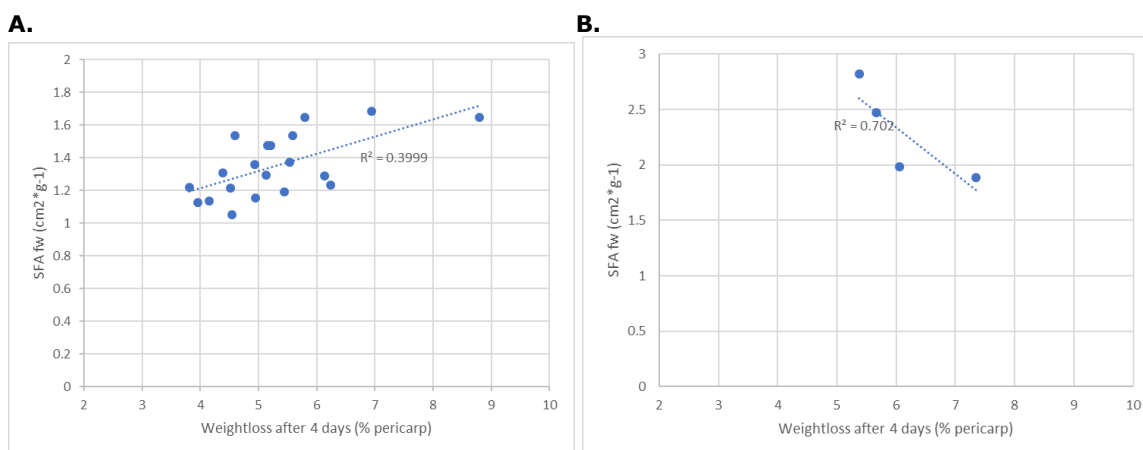


Figure 23 *Correlation between % WL and SFA fw. A) red and yellow blocky fruit, B) conicals.*

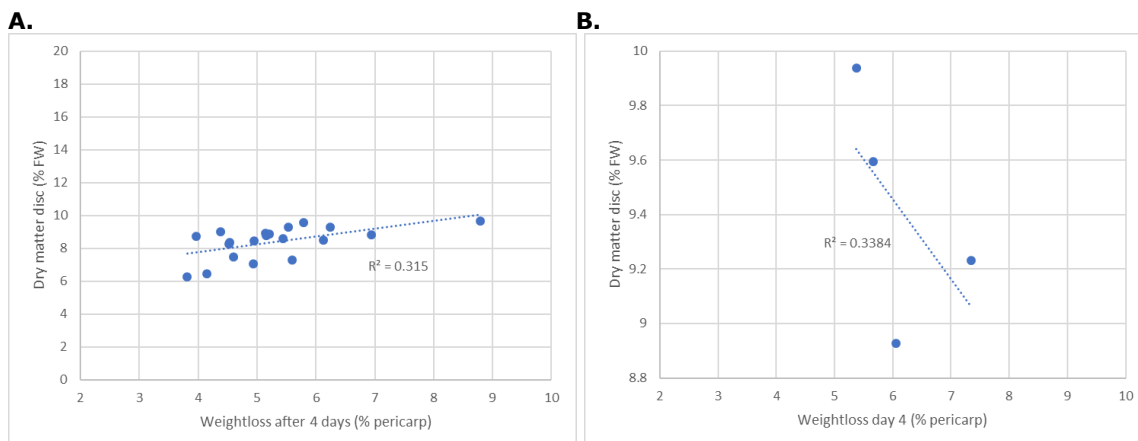


Figure 24 *Correlation between pericarp %WL and pericarp %DM. A) red and yellow blocky fruit, B) conicals.*

The %WL of the pericarp is considered a function of the transpiration (g/cm^2) and the amount of water present in the pericarp (represented by pericarp thickness). Figure 25 shows the correlation between %WL pericarp and "Transpiration/thickness". We observed a clear dependence of the %WL on both the transpiration ($\text{g}/\text{cm}^2/4$ days) and the thickness of the pericarp.

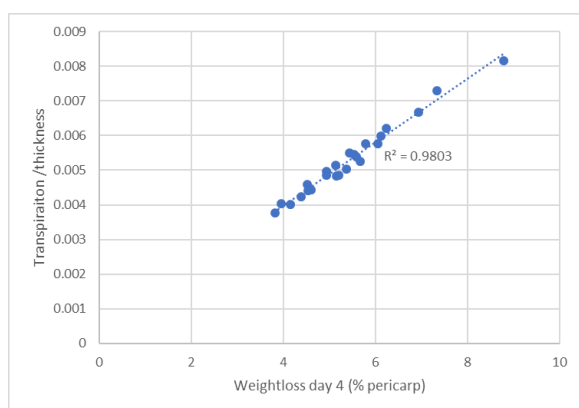


Figure 25 *Correlation pericarp %WL and "Transpiration/thickness".*

Theoretically the amount of osmotic substances can also affect the transpiration and hence the %WL. A high DM% of the pericarp might therefore indicate that there are, apart from the structural sugars (presumably about 4%), also soluble sugars. It seems that the yellow blocky peppers may contain less sugars compared to the other pepper types (not experimentally verified). However, in general, there is no tendency that high DM% would be associated with less %WL (Figure 24). If any, the association may be the other way around.

3.2.4 Quality parameters

In Figure 26 the average scores on overall quality and firmness are provided per cultivar, per harvest. Already after 7 days there are a few cultivars with below acceptable ($< \text{mark } 6$) in harvest 1: P01 and P10, P16 and P17. P15 still scores a 6 for firmness, but overall quality is < 6 . In harvest 2 the same cultivars show these low acceptable scores, this time including cultivar P7 and P8. After 14 days only P2 from harvest 1 still scores just acceptable (6).

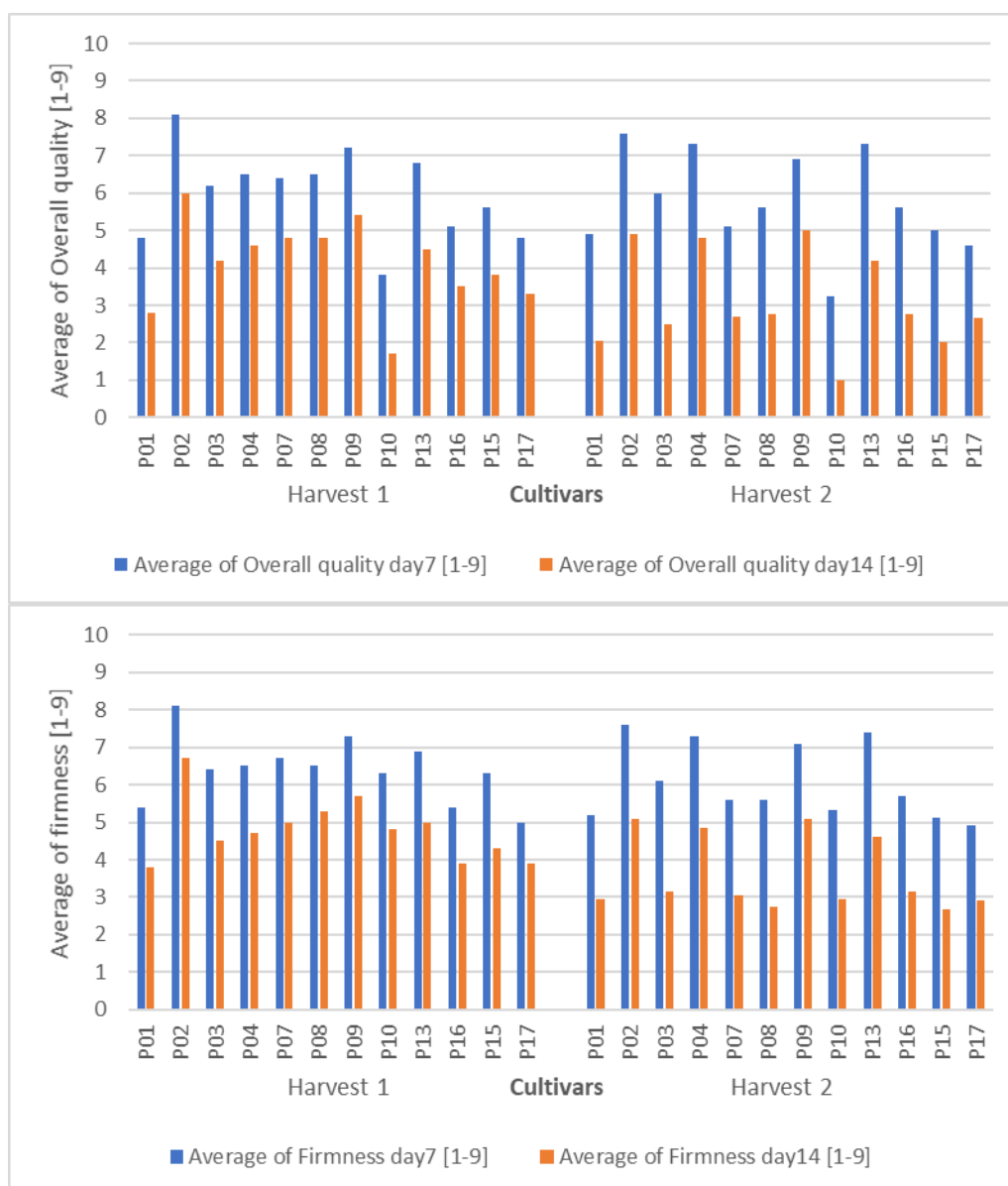


Figure 26 *The average scores on overall quality (upper panel) and firmness (lower panel) are provided per cultivar, per harvest evaluated after 7 days and after 14 days. N =10*

Apart from firmness loss, appearance of wrinkles and pitting was sometimes a reason for low quality scores. Figure 27 and Figure 28 show the results of the counting of peppers with these symptoms. Pitting was a severe issue in cultivar P10 in both harvests. Cultivars P1, 2, 3, 4, and 7 had some peppers with pitting. The cultivars with most peppers with wrinkles were P1, 3, 15, 16 and 17. Cultivars P2, 4, 10 had lowest numbers regarding wrinkles.

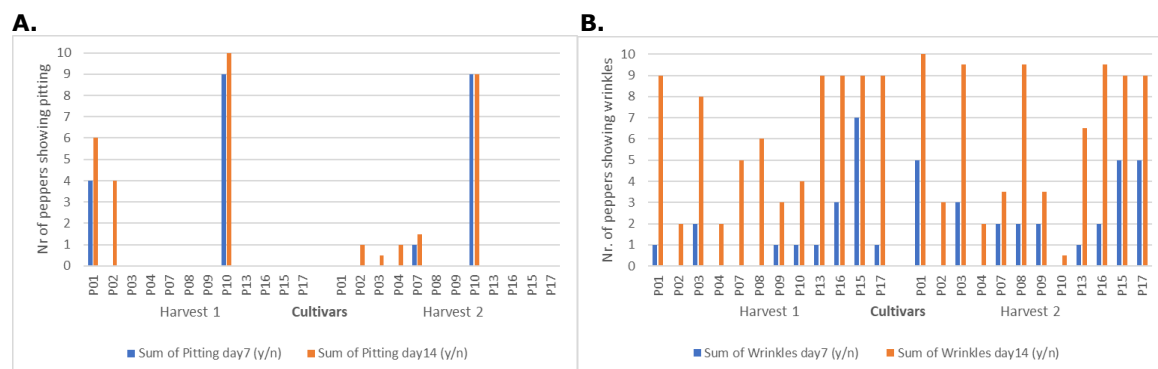


Figure 27 *Number of peppers showing pitting (A) or wrinkling (B) per cultivar per harvest*

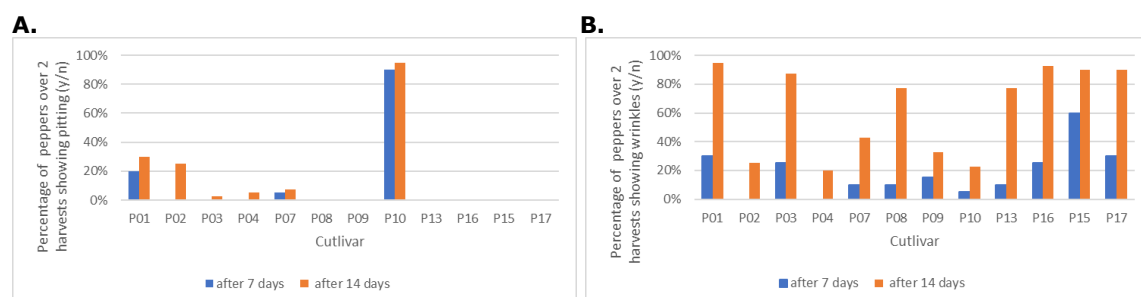


Figure 28 *Percentage of total measured peppers over 2 harvests showing pitting (A) or wrinkling (B) at day 7 and day 14.*

3.2.5 Correlations between quality parameters and water loss.

To determine the importance of water loss in relation to quality, the %WL was correlated the quality attributes (determined at several timepoints). The most relevant are shown in below table. The analyses shows that there are clear negative correlations between %WL on the one hand and both quality scores and firmness scores on the other hand. In Figure 29 the correlations are shown graphically. The cv P10 (red blocky) showed disease development after some days of shelf life; quality scores in P10 were very much influenced by these issues that apparently have no direct connection to water loss. P10 was therefore not included in the correlation graphs.

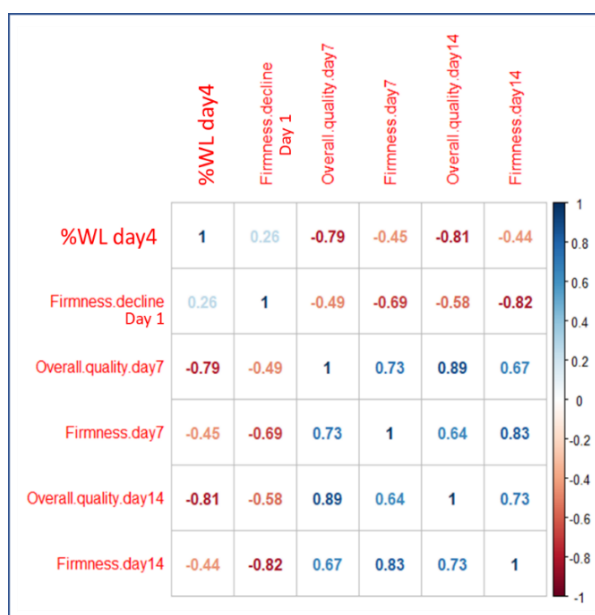
In the blocky peppers, correlations were higher when %WL was correlated to quality and firmness values measured after 1 week than after 2 weeks. In conicals this was the other way around (data not shown). Firmness scores generally correlated well with quality scores (Figure 29C). This is expected as firmness loss was the pivotal attribute of the overall quality.

Table 7 *Correlations between %WL (whole fruit) and Quality features of the fruit. Analyses was carried out on 2 harvests taken together, on red blocky, yellow blocky, red + yellow blocky, conicals and on all fruit together (A, upper panel). Correlation matrix all fruit from 2 harvests (B, lower panel)*

A.

Serie 1 + 2	
Red	
	% WL
% WL	1.00
Firmness decline after one day	0.21
Overall quality D7	-0.70
Firmness D7	-0.46
Overall quality D14	-0.65
Firmness D14	-0.38
Yellow	
	% WL
% WL	1.00
Firmness decline after one day	0.15
Overall quality D7	-0.77
Firmness D7	-0.77
Overall quality D14	-0.61
Firmness D14	-0.56
R&Y	
	% WL
% WL	1.00
Firmness decline after one day	0.18
Overall quality D7	-0.70
Firmness D7	-0.48
Overall quality D14	-0.62
Firmness D14	-0.37
Conical	
	% WL
% WL	1.00
Firmness decline after one day	0.68
Overall quality D7	-0.64
Firmness D7	-0.51
Overall quality D14	-0.74
Firmness D14	-0.70
ALL	
	% WL
% WL	1.00
Firmness decline after one day	0.23
Overall quality D7	-0.70
Firmness D7	-0.52
Overall quality D14	-0.65
Firmness D14	-0.45

B.



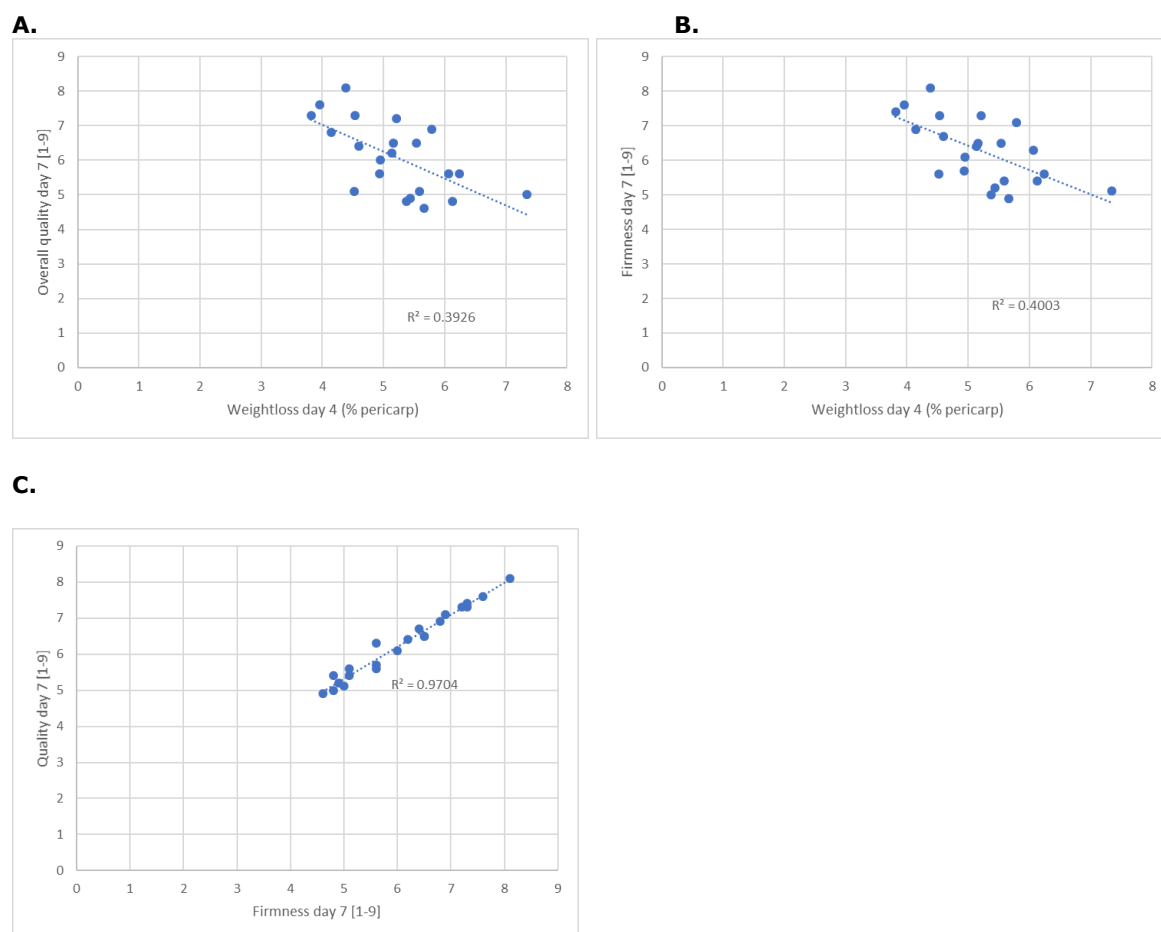


Figure 29 *Correlations between %WL of the pericarp after 4 days with overall quality and firmness ratings. A) %WL and Overall quality day 7, B) %WL and firmness d7, C) Correlation between quality and firmness on day 7. Two outliers were discarded (P10-1 and P10-2)*

3.2.6 Microscopic observations

(Fluorescence) microscopy on epidermal imprints and epidermal strips showed that the bell peppers did not have stomata. Occasionally some lenticels were seen. Also some cultivars showed clearly a greater amount of “cracks”. The influence of the cracks on water loss and quality has not yet been quantified.

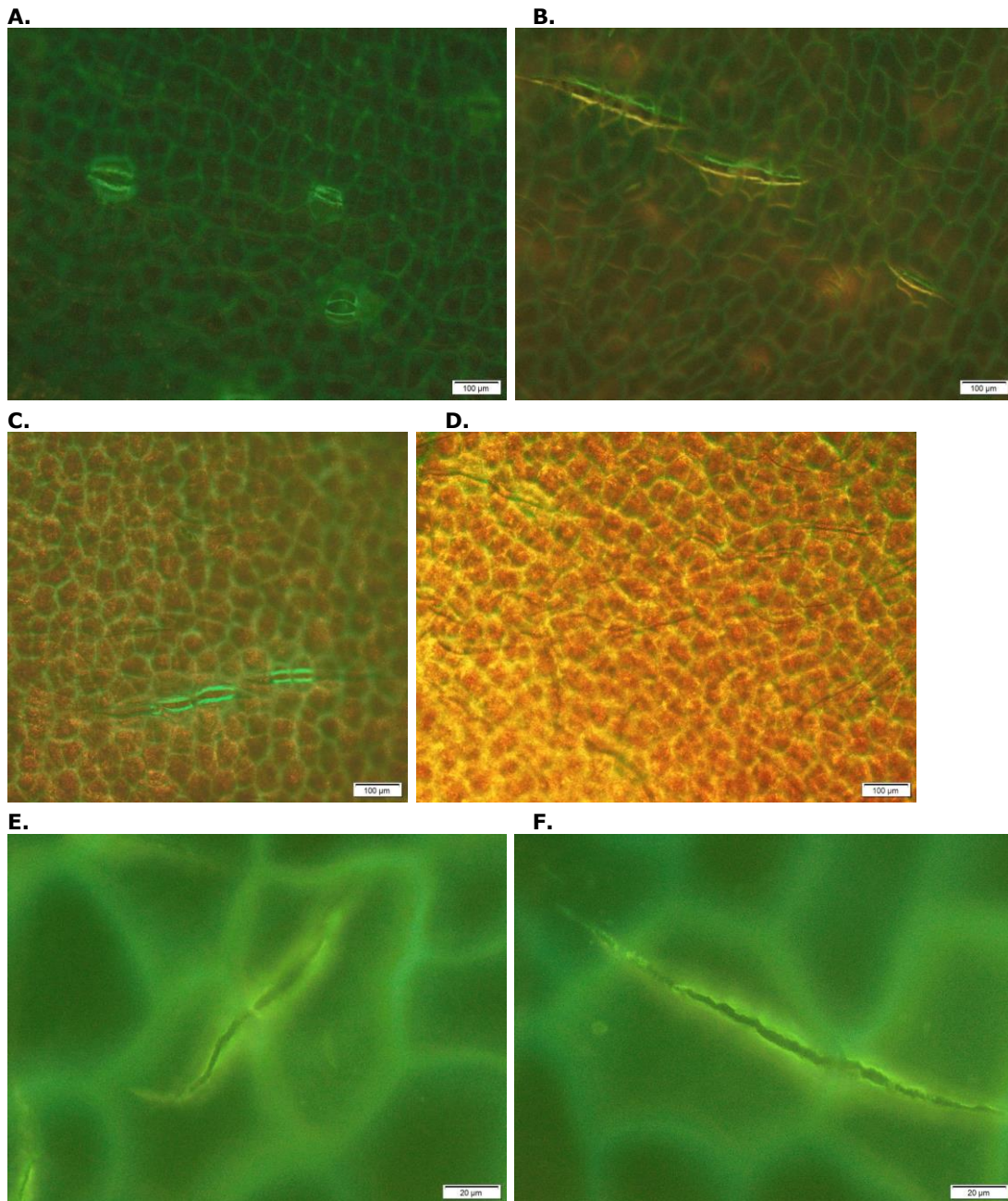


Figure 30 *Microscopic images from red bell peppers. A) individual lenticels, B and C) rows of connected lenticels that form a "crack", D) superficial cracks in cuticle, E and F) superficial cracks in higher magnification*

4 Conclusions & Discussion

Water loss is detrimental for fruit quality. Therefore cultivars with minimal water loss are of interest for breeders, growers, logistics up till consumer level. Several experiments were performed to develop a reliable and semi-throughput method to measure sub-traits related to water loss. The above described results indicate that pericarp %WL is the parameter that is responsible for visual quality loss and loss of fruit firmness. %WL in all fruit was strictly linear over the duration of the dehydration experiment (4 days). This indicates that water availability (free water) is not a restriction to transpiration. It also indicates that the resistance of the peel is not changing during the experiment. Experiments with a larger population also showed that water loss is linear, even over a period of 2 weeks (See Part B Peppers WP2). Within a period of 4 days, the %WL of the pericarp was about 5% on average. Differences between the genotypes were small, but the lowest %WL was observed in P2 and P13 (about 4%) and highest in P10 (around 8%). In general the fruit from the 2nd harvests responded similar to the dehydration treatment.

%WL is highly dependent on 2 parameters: the transpiration (g water loss/cm²) and the thickness of the pericarp. **A thicker pericarp contains more water and at a given transpiration rate it will take longer before a situation of water stress occurs.** All other measured parameters (also called sub-traits) like fruit weight, fruit area, DM% showed little or no correlations with %WL and are considered of minor importance in the explanation of differences in %WL.

Pericarp thickness

The tested cultivars vary in pericarp thickness, with the conicals P15 and P17 having a thin pericarp while the yellow blocky P13 has a thick pericarp. Pericarp thickness may also differ between harvests.

Pericarp thickness can be measured directly with a caliper. Pericarp weight/area is also an indication of its thickness (or water content) and can be measured by weighing pericarp discs with a known area. Pericarp thickness may be a selection criteria.

Transpiration

The other, presumably most important, factor determining pericarp %WL is the transpiration. Transpiration showed marked differences between cultivars, with a low value for P17 and highest values for P01, P08 and P10.

Transpiration is mostly affected by the structure and composition of the cuticle and wax layer. In addition, it may be influenced by the existence of lenticels and/or cracks in the wax/cuticle layer. In the fruit that were tested, no stomata were observed, but some fruit had small numbers of lenticels and some fruits showed superficial cracks in the cuticle on microscopic images. Currently we do not know what the contribution of lenticels and cracks are to the total transpiration. It was observed but not quantified, that P10 showed vast amounts of superficial cracks. This is in line with its relatively high transpiration. Transpiration can be a trait to select for. In addition, measurement of the relative abundance of microcracks may be a trait to select for.

Several methodologies can be applied to measure transpiration of individual fruit. For leaf transpiration a porometer is often used and maybe such a device can also be used to measure transpiration of pericarp strips. This would immediately give a value of g water/cm² area/s. Alternatively a device/methodology to measure whole fruit transpiration can be constructed. After the transpiration measurement this needs an estimation of pericarp area. The latter can be done e.g. using computer vision technology.

Annex 1: Pepper weight loss



Figure 31 Average weight loss% of 6 different cultivars with (+) and without (-) grease

Annex 2. Statistical analysis

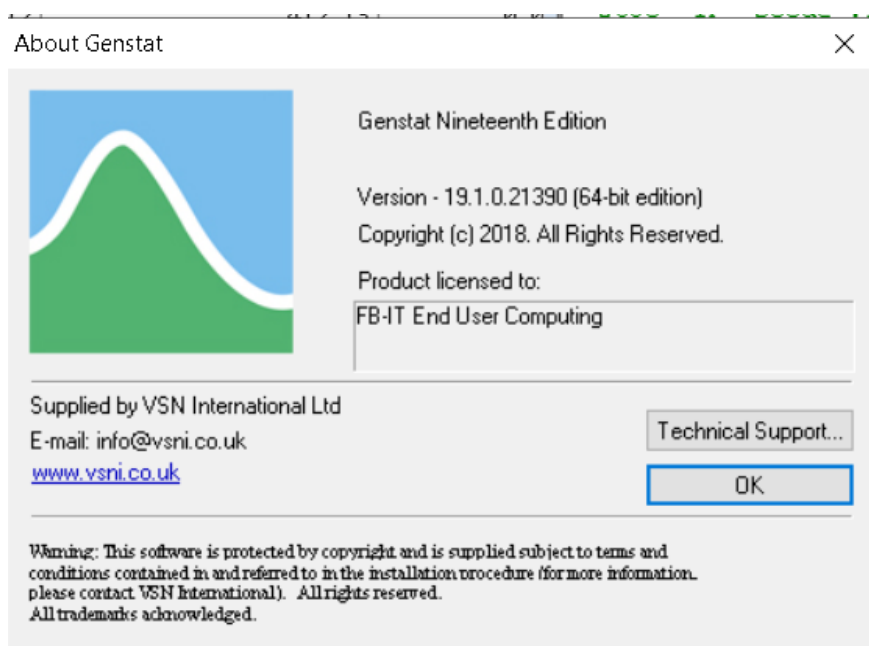


Figure 32 Information on used GenStat software

Table 8 ANOVA table, l.s.d. and Tukey post hoc test for the fresh weight (g) of starting material

ANOVA table													
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.							
CV	11		821732	74703	74.1	<.001							
Series	1		138913	138913	137.8	<.001							
CV.Series	11		93517	8502	8.43	<.001							
Residual	92	-124	92743	1008									
Total	115	-124	606326										
Least significant differences of means (5% level)													
Table	CV	Series	CV*series										
rep.	20	120	10										
d.f.	92	92	92										
l.s.d.	19.94	8.14	28.2										
Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)													
	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17	
mean	250.6	210.7	222.3	189.3	168.5	216.4	58	134.9	220.9	100.1	203.1	96.6	
	f	e	ef	de	cd	e	a	c	ef	b	e	b	
Means per c Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)													
CV	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17	
serie 1	213.9	173.2	180.4	145.6	115.1	175.4	59.5	115.4	229	95.3	190.9	88.8	
	fgh	ef	efg	cde	bcd	ef	a	bcd	gh	abc	efg	ab	
serie 2	287.3	248.2	264.1	233	221.9	257.4	56.5	154.3	212.8	104.9	215.3	104.3	
	i	hi	hi	gh	fgh	hi	a	de	fgh	abcd	fgh	abcd	

Table 9 *ANOVA table, l.s.d. and Tukey post hoc test for dry matter discs starting material*

Anova table						
Variate: DM_discs_%_FW						
Source of va	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
CV	11		104.6979	9.518	19.22	<.001
Serie	1		0.15	0.15	0.3	0.583
CV.Serie	11		6.1086	0.5553	1.12	0.354
Residual	92	-4	45.5536	0.4951		
Total	115	-4	152.0192			

Least significant differences of means (5% level)

Table	CV	Serie	CV
			Serie
rep.	10	60	5
d.f.	92	92	92
l.s.d.	0.625	0.255	0.884

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

	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
Mean	8.561	8.867	8.672	8.573	7.887	9.295	9.229	9.26	6.367	9.071	7.166	9.767
	d	ef	de	d	c	g	g	g	a	fg	b	h

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

CV	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
serie 1	8.52	8.99	8.89	8.77	7.51	9.29	8.88	9.65	6.46	8.93	7.29	9.94
serie 2	8.61	8.74	8.45	8.37	8.28	9.3	9.57	8.82	6.27	9.23	7.04	9.59

Table 10 *ANOVA table, l.s.d. and Tukey post hoc test for thickness pericarp starting material*

Analysis of variance																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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Table 11 ANOVA table, l.s.d. and Tukey post hoc test for SFA cm²/g FW of starting material

Anova table												
Variate: SFA_fw_cm2_g_FW												
Source of va	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.						
CV	11		19.66839	1.78804	109.61	<.001						
Serie	1		0.55345	0.55345	33.93	<.001						
CV.Serie	11		0.8535	0.07759	4.76	<.001						
Residual	92	-4	1.50073	0.01631								
Total	115	-4	22.57307									
Least significant differences of means (5% level)												
Table	CV	Serie	CV									
			Serie									
rep.	10	60	5									
d.f.	92	92	92									
l.s.d.	0.1134	0.0463	0.1604									
Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)												
	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
Mean	1.243	1.218	1.225	1.266	1.375	1.304	1.564	1.668	1.18	1.933	1.449	2.646
	ab	ab	ab	abc	bcd	abc	de	e	a	f	cd	g
Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)												
CV	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
serie 1	1.292	1.308	1.294	1.478	1.534	1.374	1.478	1.649	1.138	1.981	1.538	2.821
	abcde	abcde	abcde	cdefg	defg	bcdef	cdefg	fgh	ab	i	efg	k
serie 2	1.194	1.129	1.157	1.054	1.216	1.234	1.649	1.687	1.222	1.884	1.36	2.471
	abc	ab	ab	a	abc	abcd	fgh	ghi	abc	hi	bcdef	j

Table 12 ANOVA table, l.s.d. and Tukey post hoc test for SFA (cm²/g DW) of starting material

Anova table												
Variate: SFA_cm2_g_DW												
Source of va	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.						
CV	11		1696.226	154.202	71.89	<.001						
Serie	1		57.37	57.37	26.74	<.001						
CV.Serie	11		138.119	12.556	5.85	<.001						
Residual	92	-4	197.351	2.145								
Total	115	-4	2055.46									
Least significant differences of means (5% level)												
Table	CV	Serie	CV									
			Serie									
rep.	10	60	5									
d.f.	92	92	92									
l.s.d.	1.301	0.531	1.84									
Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)												
	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
mean	14.57	13.75	14.15	14.76	17.58	14.07	16.98	18.19	18.62	21.32	20.33	27.09
	a	a	a	a	b	a	b	bc	bc	d	cd	e
Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)												
CV	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
serie 1	15.2	14.54	14.56	16.85	20.48	14.79	16.68	17.25	17.61	22.25	21.1	28.37
	abcde	abcde	abcde	cdef	gh	abcde	bcdef	defg	efg	h	h	i
serie 2	13.94	12.96	13.73	12.66	14.68	13.35	17.27	19.12	19.62	20.4	19.55	25.81
	abcd	a	abc	a	abcde	ab	defg	fgh	fgh	gh	fgh	i

Table 13: ANOVA table, l.s.d. and Tukey post hoc test for area pericarp (cm²) of starting material based on SFA DW

Anova table												
Variate:	Area_pericarp_startmaterial_cm2_											
Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.						
CV	11		535719.3	48701.8	58.04	<.001						
Series	1		70660.6	70660.6	84.21	<.001						
CV.Series	11		61912.8	5628.4	6.71	<.001						
Residual	92	-124	77200.4	839.1								
Total	115	-124	401634.4									
Least significant differences of means (5% level)												
Table	CV	Series	CV*series									
rep.	20	120	10									
d.f.	92	92	92									
l.s.d.	18.19	7.43	25.73									
Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)												
	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
mean	265.7	224.8	238.9	198.9	205.3	247.9	78.4	195.9	228.3	172.2	249.6	224.9
	f	cde	ef	bcd	cd	ef	a	bc	de	b	ef	cde
Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)												
CV	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
serie 1	235.6	198.1	209.3	187.4	162.1	215.9	77.4	163.5	231.9	168.1	252.9	222.8
	efghi	bcdef	bcdefg	bcde	b	cdefg	a	b	efghi	bc	ghij	defgh
serie 2	295.9	251.5	268.6	210.5	248.6	279.9	79.5	228.3	224.6	176.3	246.2	227
	j	ghij	hij	bcdefg	ghij	ij	a	efgh	defgh	bcd	fghi	efgh

Table 14 ANOVA table, l.s.d. and Tukey post hoc test for pericarp % of whole fruit of starting material

Anova table																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
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Table 15 ANOVA table, l.s.d. and Tukey post hoc test for weight loss day 4 (%FW)

ANOVA table												
Source of va	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.						
CV	11		133.8528	12.1684	25.02	<.001						
Series	1		1.2397	1.2397	2.55	0.112						
CV.Series	11		17.1428	1.5584	3.2	<.001						
Residual	214	-2	104.0673	0.4863								
Total	237	-2	252.4333									
Least significant differences of means (5% level)												
Table	CV	Series	CV*series									
rep.	20	120	10									
d.f.	214	214	214									
l.s.d.	0.435	0.177	0.615									
Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)												
	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
mean	4.471	3.369	4.341	3.888	3.828	4.385	4.189	6.038	3.291	5.434	4.116	4.478
	b	a	b	ab	ab	b	b	c	a	c	b	b
Means per c							Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)					
CV	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
serie 1	4.704	3.49	4.321	4.263	3.905	4.19	4.046	6.598	3.46	4.969	4.387	4.359
	def	abc	bcde	abcde	abcde	abcde	abcde	h	abc	efg	bcdef	bcdef
serie 2	4.238	3.248	4.361	3.514	3.751	4.581	4.331	5.478	3.123	5.898	3.846	4.596
	abcde	ab	bcdef	abc	abcd	cdef	bcdef	fgh	a	gh	abcde	cdef

Table 16 *ANOVA table, l.s.d. and Tukey post hoc test for weight loss day45 (%FW corrected for %pericarp)*

Anova table												
Source of va	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.						
CV	11		257.4612	23.4056	35.71	<.001						
Series	1		1.6309	1.6309	2.49	0.116						
CV.Series	11		36.6128	3.3284	5.08	<.001						
Residual	214	-2	140.2663	0.6555								
Total	237	-2	429.9431									
Least significant differences of means (5% level)												
	CV	Harvest	CV. Harvest									
rep.	20	120	10									
d.f.	214	214	214									
l.s.d.	0.5046	0.206	0.7137									
Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)												
	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
mean	5.785	4.171	5.041	4.847	4.559	5.884	5.5	7.868	3.98	6.703	5.264	5.52
	e	ab	cde	bcd	abc	ef	de	g	a	f	cde	de
Means per c Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)												
CV	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
serie 1	6.13	4.383	5.137	5.156	4.601	5.531	5.207	8.796	4.147	6.059	5.592	5.373
	fgh	abcd	abcdef	bcdef	abcde	def	bcdef	i	abc	fgh	def	cdef
serie 2	5.439	3.96	4.946	4.539	4.517	6.236	5.792	6.941	3.813	7.347	4.935	5.667
	cdef	ab	abcdef	abcde	abcde	fgh	efg	gh	a	h	abcdef	defg

Table 17 *ANOVA table, l.s.d. and Tukey post hoc test for weight loss day 4 (g/cm2))*

ANOVA table												
Source of va	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.						
CV	11		0.011439	0.00104	38.18	<.001						
Series	1		0.000205	0.000205	7.51	0.007						
CV.Series	11		0.002215	0.000201	7.39	<.001						
Residual	214	-2	0.005828	2.72E-05								
Total	237	-2	0.019668									
Least significant differences of means (5% level)												
	CV	Harvest	CV. Harvest									
rep.	20	120	10									
d.f.	214	214	214									
l.s.d.	0.00325	0.00133	0.0046									
Means per cultivar and the outcome of the Tukey post hoc test (95% confidence intervals)												
	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
mean	0.04636	0.03424	0.04113	0.03884	0.03354	0.04526	0.03509	0.04697	0.03371	0.03476	0.0361	0.02097
	de	b	cd	bc	b	de	b	e	b	b	bc	a
Means per c Means per cultivar per harvest and the outcome of the Tukey post hoc test (95% confidence intervals)												
CV	P01	P02	P03	P04	P07	P08	P09	P10	P13	P15	P16	P17
serie 1	0.04738	0.03351	0.03966	0.03488	0.02993	0.04026	0.03514	0.05281	0.03644	0.03051	0.03634	0.01906
	hij	cde	efgh	cdef	bc	efgh	cdef	j	cdef	bcd	cdef	a
serie 2	0.04535	0.03496	0.04261	0.04281	0.03715	0.05026	0.03504	0.04113	0.03097	0.03902	0.03586	0.02288
	ghij	cdef	fghi	fghi	cdefg	ij	cdef	efgh	bcd	defgh	cdef	ab

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quality of life



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