Dynamics of ripening of avocado fruit

GreenCHAINge Fruit & Vegetables WP6 (BO-29-03-001-010)

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UNIVERSITY & RESEARCH

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Summary

A series of experiments was performed to gain more insight in the dynamics of ripening of avocado fruit as part of the GreenCHAINge Fruit & Vegetables program (GreenCHAINge). The main aim of work package 6 was to develop tools to better monitor the ripening of batches of fruit resulting in less variation and a higher percentage of fruit reaching the Ready to Eat (RTE) stage. In addition, it was investigated if the further ripening of RTE fruit could be slowed down to prolong the shelf life of ripe fruit.

It was found that the ripening speed of avocados depends on the initial firmness (at arrival) and on the ripening temperature. Using a mathematical model incorporating these parameters allows for better prediction of the ripening outcome. Ripening can be interrupted by bringing the fruit temporarily to a low temperature (e.g. 7°C); the firmness loss greatly slows down and will regain again if fruit are brought back to ripening temperatures. Different ripening scenarios, employing different time periods (2 to 4 days) at different temperatures (between 18 and 22°C) with or without a short period of cold storage interruption all result in RTE fruit without inducing internal quality defects.

In all the ripening experiments, heterogeneity within a batch and between batches was extremely high. Generally, after a given ripening period at temperatures between 18 and 22°C, only 50-60% of the fruit are within the perfect RTE range; at the same time part of the fruit are too firm, part of the fruit are too soft (overripe). Sorting the fruit, prior to ripening, in classes of different firmness lowered the firmness heterogeneity at the start of ripening but had no effect on heterogeneity at the RTE stage. Within each firmness class, individual fruit still show very unpredictable ripening behaviour. Pre-sorting into firmness classes appears not a viable strategy to lower fruit heterogeneity at the RTE stage. A hot water or hot air treatment prior to ripening did not affect the ripening rate and had no effect on heterogeneity of fruit at the RTE stage. Ethylene treatment during ripening slightly stimulated the softening rate and slightly lowered the heterogeneity of fruit at the RTE stage. Ethylene, however, also slightly increased the occurrence of internal defects.

The shelf life of RTE fruit can be prolonged by storing the fruit at low (e.g. 8°C) temperature. This has no negative effect on development of internal defects. Modified atmosphere packaging (MAP) did not prolong the shelf life of RTE fruit at 18°C.

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

1 Introduction

Tropical stone fruits such as avocado and mango are sourced from many different regions in the world. Generally they are harvested while still immature, transported for about 3 weeks in reefer containers and ripened at arrival to be marketed as Ready to Eat (RTE) fruit. A challenge is to assure that major part of the fruit do eventually reach the RTE stage. Due to fruit maturity heterogeneity within and between the batches at arrival, it is hard to determine the best ripening protocol.

Consequently this leads to major product losses because following a ripening process, some fruit are already overripe while some fruit are still too unripe. A common way to deal with the heterogeneity is the repeated measurement of the firmness of all fruit either by hand or using a sorting line equipped with Aweta acoustic firmness sensor. Following sorting into firmness classes, the fruit that are not yet RTE are brought back to the ripening facility to be measured again at a later time point.

A series of experiments was performed to gain more insight in the dynamics of ripening of avocado fruit as part of the GreenCHAINge Fruit & Vegetables program (GreenCHAINge). The main aim of work package 6 was to develop tools to better monitor the ripening of batches of fruit resulting in less variation and a higher percentage of fruit reaching the ready to eat stage. In addition, it was investigated if the further ripening of RTE fruit could be slowed down to prolong the shelf life of ripe fruit.

2 Materials and methods

Experiments were performed with Hass avocado fruit that we received after approximately 3 weeks of transportation. Transportation was by reefer container from Chile and Peru under low temperature (5-7°C), controlled atmosphere conditions (approximately 5% O_2 and 5% CO_2) and ethylene removal. Fruit firmness was measured with an Aweta acoustic firmness sensor. This apparatus measures firmness in a non-destructive way using acoustics. Firmness is expressed in $Hz^2 \cdot g^{2/3}$ (further referred to as Aweta units). There is a good correlation between firmness measured by Aweta and by limited compression. Freshly harvested fruit may have a firmness of about 60-70 Aweta units; during ripening, firmness decreases to 5-10 Aweta units.

The eating quality was expressed in a ready to eat (RTE) score from 1 to 5. A score of 1 means the fruit is still too unripe; a score between 2 and 4 means the fruit has just the right texture and is ready to eat; a score of 5 is too soft/overripe. The RTE scores were determined by specialists through judgement of texture and taste of the fruit. The RTE scores were correlated to Aweta values (Table 1). This showed that Aweta values between about 11 and 20 represent fruit that are RTE.

Table 1: Ready-To-Eat stage classification (source:WFBR)

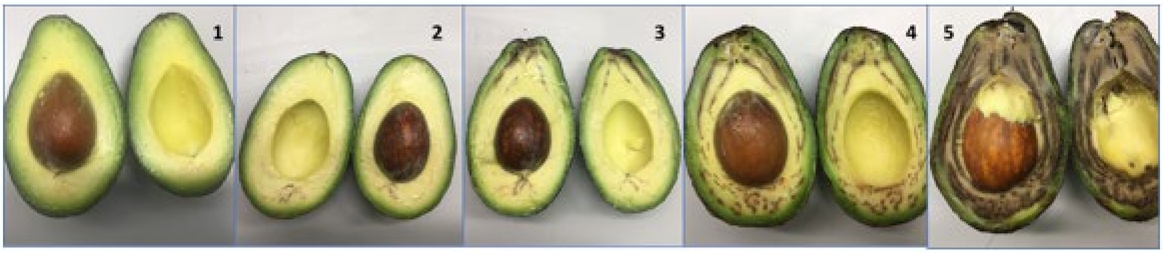
Class RTE	Description	Aweta value ($Hz^2.g^{2/3}$)
1	RTE not OK – too hard	>22
2	RTE OK, but still hard	18.3 – 22
3	RTE OK	11.3 – 18.3
4	RTE OK, but getting too soft	9 – 11.3
5	RTE not OK – too soft	<9

Internal quality of fruit was mostly determined at the end of the shelf life (fruit RTE score > 4; Aweta value < 10). Fruit were judged for occurrence of vascular or pulp browning, stem end rot and fungal infection (Figure 1).

The experimental set up, measurement parameters and results are described in more detail in WFBR reports (for internal use only). The current report summarizes the main findings and discusses the practical use of the findings for ripening and transport of avocados.

Flesh discoloration:

Vascular browning:



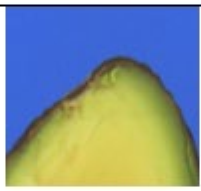
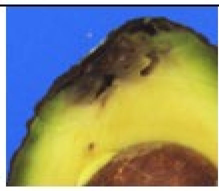
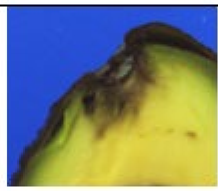
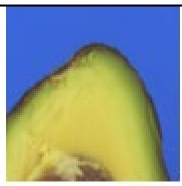
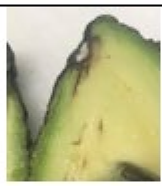
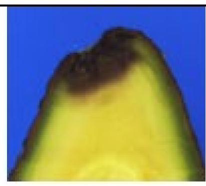
Item	Scale 1	2	3
Fungal on the stem/External Fungal (1-3)	No visible fungal on the stem		
Internal fungal (1-3)			
Stem rot (1-3)			
Sticky stone (1-2)			
Dehydration skin (1-3)	No visible dehydration skin		

Figure 1: Quality disorders scales (source: WFBR)

3 Results

3.1 Dynamics of ripening in relation to temperature and initial firmness

Avocado fruit were ripened at different temperatures and the ripening behaviour of fruit with different starting firmness was modelled. Firmness was regularly measured with Aweta firmness sensor.
It was found that fruit that were very firm at arrival needed more time to ripen (to reach the RTE firmness of about 10-20 Aweta units) than fruit that were less firm at arrival. However, the ripening speed (Aweta units firmness loss per day) was actually higher in the initially firm fruit than in the initially less firm fruit. This means that the ripening speed is dependent on the initial fruit firmness.
The temperature has a prominent effect on the softening speed, irrespective the initial firmness. There was a clear difference in softening speed between e.g. fruit that ripened at 18°C (average firmness loss = 3.7 Aweta units/day) and fruit that ripened at 22°C (average firmness loss = 6.9 Aweta units/day). Softening is severely suppressed at low temperatures. At 7°C firmness loss was almost completely stopped. The temperature effect was found to be reversible. When fruit are brought from high temperature to low temperature, the softening speed decreases but will increase again if the fruit are brought back to a higher temperature. This behaviour is very similar to what was earlier observed in mango ripening experiments. The formulas earlier developed for (prediction of) mango ripening can therefore also be applied to avocado ripening. If the initial firmness (firmness at arrival) of a number of fruit (50-100 fruit) is measured, a specific ripening temperature (between e.g. 18 and 22°C) can be chosen to have major part of the fruit ripe within a selected number of days.
Another implication of the findings is that the ripening can be stopped by bringing the fruit at any time during the ripening to a low temperature (e.g. 7°C). This may facilitate transport of partly ripe fruit to final destination. An example is given in Figure 2. Fruits were exposed to 18, 20 and 22°C for 4 days and thereafter brought back to storage temperature of 7°C for 2 days. Thereafter fruit were brought to ripening (retail) temperature of 18°C.

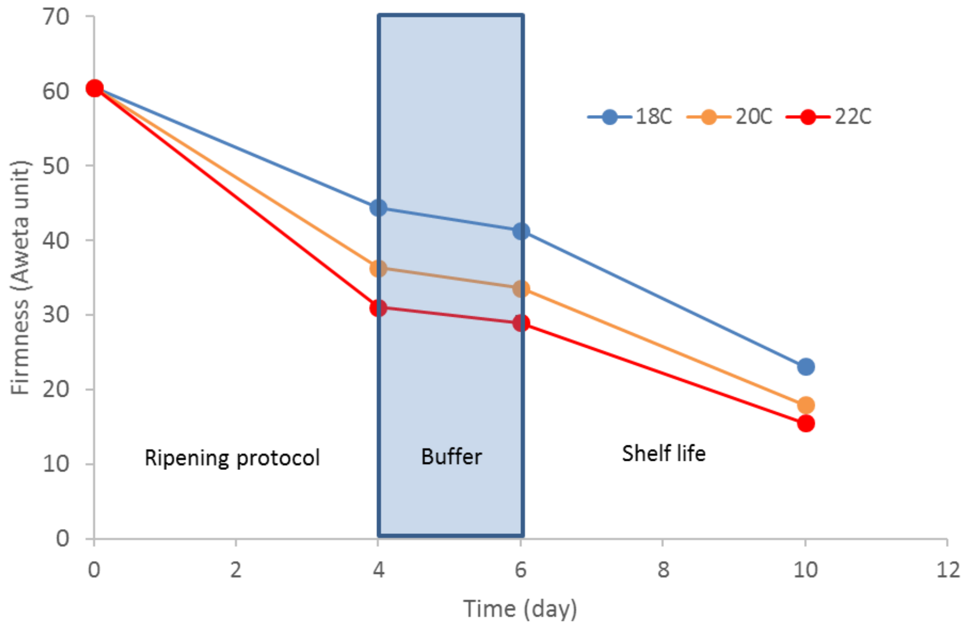


Figure 2: Average firmness of three avocado batches subjected to ripening protocol (4 days at 18, 20 and 22°C – 85% relative humidity) followed by two days buffer (7°C) and 4 days shelf life simulation at 18°C and 60% relative humidity. (n=100).

All the different ripening scenarios (ripening durations and temperatures) yielded fruit of good quality that were ready to eat (Figure 3). The abundance of internal defects such vascular and pulp browning increases along with the ripening but was in itself not affected by the ripening temperature. This indicates that ripening operations can use different ripening temperatures and can also temporarily stop the ripening of a batch of fruit to suit the required delivery time of their customers without negative effects on product quality.

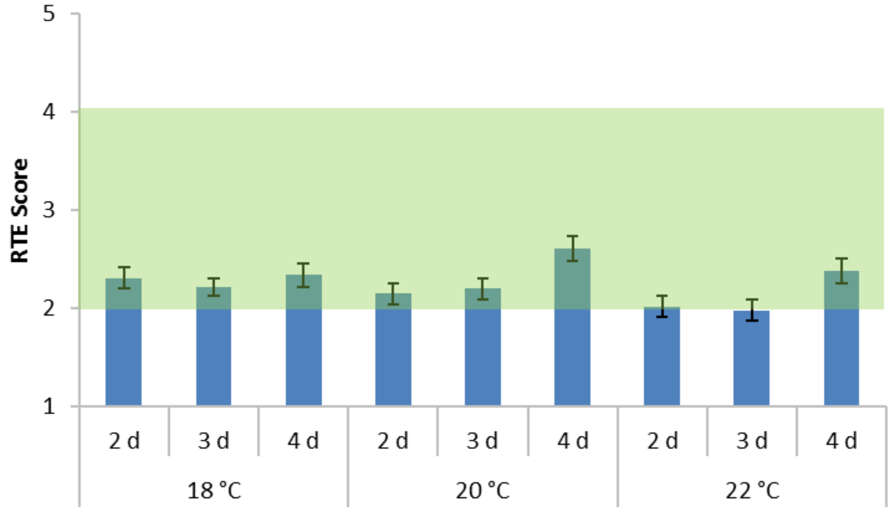


Figure 3: Ready-to-Eat (RTE) score for fruit from different ripening protocols determined at the end of a four days "shelf life" period at 18°C. Fruit were ripened at 18, 20 and 22°C for 2, 3 or 4 days. Thereafter fruit were stored for 2 days at 7°C, followed by 4 days at 18°C as shelf life simulation. The green area schematizes the optimum RTE scores. Error bar represents the standard error (n=100).

It should be noted, that there was in all ripening scenarios heterogeneity. Although on average the batch of fruit reached the RTE score of 2, within a batch about 40% of the fruit were not yet RTE (too firm) and about 10% was at the upper limit of acceptability (too soft/overripe) (Figure 4). Thus, only about 50% of the fruit were "just right". This heterogeneity is often observed in the ripening of tropical stone fruits (avocado, mango) and is a major drawback in the delivery of ready to eat products.

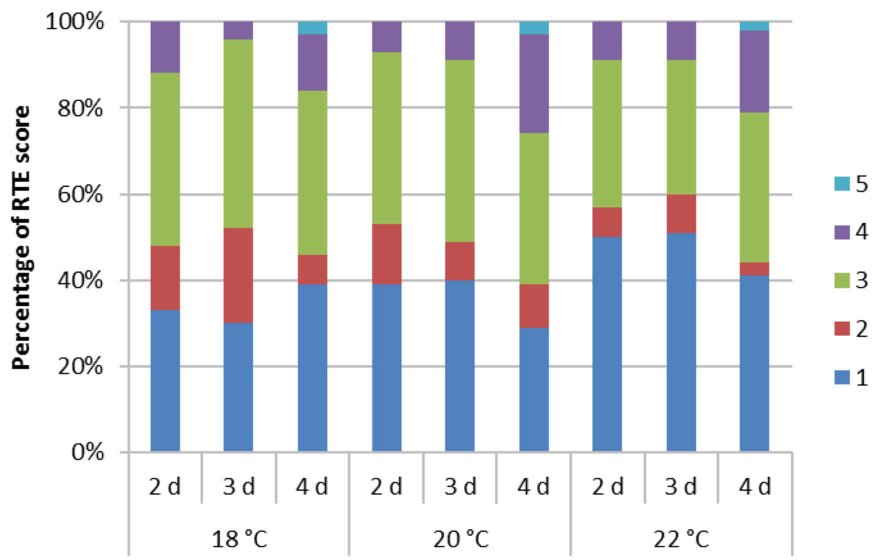


Figure 4: Distribution of RTE scores (RTE scores of 1 – 5) within avocado batches at the end of a four days shelf life period at 18°C. Fruit were ripened at 18, 20 and 22°C for 2, 3 or 4

days. Thereafter fruit were stored for 2 days at 7°C, followed by 4 days at 18°C as shelf life simulation. (n=100).

3.2 Ripening heterogeneity and methods to reduce heterogeneity

Already at harvest there is a huge difference in the firmness of individual fruit. When judged by hand, fruit are all "rock hard" but instrumental measurements show that firmness may vary between e.g. 30 and 70 Aweta units. During ripening (at a given temperature) initially firm fruit generally show faster softening than initially soft fruit. Still, it takes longer for firm fruit to reach the RTE stage than for the softer fruit. This explains that there is quite some heterogeneity following a fixed ripening period. The current solution to this problem is to apply a certain ripening protocol (e.g. 4 days at 20°C), than sort out the ripe fruit and bring the unripe fruit back in the ripening room for some days. This cycle can be repeated. This is an elaborate procedure in which the fruit have to be sorted either by hand or using sorting equipment. It is labour intensive and may lead to extra bruising of the fruit and quality loss. We investigated the evolution of firmness heterogeneity during the ripening process and tested several concepts to reduce firmness heterogeneity.

3.2.1 Sorting fruit into different firmness classes before ripening

There is huge variation in firmness of individual fruit at arrival, and there is a difference in the speed of ripening between initially firm and initially less firm fruit. Therefore, it seems a logical step to divide a batch of fruit into different firmness classes and to apply a different ripening protocol to each firmness class. Using Aweta, the firmness of all fruit was measured at arrival. Based on this, three firmness classes were made: soft = 20-40 Aweta units; medium = 40-50; hard = 50 and up. The different sub-batches were ripened at 18 and 22°C. A reference batch was made that contained equal amounts of fruit of each firmness class. The experiments confirmed that fruit at 22°C show a higher softening speed than fruit at 18°C and that softening speed of initially hard fruit is higher than of initially softer fruit (Figure 5). In this experiment there was quite some synchronisation of the ripening and, despite the differences in starting firmness of the sub-batches, at a given temperature, fruit reached the RTE firmness approximately at the same time (Figure 6).

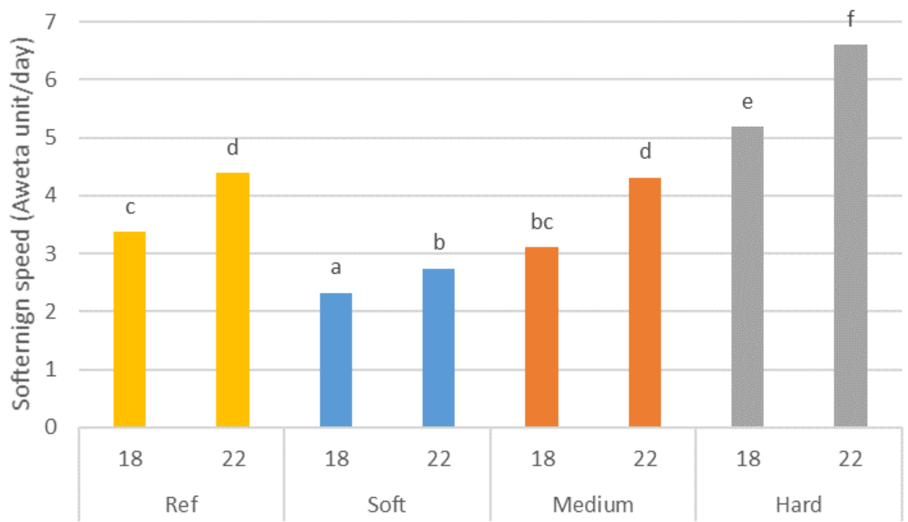


Figure 5: Softening speed measured during the ripening period per batch (Reference, Soft, Medium, Hard batches) and at 2 ripening temperatures (18 and 22°C). Bars with different letters are significant different from each other according to Fisher's protected LSD test (P<0.05, n=90).

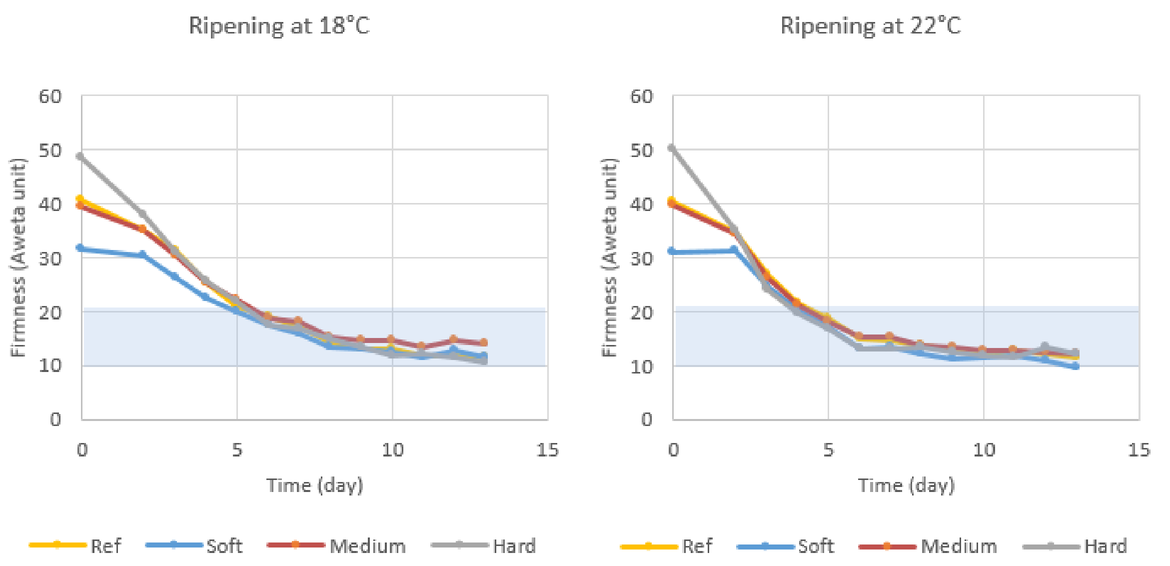


Figure 6: Average firmness per batch measured daily during the 4 days ripening at 2 temperatures (18 and 22°C) and shelf life period (18°C & 60% relative humidity). Blue area represents the ready to eat firmness. (n=80).

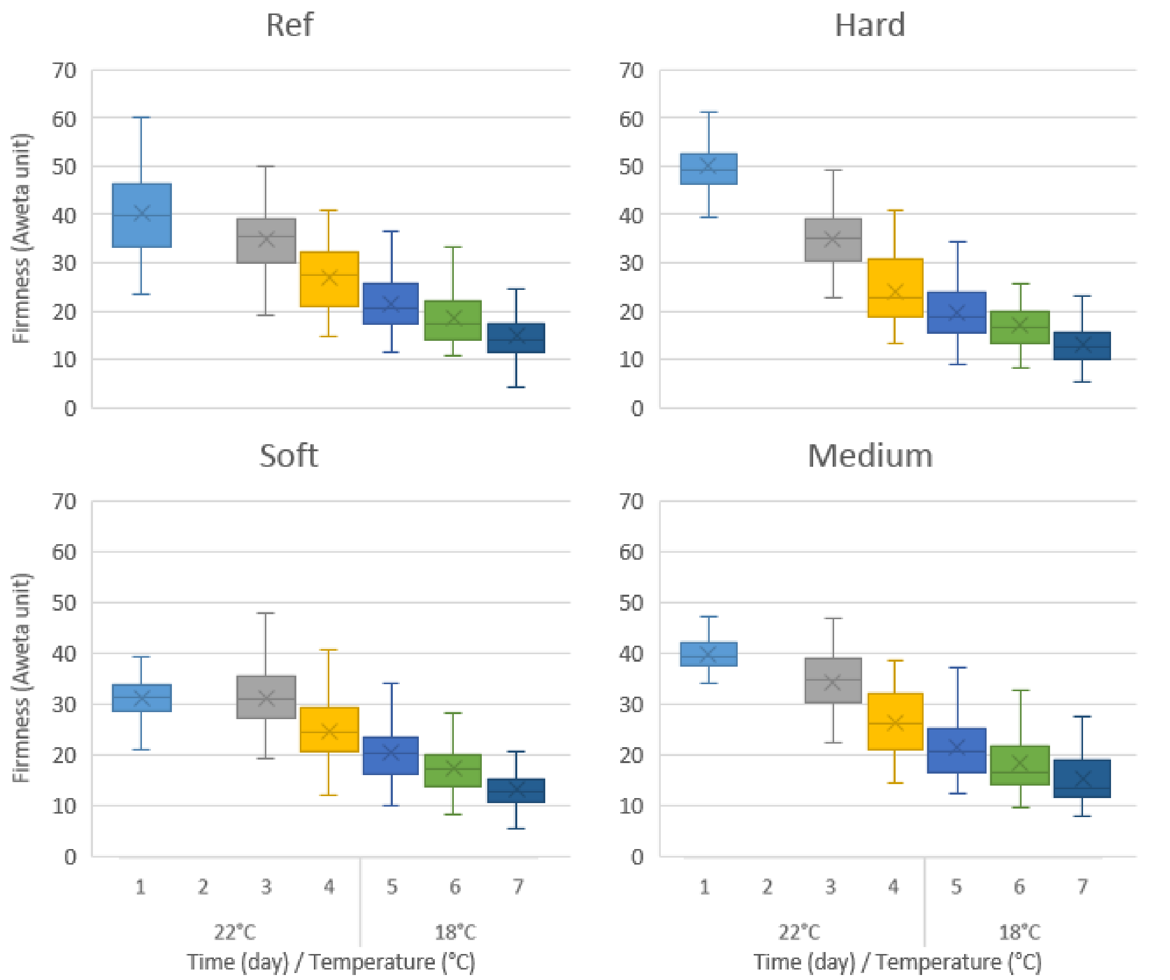


Figure 7: Boxplot representation of firmness measurements of avocado batches. Prior to ripening the fruit were divided into four batches: Reference, Soft, Medium and Hard on basis of initial fruit firmness. Avocados were ripened at 22°C for 4 days and stored at 18°C for shelf life simulation (n=90).

The effect of the pre-sorting in firmness classes on heterogeneity during ripening at 22°C is shown in the different boxplots Figure 7. Per evaluation day, the coloured square represents the firmness range of 50% of the fruit batch; the bars beneath and above the coloured square indicate the range of firmness of the 25% most soft and 25% most firm fruit, respectively. As expected, compared to the reference batch, pre-sorting significantly reduces the heterogeneity of the sub-batches at the start of ripening. However, this beneficial effect is not maintained during the ripening. At the time fruit are RTE, heterogeneity is similar in all sub-batches. This was also observed during ripening at 18°C (data not shown).

The increase in heterogeneity during ripening is also shown in Figure 8. Here the firmness distributions are shown for the reference batch and the medium firmness class on day 0, 4 and 7 during the ripening at 18°C. Heterogeneity at day zero is clearly less in the medium firmness class compared to the reference. At day 4 and 7, the medium firmness class and the reference show similar distribution of firmness. It means that within each firmness class, the individual fruit still develop such a degree of variability that pre-sorting in this way is not effective.

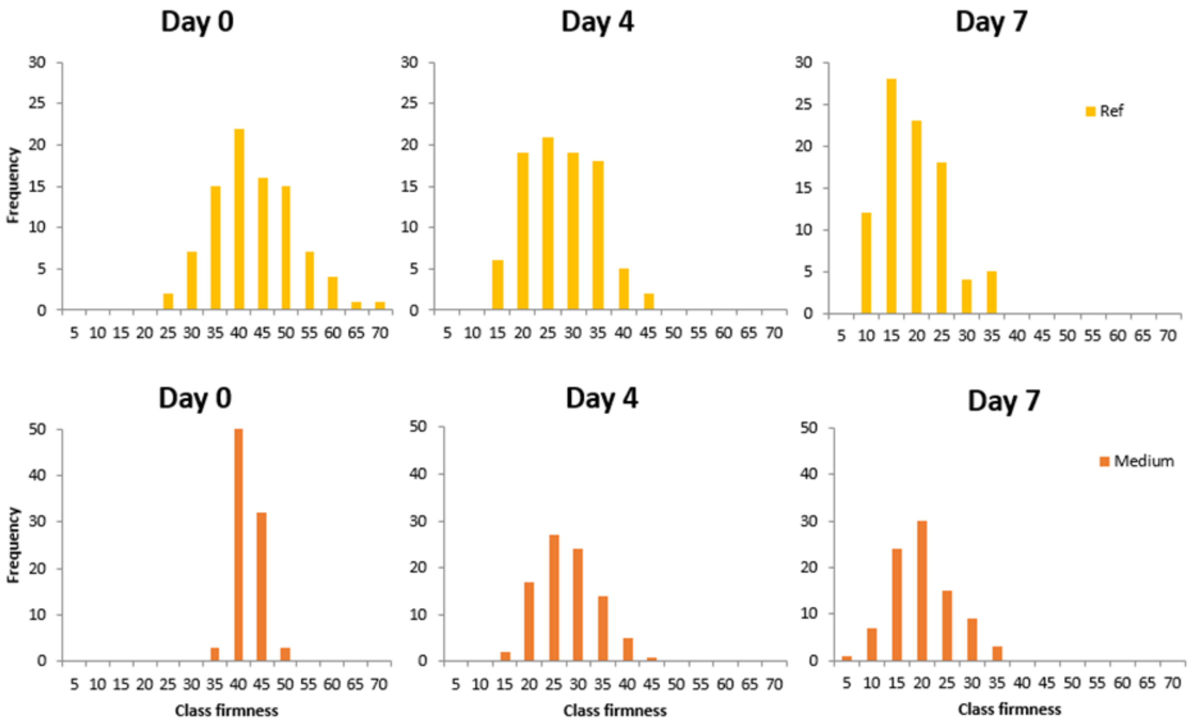


Figure 8: Distribution histogram of avocado firmness in 2 selected batches (Reference and Medium firmness batch) at three evaluation times (day 0, 4 and 7) during ripening at 18°C. (n=90). Note that scale of y-axis is different for reference and medium firmness batch.

3.2.2 Applying a heat-shock prior to ripening

It has been shown that application of heat-shock immediately after harvest/before storage can reduce the heterogeneity that occurs during ripening[1]. Here we tested whether a heat-shock applied at arrival (after storage/transport) can affect the ripening speed and firmness heterogeneity. A heat-shock was applied either using hot air or hot water. The hot air treatment consisted of a 1h exposure of the fruit to 40°C and 80% relative humidity. By the end of the treatment the pulp temperature had reached 35°C. Thereafter fruit were allowed to cool down for 2 h at 22°C. The hot water treatment consisted of immersion of the fruit for 30 min in 40°C water. By the end of the treatment the pulp temperature had reached 35°C. Thereafter the fruit were cooled down by 2h immersion in 20°C water.

Firmness measurements before and after the hot air/water treatment showed that the treatments itself had no immediate effect on the fruit firmness (data not shown). Non-heat-treated and heat-

[1] Hernández, I., Fuentealba, C., Olaeta, J.A., Poblete-Echeverría, C., Defilippi, B.G., González-Agüero, M., Campos-Vargas, R., Lurie, S., Pedreschi, R., 2017. Effects of heat shock and nitrogen shock pre-treatments on ripening heterogeneity of Hass avocados stored in controlled atmosphere. Scientia Horticulturae 225, 408-415.

treated fruit were first ripened at 22°C for 3 days; followed by 3 days at 5°C and thereafter fruit were held for 4 days under retail conditions (18°C). Compared to the non-heat-treated fruit, both the ripening speed and the firmness heterogeneity was not affected by the heat-treatment (Figure 9).

Internal quality judged when fruit were RTE was not affected by the heat treatments. A pre-ripening heat treatments does not seem to be useful to reduce firmness heterogeneity.

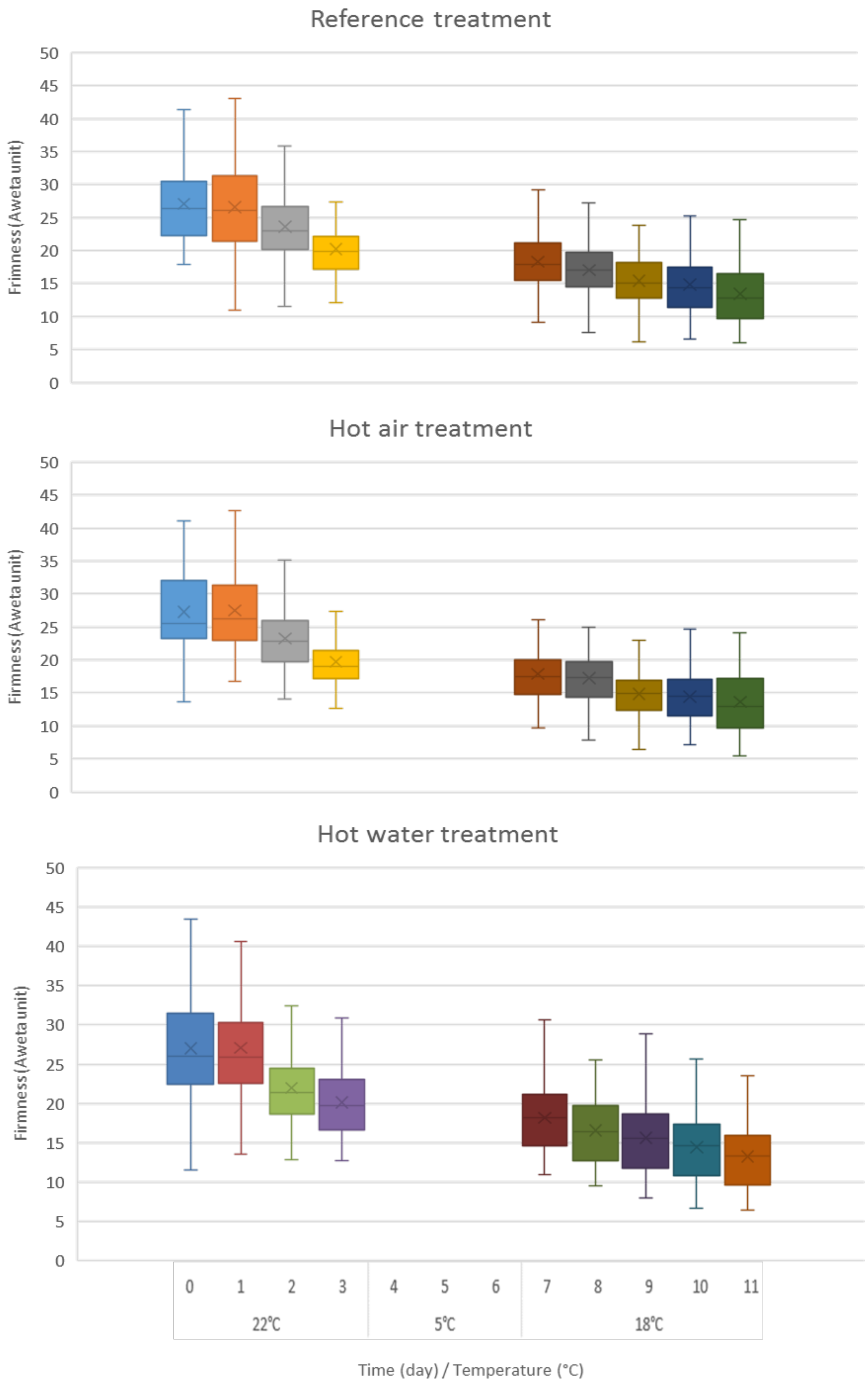


Figure 9: Boxplot representation of firmness measurements of avocado batches per evaluation day. Fruit were ripened at 22°C, thereafter held for 3 days at 5°C and thereafter placed under shelf life conditions at 18°C (n=40).

3.2.3 Applying ethylene during ripening

In commercial ripening operations, often ethylene is applied to speed up the ripening. We investigated the effect of ethylene on the ripening speed and heterogeneity of two batches of fruit from different orchards in Chile. Ripening was done at 18 and 22°C; ethylene (100 ppm) was applied during the first 48h of ripening. On average, the fruit from orchard 1 were more firm than from orchard 2 at arrival. Ripening at 22°C was faster than at 18°C (Figure 10). In both batches and at both ripening temperatures ethylene stimulated the rate of firmness loss. Especially the lag phase is shortened. Ethylene treated fruit reached the RTE firmness at maximum about 0.5 day earlier than the non-ethylene-treated fruit. The effect was clearly present in the batch from orchard 1, but minimal in the batch from orchard 2.

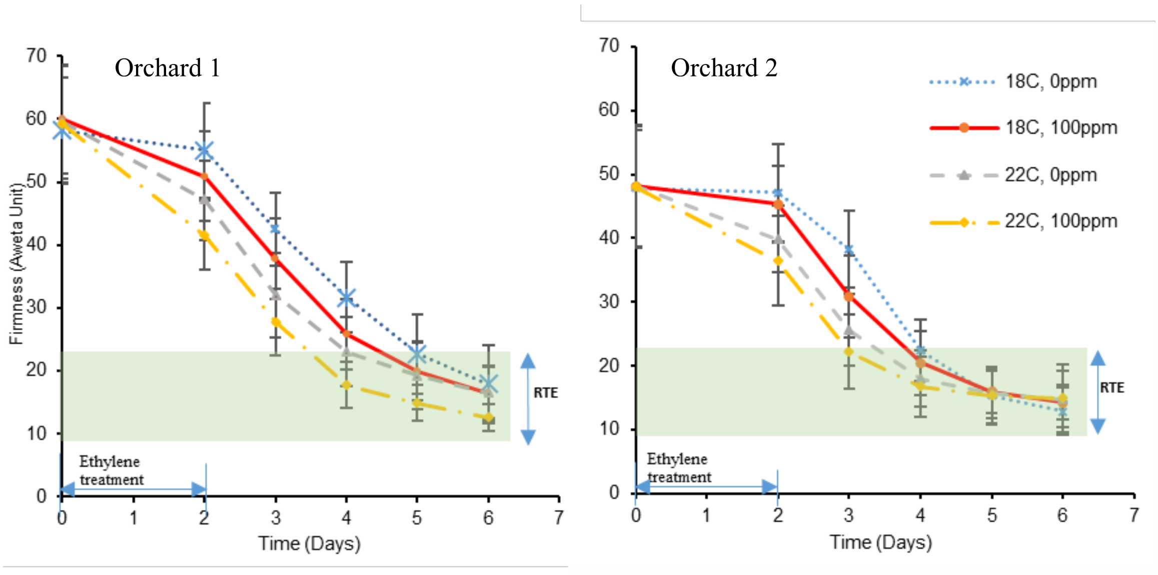


Figure 10: Avocado firmness of two batches (Orchard 1 and 2) ripened at 18 and 22°C with and without ethylene application during the first 48 hours of ripening. Error bars represents the standard error. Green area shows the RTE firmness range (n=90).

Firmness heterogeneity for the batch from orchard 1 is shown in Figure 11 and in Table 2. From both the box plots and the calculated standard deviations in Table 2, it is concluded that ethylene does indeed lower the firmness heterogeneity in the batch from orchard 1. When fruit had reached the RTE ripening stage 4 (end of shelf life) internal quality was judged. When fruit had ripened at 22°C a greater percentage of the avocado batch showed internal defects compared to fruit that had ripened at 18°C. In addition, ethylene treatment slightly increased the abundance of internal defects (Figure 12).

Table 2: Firmness average and firmness standard deviation of avocado (batch from farm 1) ripened with (100 ppm) and without (0 ppm) exogenous ethylene during the first 48 hours of the ripening (n=90). Per evaluation day, for both the average firmness and the standard deviation, different colours indicate significant difference between the treatments (p=0.05).

	Average		Standard deviation	
	0 ppm	100 ppm	0 ppm	100 ppm
day 0	58.9	59.6	4.00	3.81
day 2	51.1	46.2	4.29	3.94
day 4	27.3	21.8	4.48	3.74
day 6	17.2	14.6	5.08	2.91

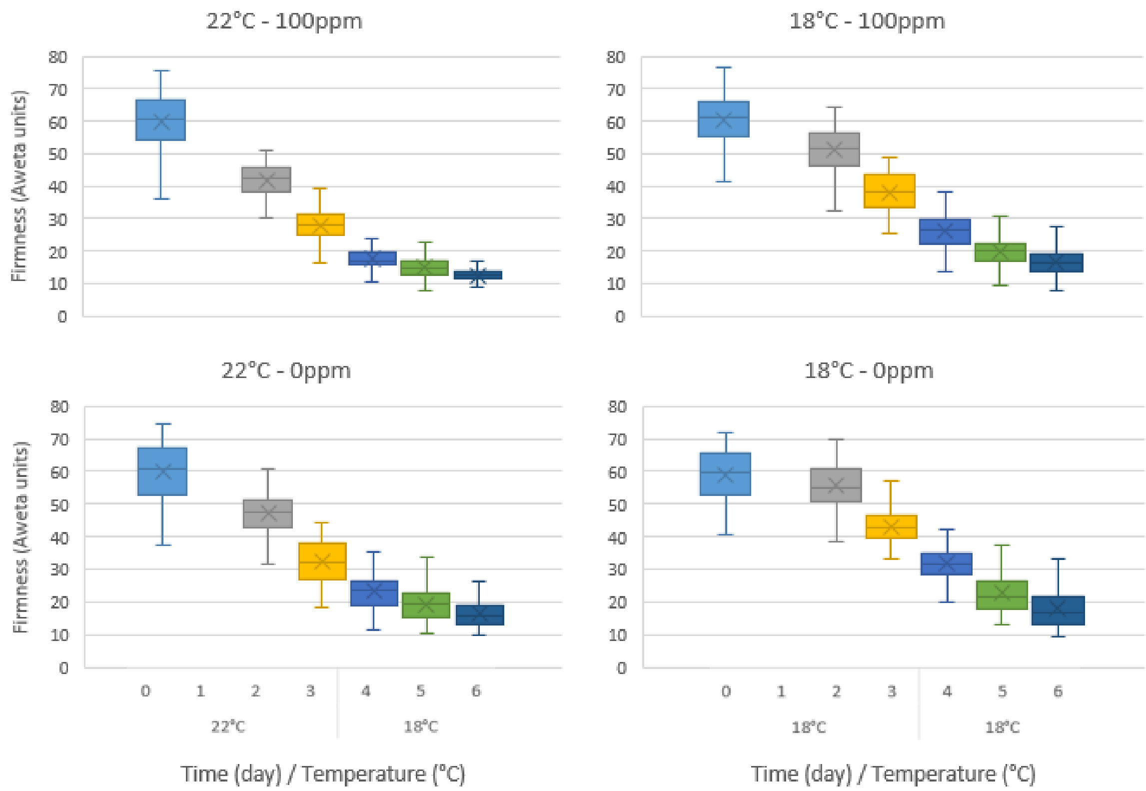


Figure 11: Boxplot of firmness measurement of avocado batch from orchard 1. Fruit were ripened 3 days at 18 and 22°C, with (100 ppm) and without (0 ppm) application of exogenous ethylene during the first 48 hours of ripening. Shelf life simulation at 18°C followed the ripening period (n=90).

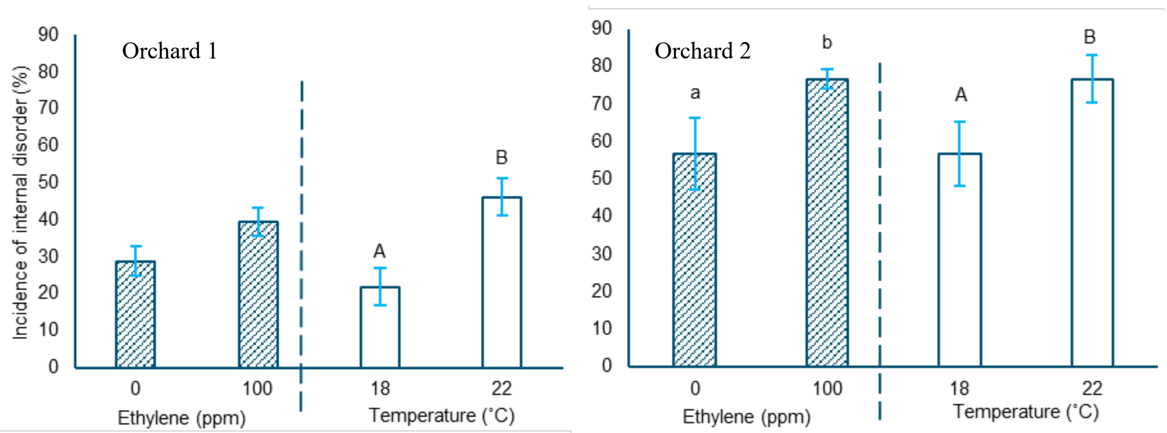


Figure 12: Percentage of internal disorder observed at the end of the individual avocado shelf life period of two avocado batches (Orchard 1 and 2). The letters on the bar represent significant levels according to the Fisher's protected test (p=0.05).

3.3 Effect of temperature and MAP on the shelf life of ripened fruit

Once fruits have reached the RTE stage 2 (fruit can be eaten but still firm), they generally rapidly develop into RTE 4 (limit of acceptability) under retail display conditions (18°C). The shelf life therefore is determined by the ripening speed. To prolong the shelf life we investigated the effect of lowering the display temperature on the softening speed and, in addition, the effect of a modified atmosphere packaging (MAP) was tested.

3.3.1 Effect of temperature on shelf life of ripe fruit

When ripe fruit were stored at low temperature (8°C), softening was greatly delayed compared to fruit stored at 18°C (Figure 13). Shelf life was at maximum about 7-8 days at 18°C but over 12 days at 8°C. An advantage of the storage of ripe fruit at 8°C is the greatly reduced weight loss (about 4% at 8°C versus 10% at 18°C over a 10 day period) resulting in a more fresh and shiny appearance. Internal defects, as measured in fruit when they reached RTE 3 (firmness between 11 and 18 Aweta value) were similar for fruit held at 18 or 8°C (Figure 14). This shows that lowering the temperature during display is an effective way to prolong the shelf life of ripe fruit.

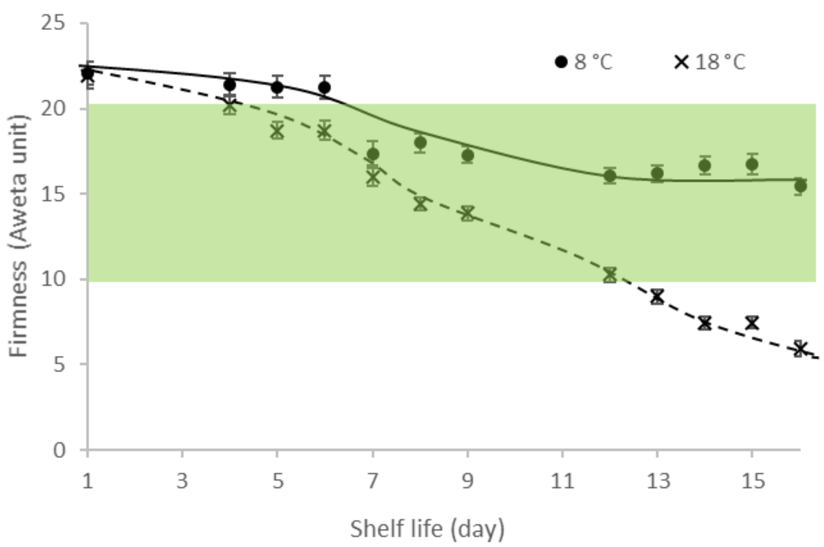


Figure 13: Firmness of avocado stored at 8 and 18°C with 60% relative humidity. Green area represents the RTE firmness range. The error bar represents the standard error (n=60).

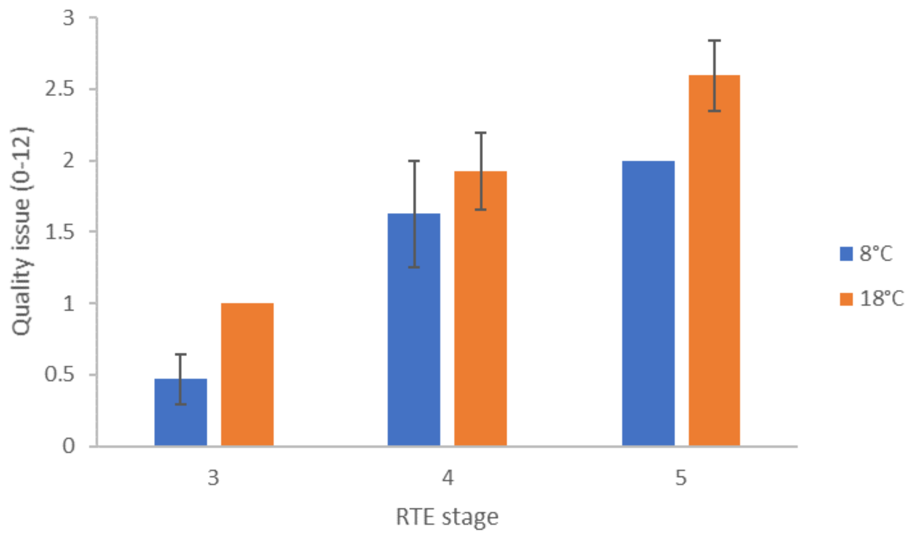


Figure 14: Quality issues (internal brown, vascular browning) of RTE avocado stored at 8 and 18°C in function of the RTE stage when the fruit were judged for quality (error bar represents standard error).

3.3.2 Effect of MAP on shelf life of ripe fruit

It was tested if the shelf life of ripe fruit could be prolonged by using modified atmosphere packaging (MAP) technology. Fruit were first ripened at 18°C. Fruit at RTE 2 stage (just ready to eat) were packed with 2 fruits in a bag of 15x26 cm BOPP (bi-oriented polypropylene, 30µm thickness), with varying number of micro perforations (6, 20 and 76 holes per bag). Micro perforations (diameter 100µm) were applied by laser technology (Perfotec, Woerden). The bags were flushed with an air mixture (5% O_2, 5% CO_2 and 90% N_2) at the start of the experiment. In addition to these treatments, an extra treatment was made by adding an ethylene absorber (10g of $KMnO_4$ containing beads) in the bags with 20 micro perforations. The bags with fruit were stored at 18°C, as a reference served fruit in a bag with macro perforations under atmospheric conditions.

Within 3 days the gas concentrations in the bags were at equilibrium. This yielded an atmosphere of 5% O_2 and 18% CO_2 in the bags with 6 perforations; 12% O_2 and 10% CO_2 in the bags with 20 perforations and 18% O_2 and 4% CO_2 in the bags with 76 perforations (Figure 15). Ethylene levels in the headspace were high in the bags with 6 perforations and low in the bags with 76 perforations. The ethylene scrubber was effective in lowering the ethylene concentration during the first 8-10 days of the shelf life (Figure 16).

At regular time intervals a number of bags were opened and fruit firmness and internal quality was measured. Fruit softening (expressed as the change in firmness between start of the experiment and the firmness on the measurement day) was not affected by the packaging nor by the addition of an ethylene absorber (Figure 17). The same was true for the development of internal disorders (Figure 18).

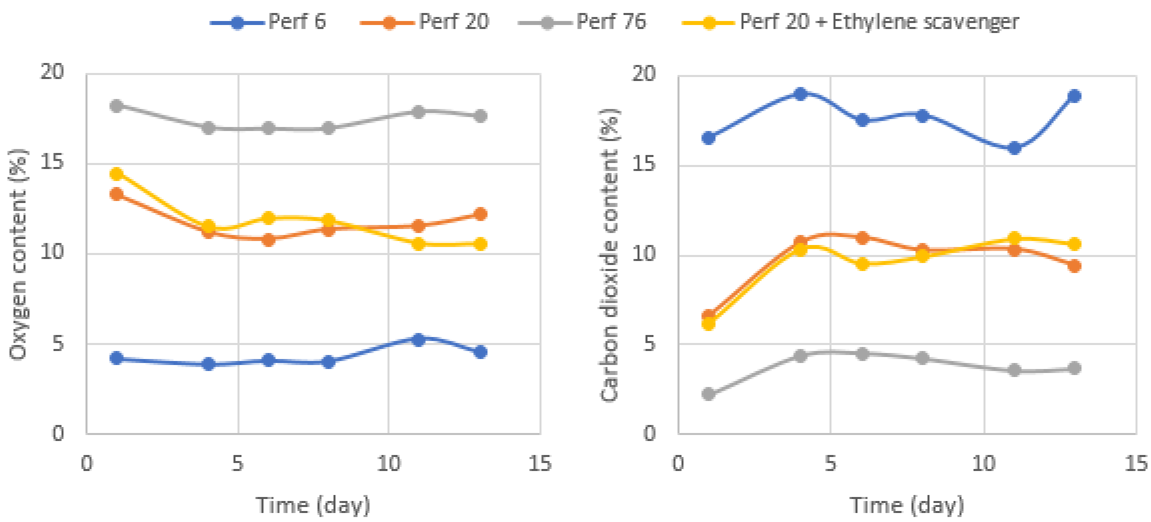


Figure 15: Oxygen and carbon dioxide concentration of packaging headspace (n=5). Bags with two RTE avocados were stored at 18°C and 60% relative humidity for a period of 14 days.

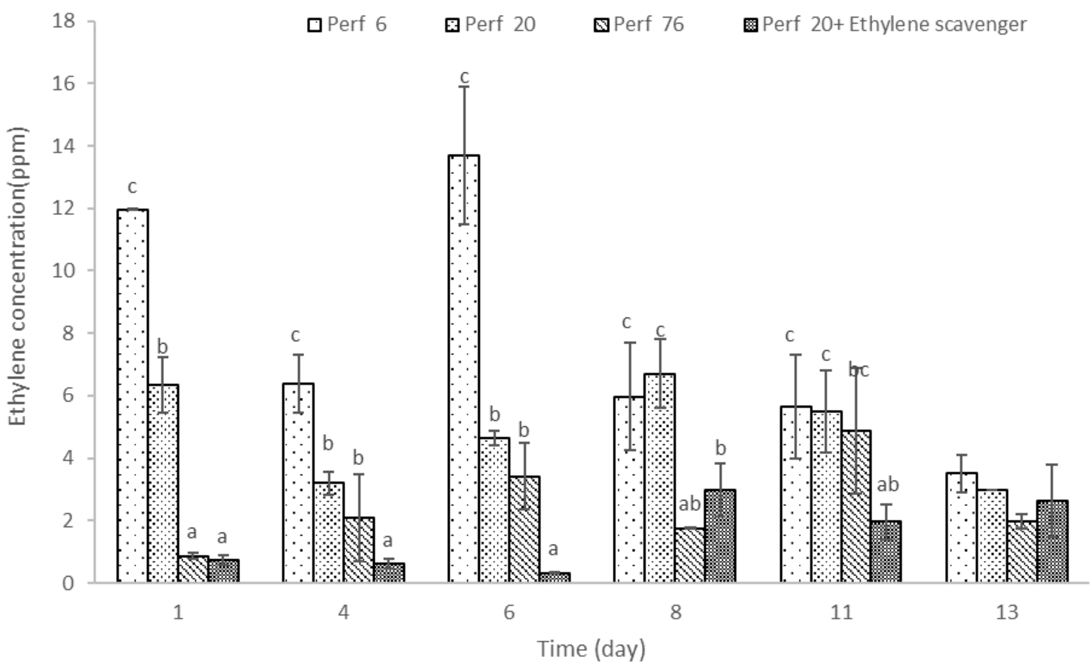


Figure 16: Ethylene concentration (ppm) measured in MAP during the shelf life of avocados among different packaging. The different letters on the same evaluation day indicate a significant difference between treatments according to Fisher's LSD test (P<0.05, n=5). The error bar represents the standard error.

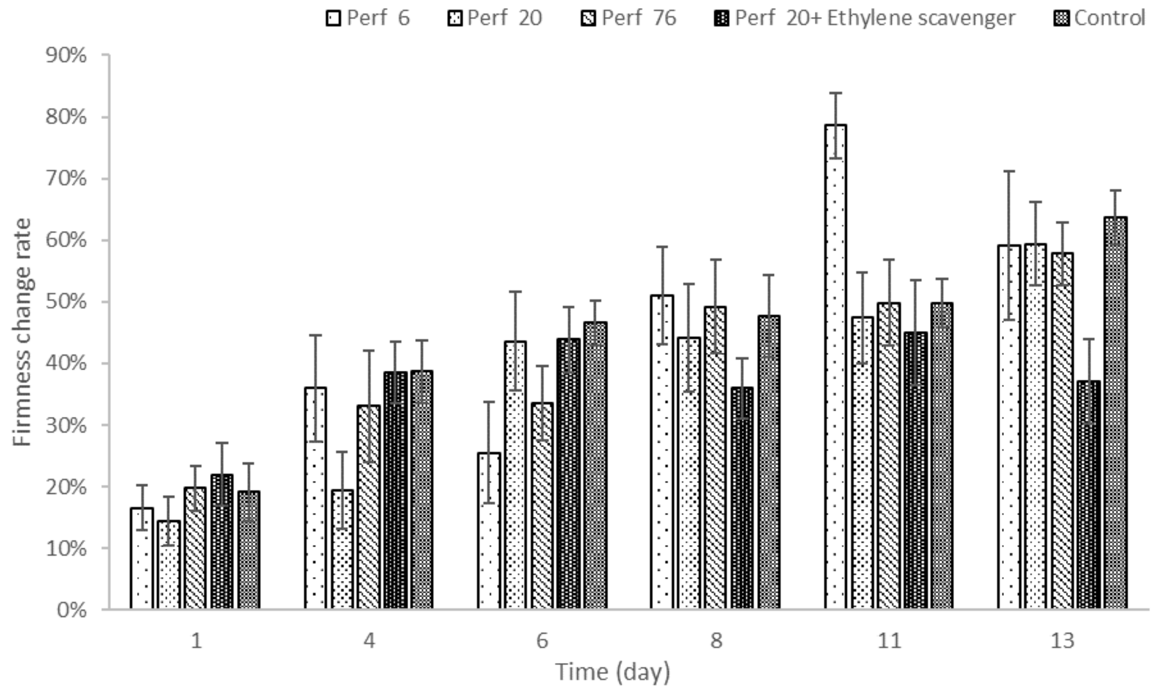


Figure 17: Percentage of firmness change during the shelf life of RTE avocado in MAP. The error bar represents standard error (n=10).

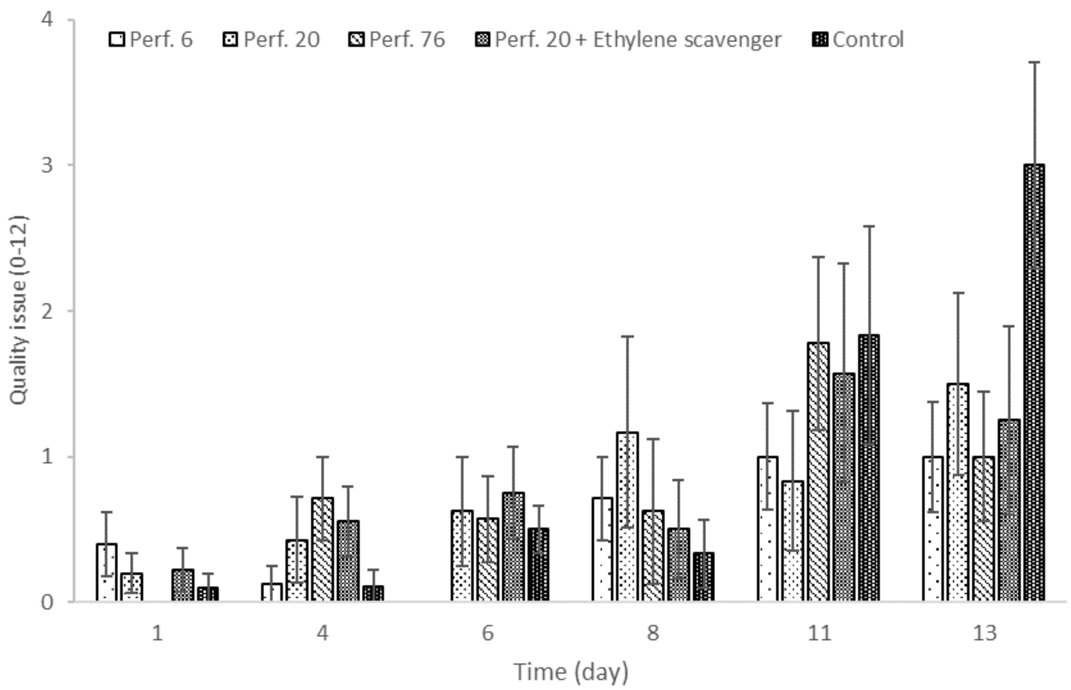


Figure 18: Overall internal quality during shelf life period at 18°C in MAP. Ripe avocados were packed per two into different modified atmosphere packaging's. Score of 0 reflects perfect internal quality, whereas score of 12 represents very bad quality. Control fruit were packed in film with macro perforations. The error bar represents the standard error.

4 Conclusions

- Ripening speed of imported avocados depends on the initial firmness (at arrival) and on the ripening temperature. Using a mathematical model[2] incorporating these parameters allows for better prediction of the ripening outcome.
- Ripening can be interrupted by bringing the fruit temporarily to a low temperature (e.g. 7°C). The firmness loss greatly slows down and will regain again if fruit are brought back to ripening temperatures.
- Different ripening scenarios, employing different time periods at different temperatures (between 18 and 22°C) with or without a short period of cold storage interruption all can result in RTE fruit without inducing major internal quality defects.
- In all the ripening experiments within batch and between batches, variation is extremely high. Generally, after a given ripening period, only 50-60% of the fruit are within the perfect RTE range; at the same time some fruit are too firm, others too soft.
- Sorting the fruit, prior to ripening, in classes of different firmness lowered the heterogeneity at the start of ripening but had no effect on heterogeneity at the RTE stage. Within each firmness class, individual fruit still show very unpredictable ripening rate.
- A hot water or hot air treatment prior to ripening did not affect the ripening rate and had no effect on heterogeneity of fruit at the RTE stage.
- Ethylene treatment during ripening slightly stimulated the softening rate and slightly lowered the heterogeneity of fruit at the RTE stage. Ethylene also slightly increased the occurrence of internal defects.
- Shelf life of RTE fruit can be prolonged by storing the fruit at low (e.g. 8°C) temperature. This has no negative effect on development of internal defects.
- MAP did not prolong the shelf life of RTE fruit at 18°C.

[2] Mathematical model was developed by WFBR and remains an intellectual property of WFBR.

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

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