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Modelling the transient effect of 1-MCP on 'Hass' avocado softening: A Mexican comparative study

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ABSTRACT

In this study the effect of 1-methylcyclopropene (1-MCP) on the softening of avocado fruit (*Persea americana* Mill.) cv. Hass was modelled. Data were collected throughout the 2006 season by sampling 40 batches of fruit from 8 different commercial orchards in the region of Michoacan (Mexico). A simplified mechanistic model was developed to analyse the experimental data. Most of the model parameters were treated as being generic for all fruit while only two of the model parameters were identified as being unique to each individual fruit. The two fruit specific parameters characterised the maturity at harvest of an individual fruit and the sensitivity of an individual fruit to 1-MCP. Monte Carlo simulations were performed. The model was able to describe the individual fruit behaviour very well explaining more than 95% of the observed variation for most of the fruit. The model successfully quantified the effect of 1-MCP on avocado softening taking into account the stochastic nature of batch behaviour. The developed model can be utilised to predict the behaviour of a specific batch of 'Hass' avocado fruit given the distribution of the two fruit specific model parameters.

1. Introduction

The avocado differs from most other fruits in that ripening normally does not take place on the tree, but only after picking. The most obvious feature of avocado fruit ripening is softening. At harvest, the mesocarp firmness of avocado fruit measured as a non-destructive compression force is generally in the range of 80–100 N and initially declines at a moderate rate. The softening rate increases with time resulting in firmness levels of less than 5 N at a full ripe state. Previous studies have shown that avocado firmness is a good predictor of ripening stage and expected storage time (Lewis, 1978; Peleg et al., 1990).

It is well established that avocado fruit softening is the result of the activity of cell wall degrading enzymes with some pre- and postharvest factors affecting the rate of softening and final quality (Awad and Young, 1979; Bower and Cutting, 1988). Unpredictable ripening during storage, transport and distribution can result in spoilage before consumption. Furthermore, firmness heterogeneity in avocado fruit batches complicates the use of postharvest treatments to maintain quality.

Ethylene is believed to take a central role in regulating fruit ripening of avocado (Jeong and Huber, 2004). The application of 1-MCP is known to block ethylene binding sites (Sisler and Serek, 1997) and has been shown to be effective in inhibiting the ripening of avocado (Adkins et al., 2005). Depending on the timing of application relative to the climacteric development, the treatment can be more or less effective (Hershkovitz et al., 2005) as, once triggered, the ripening process is hard to stop.

If avocado fruit softening can be characterised during storage, an objective measurable at-harvest criterion can be developed to determine the potential postharvest life of avocado. This would allow early segregation of fruit into fast and slow ripening fruit enabling the industry to apply the appropriate marketing strategy to the different groups of fruit. This would facilitate inventory management and reduce fruit losses. The ability to predict the rate of fruit softening would benefit growers, packers, shippers, retailers and consumers alike.

The aim of this work is to characterise and quantify the transient effect of 1-MCP on 'Hass' avocado softening taking into account the inevitable variation introduced by batches coming from different harvests, growers and dry matter contents throughout the season.

2. Material and methods

2.1. Produce

Experimental data were collected during studies in 2006 which assessed the response of avocado fruit (*Persea americana* Mill.,

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cv. Hass) from commercial orchards in the region of Michoacan (Mexico) to 1-MCP treatment. During the 2006 season, fruit were collected at 14 different sample times from February to October, from 8 different locations in the region resulting in 40 different batches of 40 or 60 avocado fruit (Table 1). Fruit from all batches were of export quality (count size 20) and commercially packed into single-layered trays. Fruit were couriered to Michoacan University and placed in 117.3 L gas-tight containers (Rubbermaid®) at 5 °C and subsequently treated with 0, 200 or 300 nL L^{-1} 1-MCP for 12 h (Table 1). 1-MCP was released by adding a buffering agent to a calculated number of SmartFresh™ research tablets (a.i. 25 μg; Agrofresh Ltd.) according to the manufacturer's instructions. A circulating system ensured rapid diffusion of the gas. After the 1-MCP treatment, fruit were kept at 5 °C and 90% relative humidity for 18 to 32 d depending on the batch (Table 1).

2.2. Quality measurements

After the 1-MCP treatment and the subsequent storage period, both at 5 °C, fruit were moved to shelf-life conditions at 22 °C (±1 °C). During this shelf-life period, non-destructive fruit firmness was measured with a Fruit Texture Analyzer (Model GS-14) fitted with a convex tip (8 mm diameter), trigger threshold of 0.50 N and measuring speed of 10 mm s^{-1}. The compression force was recorded in newtons (N) at 2 mm deformation and was determined at three equidistant points on the equatorial region of each whole fruit. Repeated measurements were taken from the same fruit every day until they reached the full-ripe stage. Depending on the actual batch and the 1-MCP treatment applied shelf-life covered a period of between 3 and 9 d.

2.3. Data analysis

As indicated above, the experimental design was the same for all batches except for some details on timing and shelf-life temperature. However, data from all batches were analysed taking into account these batch-to-batch differences in terms of the length of the actual cold storage period (ranging from 18 to 32 d) and the actual length (ranging from 3 to 9 d) and temperature (ranging from 20.7 to 23.4 °C) of the shelf-life period. In this way, these variations in the experimental setup cannot obscure the statistical outcome of the analysis.

The developed model was implemented and model parameters were estimated using OptiPa (Hertog et al., 2007a; http://perswww.kuleuven.be/~u0040603/optipa/), a dedicated optimisation tool which was developed for use with Matlab (Matlab R2006b, The MathWorks, Inc., Natick, MA, USA). Monte Carlo simulations were performed using OptiPa as well; generating values for the stochastic Monte Carlo model parameters (t_{age} and t_{MCP}) based on the previously collated distributions of the estimated values of these parameters. The technique used for random generation of correlated non-Gaussian model parameters was described in detail by Hertog et al. (2008).

3. Model development

3.1. Modelling softening

Softening of avocado generally follows a logistic trend and has been modelled using a simple logistic model (Hertog et al., 2003). Comparable model equations have been previously applied successfully to describe both colour and firmness changes as a function of time (Schouten et al., 1997; Tijskens et al., 2000; Hertog, 2002; Hertog et al., 2003, 2004, 2007b). Such a logistic model can be interpreted as a simplified representation of an autocatalytic process as

Table 1
Overview of all 40 batches involved (ID) showing their origin, the dry matter content, the start date of the 1-MCP treatment, the 1-MCP levels applied and the duration of the cold storage period (5 °C) preceding shelf-life at 22 °C

ID	Growing region	Dry matter content (%)	Start 1-MCP application	1-MCP levels applied (nL L^{-1})	Length of cold storage (d)	ID	Growing region	Dry matter content (%)	Start 1-MCP application	1-MCP levels applied (nL L^{-1})	Length of cold storage (d)
1	San Juan Nuevo	32.8	10/02/2006	200–0	21	21	Tacambaro	36.9	2/06/2006	300–0	23
2	Tancitaro	32.9	10/02/2006	200–0	21	22	Tancitaro	33.6	2/06/2006	300–0	23
3	Tacambaro	35.3	10/02/2006	200–0	21	23	Uruapan	30.5	2/06/2006	300–0	23
4	Uruapan	33.6	24/02/2006	200–0	23	24	Tancitaro	29.1	16/06/2006	300–0	21
5	Tancitaro	30.5	24/02/2006	200–0	23	25	San Juan Nuevo	38.7	16/06/2006	300–0	21
6	Salvador Escalante	35.3	24/02/2006	200–0	23	26	Uruapan	33.4	16/06/2006	300–0	21
7	Ario de Rosales	34.4	10/03/2006	300–0	21	27	Uruapan	33.6	30/06/2006	300–200–0	23
8	Tancitaro	30.2	10/03/2006	300–0	21	28	Tancitaro	32.0	30/06/2006	300–200–0	23
9	Tacambaro	34.6	8/04/2006	200–0	20	29	Tancitaro	35.1	13/07/2006	300–200–0	16
10	Uruapan	33.8	8/04/2006	200–0	20	30	Acuitzio	32.6	13/07/2006	300–200–0	16
11	Tancitaro	31.9	8/04/2006	200–0	20	31	Salvador Escalante	35.7	13/07/2006	300–200–0	16
12	Tancitaro	38.3	20/04/2006	200–0	24	32	Periban	26.1	29/09/2006	300–200–0	23
13	Uruapan	35.9	20/04/2006	200–0	24	33	Uruapan	25.7	29/09/2006	300–200–0	23
14	Salvador Escalante	34.8	20/04/2006	200–0	24	34	Ario de Rosales	25.2	29/09/2006	300–200–0	23
15	Tancitaro	37.8	5/05/2006	200–0	21	35	Salvador Escalante	30.3	13/10/2006	300–200–0	24
16	San Juan Nuevo	36.8	5/05/2006	200–0	21	36	Periban	26.0	13/10/2006	300–200–0	24
17	Ario de Rosales	36.7	5/05/2006	200–0	21	37	Uruapan	25.4	13/10/2006	300–200–0	24
18	Uruapan	35.7	19/05/2006	200–0	21	38	Uruapan	27.4	27/10/2006	300–200–0	31
19	Tancitaro	39.0	19/05/2006	200–0	21	39	Ario de Rosales	28.1	27/10/2006	300–200–0	31
20	Tancitaro	36.6	19/05/2006	200–0	21	40	Tancitaro	24.9	27/10/2006	300–200–0	31

a consequence of two parallel processes; on one hand an exponential increase in enzyme activity during ripening and on the other the action of this enzyme system on firmness. Knowing that fruit softening is the result of a complex interplay of various enzymes, the current modelling approach summarises the concerted action of these various enzymes as the action of a single enzyme system (E in arbitrary units) breaking down firmness (F in N) following:

$$F + E \xrightarrow{k_f} E \tag{1}$$

with rate constant k_f (in d^{-1}).

Most likely, ethylene, inducing the climacteric response of the fruit during fruit ripening, can be held responsible for the autocatalytic character of the overall firmness change. Instead of modelling the autocatalytic effect of ethylene in full detail, a simplified approach was taken representing how the responsible enzyme system autocatalytically induces its own activation from a limited resource of inactive precursor (E_{pre}) following:

$$E_{pre} + E \xrightarrow{k_e} 2 \cdot E \tag{2}$$

with rate constant k_e (in d^{-1}). Thus, ethylene production as such was not modelled, only the autocatalytic effect it has on the activation of the enzyme system subsequently inducing softening.

The simplified reaction scheme from Eqs. (1) and (2) results in the following ordinary differential equations (ODEs) describing the changes in F and E with time (t in d):

$$\begin{cases} \dfrac{dF}{dt} = -k_f \cdot E \cdot (F - F_{fix}) \\ \dfrac{dE}{dt} = k_e \cdot E \cdot E_{pre} \\ \dfrac{dE_{pre}}{dt} = -k_e \cdot E \cdot E_{pre} \end{cases} \tag{3}$$

with the initial values at harvest ($t = 0$ d) of the ODEs defined following:

$$\begin{cases} E(0) = E_0 \\ F(0) = F_0 \\ E_{pre}(0) = E_{tot} - E_0 \end{cases} \tag{4}$$

This formulation (Eq. (3)) takes into account that avocados do not soften to absolutely zero but to some fixed end value (F_{fix} in N) and it assumes (Eq. (4)) that the total pool of available enzyme (either activated or not) is fixed (E_{tot} in arbitrary units). Both rate constants from Eq. (3) are assumed to depend on temperature (T in K) according Arrhenius' Law (Arrhenius, 1889) following:

$$k_i = k_i^{ref} \cdot e^{(E_a/R)\cdot((1/T_{ref})-(1/T))} \tag{5}$$

with k_i referring to either k_e or k_f; k_i^{ref} the value of the concerning rate constant at an arbitrary chosen reference temperature, T_{ref} (in this case 283.15 K); E_a (J mol^{-1}) the energy of activation; R the universal gas constant (8.314 J mol^{-1} K^{-1}).

3.2. Introducing the effect of 1-MCP

It is generally assumed that 1-MCP binds permanently to the ethylene receptors present at the time of application. However, due to *de novo* synthesis of new receptor sites the effect of 1-MCP tends to wear off. While this may be true, this is largely based on indirect evidence on intact fruit responses (Blankenship and Dole, 2003). The alternative explanation would be that 1-MCP comes off the receptor after a period of time.

To include the effect of 1-MCP when modelling softening of avocado it was assumed that 1-MCP, assuming it is inhibiting only the autocatalytic part of the ripening process, results in temporarily shutting down the activation of the enzyme system from its precursor (Eq. (2)). In other words, the rate constant k_e is temporarily set to a value of zero. By doing so, softening will still continue following Eq. (1), depending on the amount of active enzyme already present. By the time the effect of 1-MCP wears off (captured by the parameter t_{MCP} in d), new receptors have been formed (or 1-MCP was released from the receptors) and the rate constant k_e is restored to its original value allowing the autocatalytic part to continue. The effect of 1-MCP on softening is thus approached as a simplified discrete process temporarily turning off the autocatalytic part of the ripening process following:

$$\begin{aligned} &\text{if } 0 < t < t_{MCP} \\ &\quad k_e = 0 \\ &\text{end} \end{aligned} \tag{6}$$

thus, ignoring the probably more subtle transitions accompanying the inactivation and possible *de novo* synthesis of receptor sites occurring *in vivo*.

3.3. Introducing biological variation

The current work covers avocados coming from various growing regions throughout Mexico during the 2006 season. Besides the obvious batch differences, individual fruit will largely vary in their maturity at harvest and how they response to the storage conditions applied (time, temperature and 1-MCP). This will be reflected in some of the model parameters to be generic or to vary from batch-to-batch or to vary from fruit-to-fruit.

The two parameters most obviously prone to fruit-to-fruit variation are the initial maturity at harvest and the response of individual fruit to the 1-MCP treatment. The latter is going to be reflected in the value of t_{MCP} defining the length of the period during which the rate constant k_e will be set to zero.

During time, fruit development is reflected in various quality aspects (colour, brix, firmness, aroma, etc.) but also the activation of certain metabolic pathways (ethylene production, respiration rate, etc.) which on their turn will be mirrored in the related enzyme activities. So the actual values of these variables can be taken as a measure of the state the fruit is in. Therefore, under the current model approach, it is plausible to assume that at harvest variation in initial maturity is most likely reflected in both the initial firmness (F_0) and in the initial activity of the enzyme system (E_0). To deal with these two sources of variation in the model, the concept of biological age (t_{age} in d) is introduced, equivalent to what was done previously by several authors (Tijskens and Evelo, 1994; Hertog et al., 2004, 2007b,c; Tijskens et al., 2005, 2007; De Ketelaere et al., 2006; Schouten et al., 2007a,b).

Biological age is in this case defined as the (virtual) time taken for an individual fruit to develop during the preharvest phase from some arbitrary reference point (with certain reference firmness, F_{ref}, and some reference amount of active enzyme, E_{ref}) to the situation observed at harvest. Applying the model to the preharvest phase the ODE-formulation can be solved to express both F_0 and E_0 as a function of biological age (t_{age}) following:

$$\begin{cases} F_0 = F_{fix} + \dfrac{(F_{ref} - F_{fix}) \cdot (E_{pre,ref} + E_{ref} \cdot e^{t_{age} \cdot k_e \cdot E_{tot}})^{-k_f/k_e}}{E_{tot}^{-k_f/k_e}} \\ E_0 = \dfrac{E_{tot} \cdot E_{ref} \cdot e^{t_{age} \cdot k_e \cdot E_{tot}}}{E_{pre,ref} + E_{ref} \cdot e^{t_{age} \cdot k_e \cdot E_{tot}}} \end{cases} \tag{7}$$

with E_{ref} and $E_{pre,ref}$ the amount of, respectively, the active enzyme system and its inactive precursor at the arbitrary reference point during fruit development as defined by the reference firmness. The benefit of this approach is that, under the assumptions of the proposed model, the two stochastic parameters (F_0 and E_0) can be interpreted as the direct result of one single stochastic model parameter (t_{age}) thus reducing the complexity of the model. The fact that F_0 and E_0 are both governed by t_{age} is easy to understand if one realises that F_0 and E_0 are determined from the ODEs from Eq. (3). These parallel ODEs are coupled through the factor time and thus t_{age}. With time progressing, F will decline and E will increase in parallel. As a consequence, any variation in t_{age} (preharvest time) induces comparable variation in both F_0 and E_0 at harvest.

This approach of introducing t_{age} to explain at-harvest differences in firmness might sound contradictory to the earlier statement that avocado fruit generally do not ripen on the tree. However, at-harvest avocado fruit do show significant variation with compression firmness ranging between 50 and 90 N. As compared to the overall softening this is largely part of the initial slow softening phase before the fast autocatalytic part of the softening process starts. Most likely, fruit do not go into the autocatalytic climacteric part of the softening process while on the tree, resulting in hand-firm fruit at harvest, but the initial slow softening phase might be initiated already. The small changes induced during this initial slow softening phase can be enough to induce the variation in F_0 and E_0 at harvest.

3.4. Model assumptions

Throughout the analysis, several *a priori* assumptions were made concerning some of the model parameters to prevent over parameterisation of the model. As already indicated, the reference temperature (T_{ref}; Eq. (5)) was selected at 283.15 K. The energies of activation (E_a; Eq. (5)) for both k_e and k_f were chosen to be equal. As the model is calibrated on firmness data it is almost impossible to estimate separate energy of activation values for the two parallel reactions underlying softening, also given the fact temperature was not an important experimental factor with only two levels observed (a storage temperature of 5 °C and shelf-life temperature of around 22 °C) with the actual firmness data only collected during shelf-life.

The total pool of available enzyme (E_{tot}; Eq. (4)) was fixed at an arbitrary value of 100% with the amount of active enzyme system at the reference point (E_{ref}; Eq. (7)) chosen at a negligible starting value of 0.001% (resulting in a value for $E_{pre,\,ref}$ of 99.999%). The reference firmness F_{ref} was set to a value of 89 N based on the maximum firmness value observed for independent measurements on avocados harvested rock firm.

Assuming the kinetic parameters (E_a, k_e^{ref} and k_f^{ref}) are lumped properties of the enzyme systems involved, these parameters were kept in common for all batches as they all relate to the same mechanism of fruit softening.

To calculate F_0 and E_0 following Eq. (7) the preharvest growth temperature (T_{growth}) is required. In the ideal situation real dynamic growing temperatures would have been collected for each and every one of the 40 batches studied, however this was out of the scope of the current research. Therefore, based on climacteric data for Michoacan, a common average growth temperature of 22.2 °C was assumed (data obtained from *Servicio Meteorológico Nacional*, Mexico).

3.5. Model calibration

Recently, emphasis has been drawn to various ways to deal with sources of variation when analysing biological data (Hertog et al., 2007c and references therein). However, the current combination of an ODE based model describing dynamic temperature conditions in combination with a discrete time effect is too complex to analyse using these suggested techniques. Therefore, based on preliminary analysis of the data, it was decided for each of the model parameters whether they would be treated as generic (T_{ref}, T_{growth}, E_a, k_e^{ref}, k_f^{ref}, E_{tot}, E_{ref}, $E_{pre,\,ref}$, F_{ref}, F_{fix}), or fruit dependent (t_{age}, t_{MCP}), some of them to be fixed at *a priori* values as indicated in the text (T_{ref}, T_{growth}, E_{tot}, E_{ref}, $E_{pre,\,ref}$, F_{ref}) and others to be estimated from the experimental data (E_a, k_e^{ref}, k_f^{ref}, F_{fix}, t_{age}, t_{MCP}). Subsequently, these parameters were estimated on appropriate subsets of the data to obtain distributions of the stochastic model parameters as outlined below.

In the first analysis only data from the control fruit were used treating the individual fruit data as ordinary replicate measurements. Based on these data, the generic model parameters E_a, k_e^{ref}, k_f^{ref}, F_{fix} were estimated in common over all batches estimating a different average value for t_{age} for every single batch of fruit while t_{MCP} was set to zero (no 1-MCP applied). In a second run, data from the individual fruit were analysed one by one keeping the generic model parameters at their appropriate estimated values, this time only estimating the value of the fruit specific parameters t_{age} and t_{MCP}. Subsequently distributions of these estimated fruit specific parameters were collated.

4. Results and discussion

4.1. General product behaviour

Fruit from different batches coming from different locations in the region and harvested throughout the year softened at different rates during storage and responded differently to the applied 1-MCP levels. As illustrated for two of the batches (Fig. 1) control fruit from batch 28, as compared to batch 37, softened more during the first 23 d of storage at 5 °C resulting in softer fruit during shelf-life even though the initial firmness at harvest was comparable (data not shown). By applying 200 or 300 nL L^{-1} of 1-MCP, firmness was maintained better during the preceding storage at 5 °C, resulting in firmer fruit during the observed shelf-life, taking increasingly more time to soften. The difference between the two 1-MCP levels applied was not that pronounced, which is in agreement with Kluge et al. (2002) who tested application levels of 0, 30, 90 and 270 nL L^{1} 1-MCP. Although increasing 1-MCP application from 90 to 270 nL L^{-1} still increased its effect on softening, the added effect was not very large.

The efficiency of 1-MCP strongly varies between batches and also between individual fruit within a batch as can be seen for the two batches represented in Fig. 1. In the case of batch 28 the variation between the fruit remained comparable between the 1-MCP treatments but with a visible increase in the efficiency of 1-MCP with increasing application levels. The 300 nL L^{-1} treated fruit from batch 28 clearly showed a delay in the softening during shelf-life while the 200 nL L^{-1} treated fruit only showed initial higher firmness levels followed by a rapid softening period. In the case of batch 37, larger fruit-to-fruit variation was observed which was only aggravated by the 1-MCP treatment. Some of the fruit responded stronger to the applied 1-MCP levels than others, resulting in a clear separation in two groups. The main difference between the 200 and 300 nL L^{-1} 1-MCP treatment was the number of fruit found in these two response groups (Fig. 1).

Over all 40 batches studied, the efficiency of the 1-MCP treatment varied strongly from batch to batch, sometimes resulting in almost no difference between the control and the 200 nL L^{-1} treatment and sometimes resulting in almost no difference between the 200 nL L^{-1} and 300 nL L^{-1} treatments. The raw data for this are not presented, but these effects will also become visible from the model analysis. This large variation existing between batches of the same variety makes one wonder about differences that have been reported between cultivars. Hershkovitz et al. (2005) compared the effectiveness of 1-MCP for three different varieties showing the same kind of variation in response between the varieties as this study revealed for a single variety. Whether this variation in

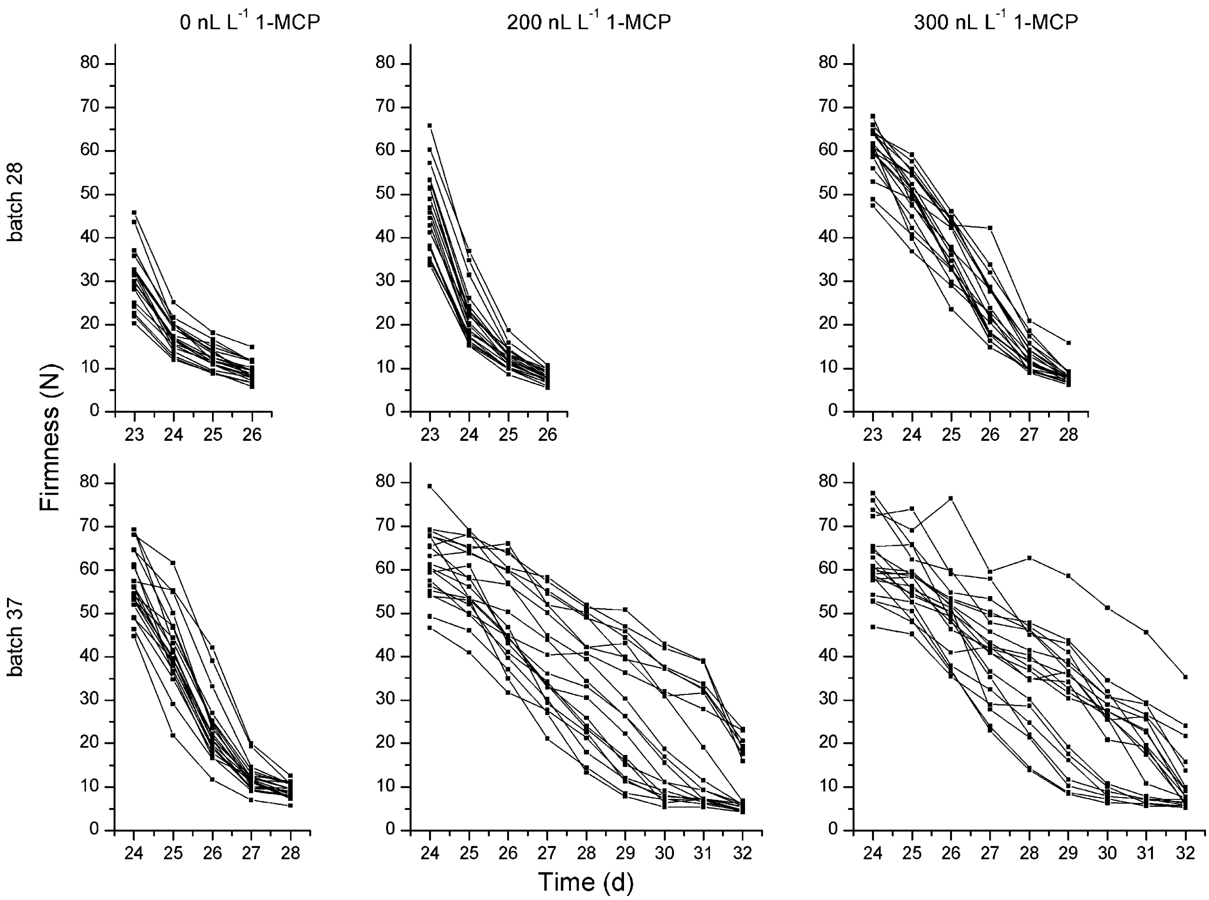

Fig. 1. Firmness measurements (in N) for two batches of 'Hass' avocado followed during shelf-life at 22 °C after initial storage at 5 °C. At the start of storage, fruit were treated with either 0, 200 or 300 nL L^{-1} 1-MCP for 12 h. The dots represent the measured firmness with the lines connecting the measurements taken on the same fruit.

responsiveness is due to the variation in physiological sensitivity of the tissue for 1-MCP (related to fruit maturity and climacteric stage) or whether it is due to variation in physical properties (related to permeance of the skin as affected by growth conditions) is not known. One other explanation could be the oil content in relation to the maturity of the fruit. As 1-MCP is known to also bind to non-target materials such as lipids (Dauny et al., 2003), this might account for the varying efficiency of 1-MCP between the 40 batches studied.

4.2. Model analysis

The experimental data were analysed as outlined in Section 2.3 using the developed ODE based model describing the dynamic temperature conditions in combination with a discrete time effect to describe the effect of 1-MCP under the model assumptions indicated in Section 3.4. This resulted in estimates for both the generic and fruit specific model parameters.

4.2.1. Generic parameters

Based on the data from the control fruit the generic kinetic model parameters E_a, k_e^{ref} and k_f^{ref} were estimated in common under the assumption that each batch was exposed to the same growing temperature T_{growth}. Even though this analysis ignored the large fruit-to-fruit variation it was able to explain 92% of the variation observed in the control fruit (Table 2) with the overall model showing no structural deviations from the measured data (data not shown). The approximate standard errors for the estimates of the kinetic parameters were less than 10% of the estimated values and none of the parameters showed high correlations to any of the other parameters (data not shown) indicating that the model was not over-parameterised and that the statistical approach was successful. Still, this is no guarantee that these values reflect physiologically relevant numbers as the model is a lumped model in which the individual variables often represent the net result of a more complex subsystem.

4.2.2. Fruit specific parameters

Using the generic model parameters from Table 2, data were analysed fruit-by-fruit to estimate the remaining fruit specific

Table 2
Generic model parameters estimated based on the dataset obtained from the control fruit (0 nL L^{-1} 1-MCP treated fruit) only ignoring fruit to fruit variation

Generic parameters[a]	Estimate (s.e.)[b]	General statistics[c]	
k_f^{ref}	0.0025 (5.9)	R^2_{adj}	92
k_e^{ref}	0.0018 (2.7)	n	4000
E_a	10,4220 (1.3)		
F_{fix}	6.7 (1.1)		

[a] k_f^{ref} value of rate constant k_f defining the rate of enzymatic firmness breakdown (in d^{-1}) valid at reference temperature, T_{ref}; k_e^{ref} value of rate constant k_e defining the rate of enzyme activation (in d^{-1}) valid at reference temperature, T_{ref}; E_a the energy of activation (in J mol^{-1}) applicable to both k_f and k_e; F_{fix} the bottom firmness level (in N) avocados soften to.

[b] The standard errors are the approximate standard errors calculated by the non-linear parameter estimation procedure and expressed as percentage of the estimated value.

[c] R^2_{adj}: percentage explained part; n: number of observations

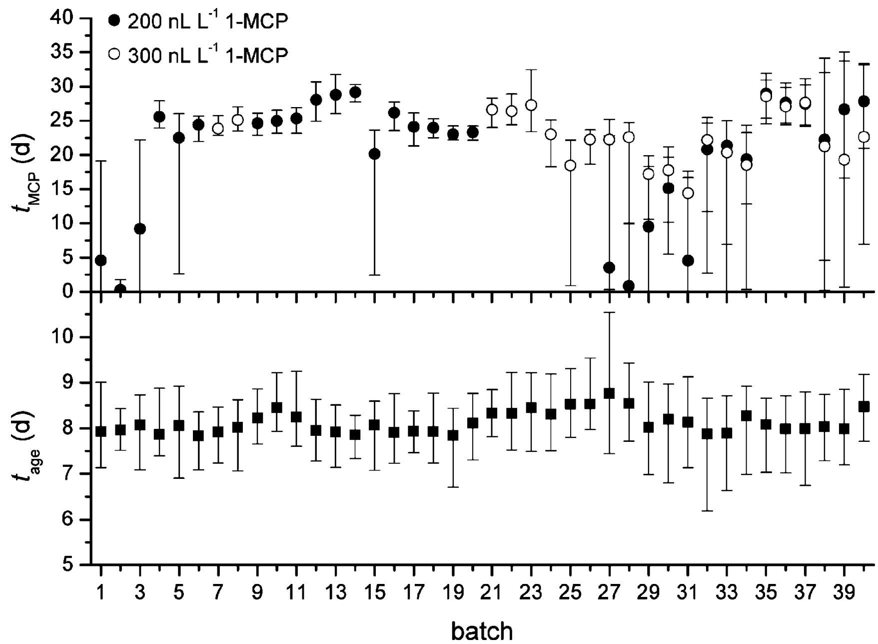

Fig. 2. Estimates of the fruit specific parameters t_{age} (the biological age of the fruit) and t_{MCP} (the efficiency of the 1-MCP treatment; both in d). The symbols indicate the average estimated value per batch while the bars enclose the whole range of values estimated per batch. Values were estimated per individual fruit keeping the remaining model parameters at the values indicated in Table 2.

parameters t_{age} and t_{MCP}. Per batch the distribution of the estimated t_{age} values for the individual fruit was checked for normality through their coefficients of correlation (D'Agostino and Stephens, 1986). For 70% of the batches t_{age} showed a normal distribution. The distributions for t_{MCP} were checked per (batch × treatment) combination. Only in 50% of the cases t_{MCP} was normally distributed. To give an idea about the variation in t_{age} and t_{MCP} between and within batches the ranges of estimated values were plotted in Fig. 2.

For fruit stored at 0 nL L^{-1} 1-MCP, t_{MCP} by definition was set to zero. The estimated t_{MCP} values estimated for the 200 nL L^{-1} 1-MCP and 300 nL L^{-1} 1-MCP treated fruit (Fig. 2) represent the varying efficiency of the 1-MCP application. For some batches the average effect of 200 nL L^{-1} 1-MCP was very low (e.g., batch 1–3). On the other hand, for batch 5 and 15 for instance, there was a clear effect of 200 nL L^{-1} 1-MCP but with a large variation between the individual fruit, with some fruit showing only limited effect. In contrast, some other batches showed only limited variation in

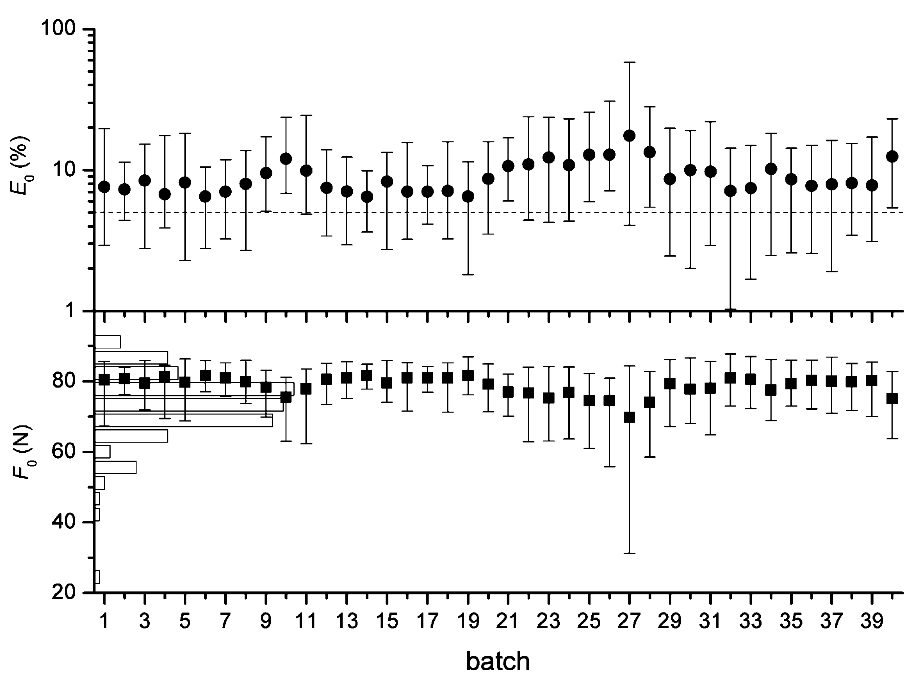

Fig. 3. Calculated values for firmness (F_0) and active enzyme level (E_0) at harvest calculated following Eq. (7) using the fruit specific parameters t_{age} and t_{MCP} from Fig. 2. The symbols indicate the average calculated value per batch while the bars enclose the whole range of values calculated per batch. The histogram represents the measured initial firmness levels at harvest based on 5 fruit measured per batch.

their t_{MCP} values (e.g., batch 19 and 20). Most of the time the effect of 300 nL L^{-1} 1-MCP treatment resulted in a comparable delay of softening to the 200 nL L^{-1} 1-MCP treatment with about 20–30 d (e.g., batches 32–37) while some batches showed on average a considerable lower effect of 200 nL L^{-1} 1-MCP treatment as compared to the 300 treatment (e.g., batch 27–29). In summary, t_{MCP} varied largely between and within batches often showing skewed distributions for fruit within a (batch × treatment) combination. The difference between the 200 nL L^{-1} 1-MCP and 300 nL L^{-1} 1-MCP treatment was not consistent.

As far as t_{age} is concerned, this parameter did not vary as much between batches (Fig. 2) and seemed to be more consistent in terms of the amount of variation within a batch. The estimated values of t_{age} are valid for an assumed T_{growth} = 22.2 °C. Depending on the choice of T_{growth} the estimated values for t_{age} will change as t_{age} is inversely related to T_{growth}. Most likely the actual temperature data for the various orchards will be different from T_{growth} = 22.2 °C affecting the estimated values for t_{age}. Ultimately, the maturity at harvest is given by the combined effect of T_{growth} and the fruit specific t_{age}. Using these two parameters, the firmness (F_0) and active enzyme level (E_0) at harvest were calculated following Eq. (7) (Fig. 3). In general, the simulated F_0 ranged between 53 and 89 N resembling fruit being firm at harvest. This is in agreement with the observation that the autocatalytic climacteric softening process of most avocado varieties (including 'Hass') only starts after harvesting, as they lack the ability to ripen on-tree (Sitrit et al., 1986). The simulated F_0 values were compared to the limited number of at-harvest measurements taken (five fruit per batch) showing general agreement.

The simulated E_0 values seem to be quite comparable between the batches as well (Fig. 3). However one should realise that the effect of E_0 on softening is not a linear effect. This is illustrated in Fig. 4 showing an artificial chain of 1-MCP treated fruit stored for 21 d at 5 °C followed by shelf-life at 21 °C. The different simulations were based on increasing t_{age} values resulting in increasingly softer fruit (lower F_0) with increasingly higher E_0 values. Fruit with higher F_0 and lower E_0 values took longer to soften. This is illustrated in Fig. 4 by the time needed to reach an arbitrary firmness level F_{crit} = 22 N. With E_0 dropping below 5%, an increasing gain in keeping quality is realised. Whether fruit have an E_0 of 5, 10 or 15% does not make much of a difference in terms of the time to reach F_{crit}.

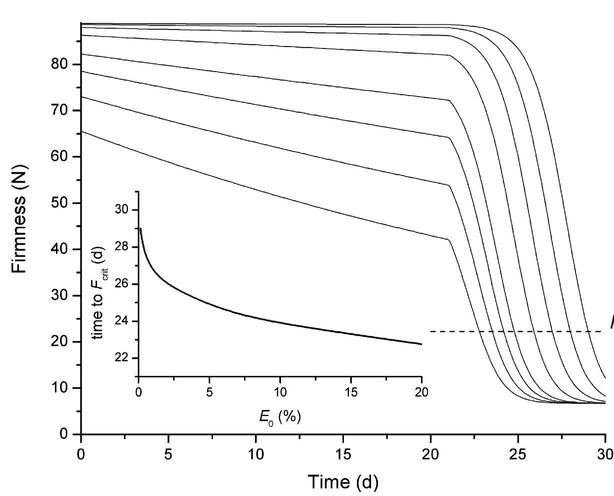

Fig. 4. Simulated firmness of 1-MCP treated 'Hass' avocado fruit exposed to an artificial handling chain 21 d storage at 5 °C followed by shelf-life at 21 °C. Fruit of different maturities (t_{age}) were used to show the effect of the initial level of E_0 on the time taken to reach an arbitrary firmness level F_{crit} = 22 N (inset figure).

Batches with on average low E_0 values (Fig. 2, batch 37) will have better storage potential than batches with on average high E_0 values (Fig. 2, batch 28, although the final performance also depends on the efficiency of the 1-MCP treatment as expressed by t_{MCP}.

4.3. Model fitness

In general, the model was able to describe the individual fruit behaviour quite well explaining in 80.4% of the cases more than 95% of the observed variation, in 14.2% of the cases between 90% and 95% of the observed variation and for only 5% of the fruit the model explained less than 90% of the observed variation.

As an illustration, the model fit is shown for two of the batches (Fig. 5). The model fit showed no obvious outliers resulting in straight lines when plotting the measured and modelled values against each other (Fig. 5). The results from Fig. 5 are exemplary for all of the 40 batches studied. The main limitation of the model can be seen when a batch of fruit reaches its lowest firmness level F_{fix}. The model assumes no variation on this value of F_{fix} while the actual fruit do show some small variation. However, the added value of including this source of variation in the model does not outweigh the increased complexity it would bring.

4.4. Monte Carlo simulations

The original aim of the developed model is to gain insight into the quantitative effects of 1-MCP on avocado softening. However, the developed model could be utilised for predictive purposes as well. To predict the behaviour of a representative batch of fruit, Monte Carlo simulations can be applied. Monte Carlo simulations consist of a large number of simulations using randomly drawn values for the different unknown model parameters from their expected distributions (Metropolis and Ulam, 1949; Eckhardt, 1987). So, each of the Monte Carlo model runs is based on a different set of random model parameter values. The Monte Carlo simulation results in confidence intervals for the predicted model behaviour.

To perform Monte Carlo simulation, one needs values for the fruit specific parameter values t_{age} and t_{MCP} given the value for T_{growth}. Starting from a T_{growth} of 22.2 °C, 10,000 parameter combinations were generated (Fig. 6) for the fruit specific parameters t_{age} and t_{MCP} taking into account the original observed variation (Fig. 2) and correlation (=0.18) in t_{age} and t_{MCP} values. The generated range and distribution in t_{age} values (Fig. 6) matched the observed values with an average t_{age} of 8.1 d. For t_{MCP} the generated values (Fig. 6) were based on the original values (Fig. 2) not discriminating between the 200 and 300 nL L^{-1} treatment as these showed no consistent differences. However, those t_{MCP} values estimated as not significantly different from zero were excluded. As a result the generated t_{MCP} values (Fig. 6) cover a wide range of 1-MCP effects ranging from 5 to 40 d with the main emphasis on t_{MCP} values around 24 d. Using the generated parameter values from Fig. 6, an artificial chain of either or not 1-MCP treated fruit stored for 21 d at 5 °C followed by 9 d shelf-life at 21 °C was simulated for two values of T_{growth} (21.2 and 22.2 °C) to generate different maturities at harvest. Based on these simulations, confidence intervals and mean, median and modus values were determined (Fig. 7).

For an increasing value of T_{growth} the firmness at harvest lowered from in average 86–79 N and showed an increased variation. This range in F_0 levels is similar to what was observed in the experimental data (see experimental data from Fig. 5) and is simulating the situation of fruit not always being harvested at exactly the same maturity and firmness level. Throughout shelf-life, the main

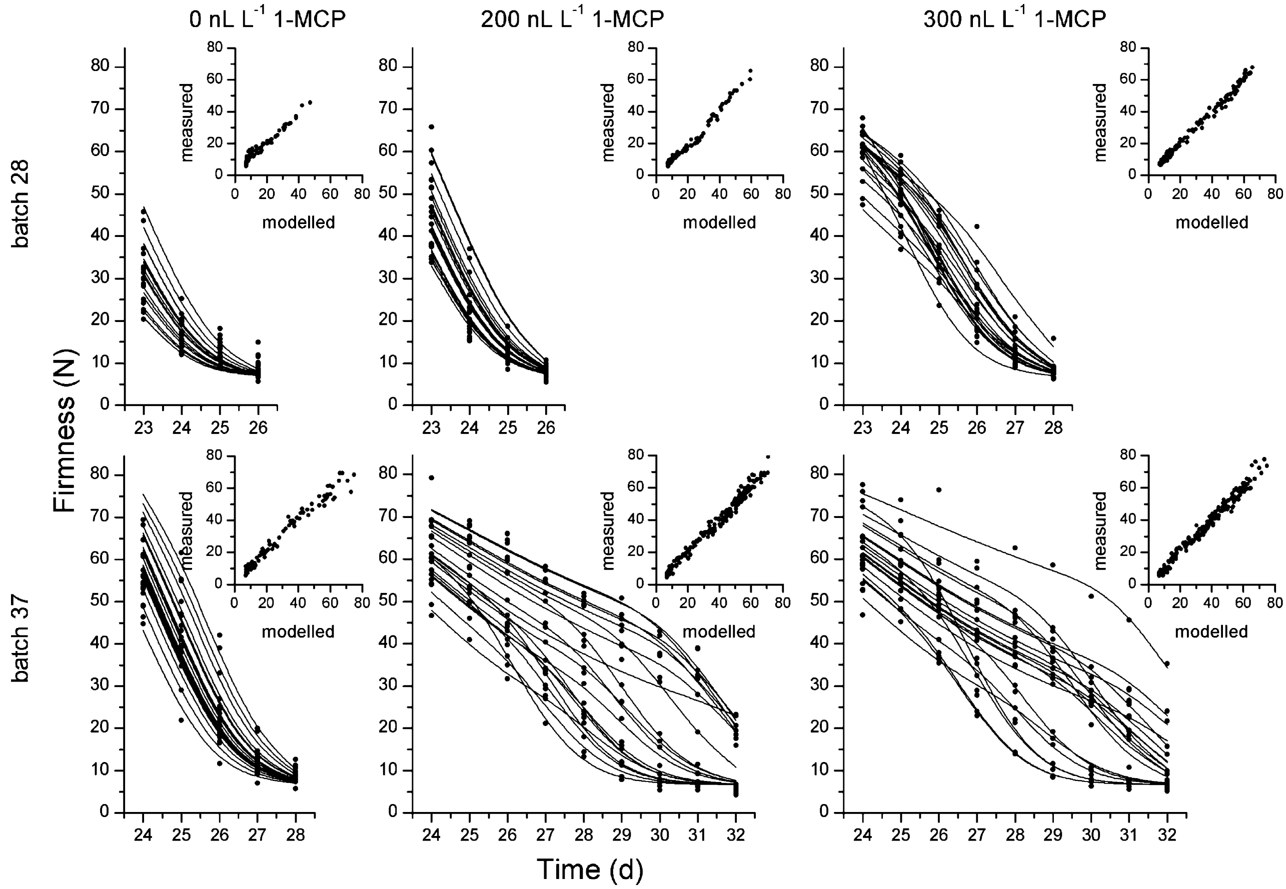

Fig. 5. Model fit of the firmness model for two batches of 'Hass' avocado followed during shelf-life at 22 °C after initial storage at 5 °C. At the start of storage, fruit were treated with either 0, 200 or 300 nL L^{-1} 1-MCP for 12 h. The dots represent the measured firmness while the lines represent the model outcome for each of the individual fruit from the batches. The generic model parameters used are given in Table 2 while the fruit specific model parameters t_{age} (the biological age of the fruit) and t_{MCP} (the efficiency of the 1-MCP treatment; both in d) where set to the values estimated for these particular avocados.

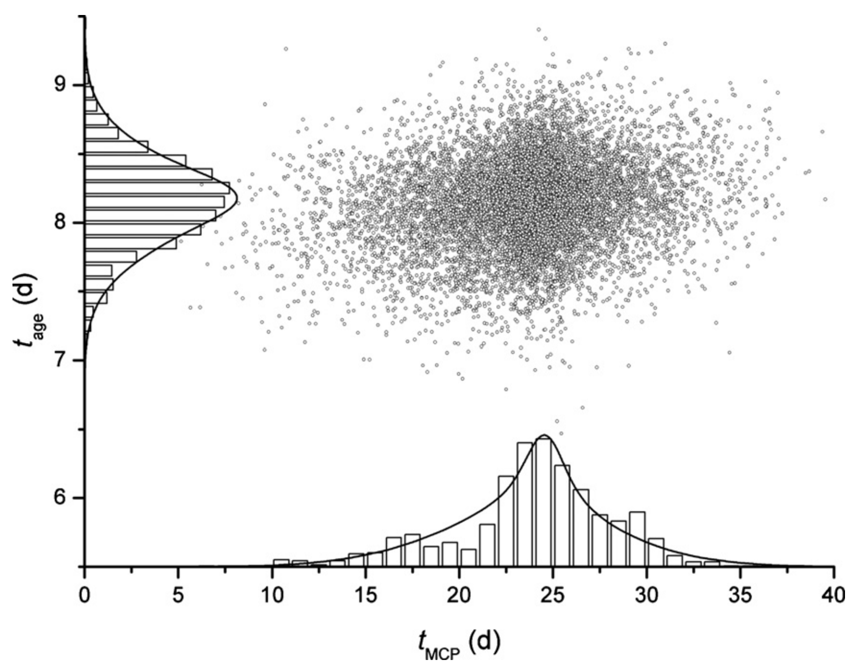

Fig. 6. Fruit specific model parameter values of t_{age} (the biological age of the fruit) and t_{MCP} (the efficiency of the 1-MCP treatment; both in d) generated for the Monte Carlo simulations. The bars represent the frequency distributions of the original 930 parameter value pairs estimated on the experimental individual fruit data and subsequently used for generating the Monte Carlo parameters. The curves represent the frequency distributions of the 10,000 newly generated parameter value pairs used for Monte Carlo simulations. The dots represent the actual 10,000 value pairs generated.

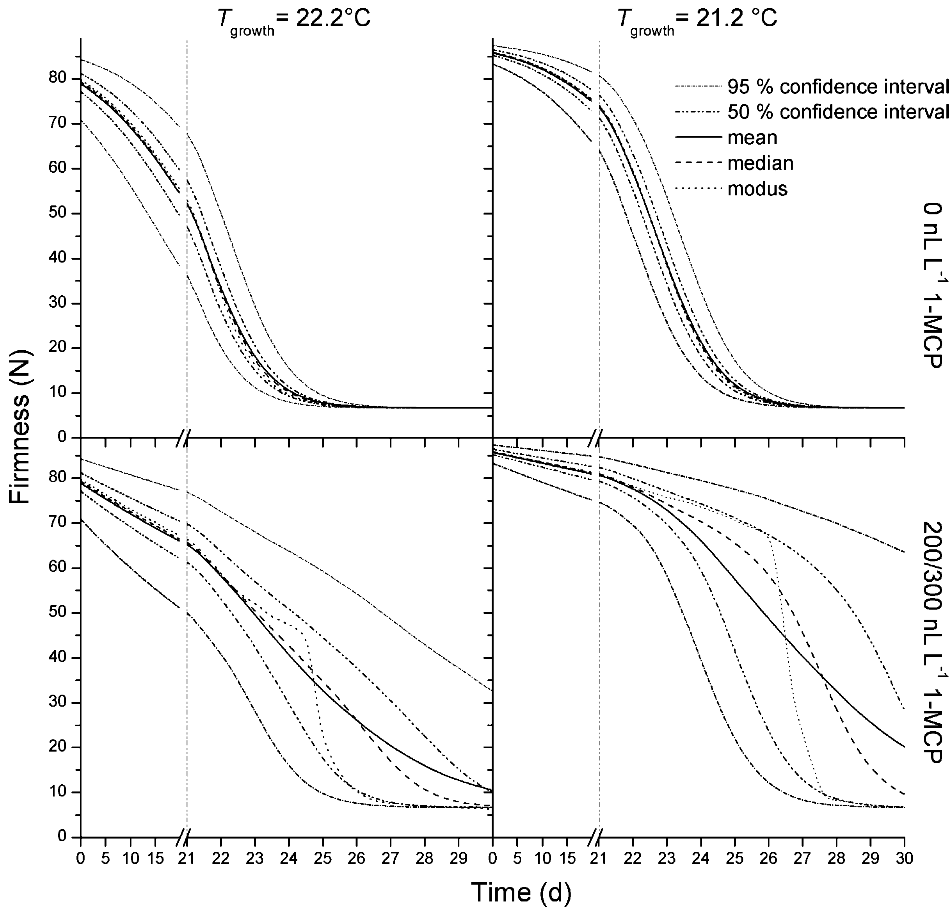

Fig. 7. Outcome of the Monte Carlo simulations of an artificial chain of either or not 1-MCP treated fruit stored for 21 d at 5 °C followed by 9 d shelf-life at 21 °C simulated for two values of T_{growth} (21.2 and 22.2 °C). The 4 × 10,000 Monte Carlo simulations are summarised by their 95% confidence interval, their 50% confidence interval and their mean, median and modus as indicated in the graph. The two values of T_{growth} (21.2 and 22.2 °C) were used to generate batches with different maturity levels at harvest.

part of the un-treated fruit (as indicated by the 50% confidence interval; Fig. 7) stayed within a relative narrow range of 10 N maximum while the 1-MCP treated fruit showed, depending on T_{growth}, a much wider firmness range. Especially for the treated fruit the mean, median and modus values do not always overlap and the confidence intervals are not symmetric, indicating that firmness is not normally distributed throughout time. For the fruit picked at the highest firmness (Fig. 7; T_{growth} = 21.2 °C) the gain in retaining firmness by applying 1-MCP is most rewarding though coming at the cost of an increasing amount of variation in firmness levels during shelf-life.

The modus of the simulated firmness shows an unexpected behaviour indicating a switch in peak location around 25–27 d. This is also illustrated by Fig. 8, showing the distribution in firmness levels of the treated fruit at three different days (day 22, 24 and 26). At day 22 firmness showed a unimodal distribution which by day 24 has turned into a bimodal distribution. Going from day 26 onwards, firmness slowly turns into a unimodal distribution again collecting all fruit into a single peak near the value of F_{fix}. The existence of the two peaks indicates the presence of two groups of fruit as was also observed in the experimental data (Fig. 5). Still the simulations were based on unimodal distributions for t_{age} and t_{MCP} (Fig. 6). This phenomenon is an inherent part of the softening process and is affected by the amount of correlation between t_{age} and t_{MCP}. In the case of a strong negative correlation, firm fruit (low t_{age}) would respond more strongly to 1-MCP (high t_{MCP}) retaining their firmness longer than initial soft fruit (high t_{age}) which would not respond as much to 1-MCP (low t_{MCP}). In such a case, any batch of avocado fruit would naturally develop a strong dichotomy in terms of firmness distribution in response to the 1-MCP treatment. In the case of a strong positive correlation between t_{age} and t_{MCP} such dichotomy would be leveraged out as initial firm fruit (low t_{age}) would not respond as much to 1-MCP (low t_{MCP}) and thus soften

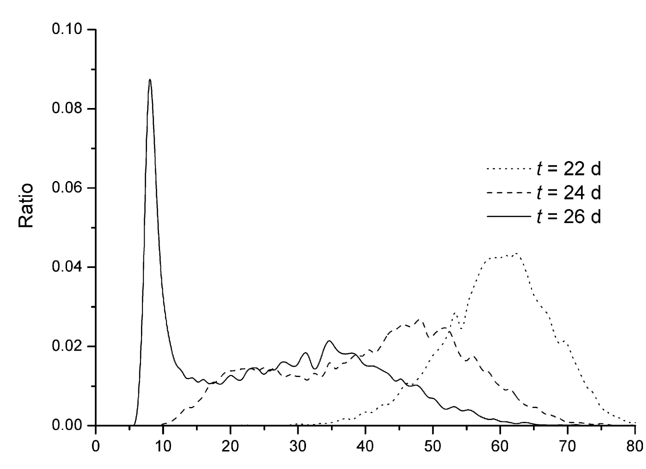

Fig. 8. Distribution of the firmness of 'Hass' avocados at days 22, 24 and 26 based on the Monte Carlo simulation from Fig. 7 for 1-MCP treated fruit with T_{growth} = 22.2 °C.

faster, overtaken initial soft fruit (high t_{age}) that would respond stronger to 1-MCP (high t_{MCP}). The current situation of almost no correlation (=0.18) between t_{age} and t_{MCP} results in the intermediate behaviour shown. Although the data showed no direct correlation between developmental stage (as expressed by t_{age}) and the efficiency of 1-MCP treatment (as expressed by t_{MCP}), one can imagine that the observed large variation in the efficiency of the 1-MCP treatment can be related to fruit properties such as the connectivity of the intercellular spaces and permeance of the skin affecting the accessibility of the tissue for 1-MCP.

4.5. Practical applications

To apply the model to actually predict the behaviour of a specific batch, specific values for the fruit parameters t_{age} and t_{MCP} and the batch parameter T_{growth} need to be obtained. As the model parameter t_{age} is a virtual parameter it cannot be measured directly. In the current research, dry matter content was determined for each of the batches studied. However, no relationship could be established between dry matter content and the average batch value for t_{age} (data not shown). This might be due to the fact that dry matter content was measured destructively on just a small subsample of each batch ignoring fruit to fruit variation. Also t_{age} and t_{MCP} did not show any clear trends as a function of harvest time. This might be due to the fact that the data came from different regions and the region effect might interact with a possible seasonal effect.

On the other hand, when firmness of the individual fruit at harvest is known, t_{age} of the individual fruit can be back-calculated using Eq. (7) given the value for T_{growth}. By combining this information on t_{age} with an expected distribution for t_{MCP} based on the variation observed in the current work, it is possible to make a prediction on the softening of a batch of fruit. Such an approach already enables batch-dedicated optimisation of the avocado handling chain based on the expected softening behaviour.

5. Conclusions

This paper has presented an accurate model for characterising the transient effect of 1-MCP in avocado fruit softening. The model allowed quantification of the variation in the efficiency of the 1-MCP treatment within and between batches of avocado fruit. However, these differences in 1-MCP response did not show a clear trend as a function of time of the year or dry matter content. Still, the variation between and within batches could be captured by the variation in developmental stage (as expressed by t_{age}) and efficiency of the 1-MCP treatment (as expressed by t_{MCP}). Starting from these two sources of variation the actual variation in the fruit response to 1-MCP could be described for over 95%.

The model can be used to predict postharvest softening of avocado facilitating the optimisation of the 1-MCP treatment to increase successful export of 1-MCP treated avocado fruit. However, both the experimental data and the model simulations showed that the gain in retaining firmness by applying 1-MCP comes at the cost of an increased variation in firmness during shelf-life. This can both be seen as a positive side effect, in that the market can provide avocados from different maturity levels at any given time, or as a negative side effect, in that the market cannot provide a homogeneous product and the logistic chain has to adapt itself to handling a wide variety of maturity levels. Therefore, the first prerequisite to extent shelf-life and to allow exporting to distant markets would be to pick the fruit at high enough firmness levels before considering the application of 1-MCP.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.postharvbio.2008.06.002.

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