Towards flexible management of postharvest variation in firmness of three apple cultivars

Gwanpua S.G.^a, Verlinden B.E.^b, Hertog M.L.A.T.M.^a, Van Impe J.^c, Nicolai B.M.^{a,b}, Geeraerd A.H.^a

^a *Division of Mechatronics, Biostatistics and Sensors (MeBioS), Department of Biosystems (BIOSYST), KU Leuven, W. de Croylaan 42, bus 2428, B-3001 Leuven, Belgium*

^b *Flanders Centre of Postharvest Technology, W. de Croylaan 42, 3001 Leuven, Belgium*

^c *Chemical and Biochemical Process Technology and Control Section (BioTeC), Department of Chemical Engineering, KU Leuven, W. de Croylaan 46, B-3001 Leuven, Belgium*

Abstract

Stochastic modeling provides a useful tool in managing biological variation in the postharvest chain. In the current study, the fruit-to-fruit variability in the postharvest firmness of apples was modeled. Apples from three cultivars (Jonagold, Braeburn, and Kanzi) were harvested at different levels of maturity, and stored at different temperatures and controlled atmosphere (CA) conditions. By using a kinetic model describing firmness breakdown as a function of time, temperature, controlled atmosphere conditions and endogenous ethylene concentration, the main stochastic variables were identified as the initial firmness and the rate constants for firmness breakdown and ethylene production. Treating these variables as random model parameters, the Monte Carlo method was used to simulate the propagation of the fruit-to-fruit variability in flesh firmness within a batch of apples during storage under different CA conditions and subsequent shelf life exposure. The model was validated using independent data sets from apples picked in a different season. The model developed in this study can be used to predict the probability of having apples of certain firmness after long term storage for different scenarios of temperatures and CA conditions.

Keywords: *Malus x domestica* (Borkh.); Biological variation; Stochastic modeling; Monte Carlo; Controlled atmosphere

1. Introduction

Consumers prefer products with uniform quality, based on important quality indicators such as color, size, soluble solids content and firmness. Postharvest handlers are usually faced with the difficult task of dealing with large variations in product quality due to inherent biological variations. Biological variation is a consequence of several factors related to both pre- and post-harvest treatments. For instance, apples picked on different dates will ripen differently during storage due to differences in maturity at harvest. Also, even for the same batch of produce, there is quite often fruit-to-fruit variation resulting from factors such as differences in shading, orientation of the fruit on the tree, types and levels of fungicides, etc. (Kingston, 1991). As a result, there is always a large variation in product's quality in a postharvest food chain. As illustrated by Carroll (2003) and Tijskens et al. (2003), biological variation cannot simply be treated as disturbances; rather it needs to be properly managed. It is, therefore, important to be able to predict how the initial variations in quality propagate along the whole chain.

Over the last decade, many authors have developed quality models incorporating biological variance to explain the propagation of biological variation in fruits (Tijskens et al., 2000; Hertog, 2002; Hertog et al., 2004b; Scheerlinck et al., 2004; Nicolai et al., 2006; Hertog et al., 2007; Mziou et al., 2009). Several approaches have been used in developing these models, such as mixed effects models (Lammertyn et al., 2003a; De Ketelaere et al., 2004), stochastic kinetic models (Hertog, 2002; Schouten et al., 2004; Tijskens et al., 2005; Tijskens et al., 2008), Monte Carlo simulations (De Ketelaere et al., 2004; Hertog et al., 2009), numerically solving the Fokker – Planck equation (Scheerlinck et al., 2004) and the variance propagation algorithm (Nicolai et al., 1998; Scheerlinck et al., 2004; Mziou et al., 2009). The use of mixed effects models and stochastic kinetic models depends on the availability of an analytical solution of the model equations describing the process. However, biological systems are usually so complex that even when the process is simplified, the resulting model equations usually do not have an analytical solution. Another prerequisite for obtaining an analytical solution of a set of differential equations is the assumption of constant boundary conditions, such as temperature and gas conditions, which is not realistic for a complete postharvest food chain.

Numerically solving the Fokker – Planck equation allows to calculate the propagation of the probability density function of random variables of a stochastic system. However, for equations describing complex systems, such as most biological processes, it is nearly impossible to solve the Fokker – Planck equation, even using numerical methods. In the variance propagation algorithm, which is a first order approximation of the Fokker – Planck equation, only the propagation of the mean and variance is predicted. Further, a common practice is to assume that for a linear system, if the initial stochastic variables are normally distributed, then the distribution remains normal, such that a normal probability density function can be used to predict the propagation of the probability density function (Mziou et al., 2009; Gwanpua et al., 2012a). In most cases, neither the stochastic variables are initially normal, nor are the equations describing the system linear. The Monte Carlo method is a more robust method when it comes to modeling a stochastic system. It involves repeated simulation of a process characterized by a system with random model parameters, using new values for the random model parameter(s) for each run of the simulations. Although the Monte Carlo method demands a relatively high computation time, it has an advantage over the other methods in that it is neither limited by the number of stochastic variables involved, nor by the complexity of the model equations describing the process. This method has successfully been used extensively in food engineering (Nicolai et al., 1999; Poschet et al., 2003; Pouillot and Delignette-Muller, 2010; Busschaert et al., 2011; Hoang et al., 2012). In postharvest science, Hertog et al. (2009) used the Monte Carlo method to model variability in the Hue color in tomatoes during different postharvest regimes, while De Ketelaere et al. (2004) used it to predict shelf life of tomatoes. The Monte Carlo method was also used to predict the firmness of mangoes during storage by De Ketelaere et al. (2006) and to explain softening of individual avocado fruits by Ochoa-Ascencio et al. (2009).

Flesh firmness is one of the most important quality indicators used in apple grading. Like other quality aspects of biological products, there is also a large variation in flesh firmness of apples at harvest. It is very important to be able to understand and predict how this initial variation in the firmness propagates throughout the apple cold chain. Most of the current models for apple firmness are only able to explain the evolution of the mean firmness (Hertog et al., 2001; Johnston et al., 2001;

Gwanpua et al., 2012b). The objective of this study is to use the Monte Carlo method to model and explain the fruit-to-fruit variability in flesh firmness within a batch of apples for three cultivars (Jonagold, Braeburn, and Kanzi) during controlled atmosphere (CA) storage and subsequent exposure to shelf life conditions. Furthermore, the model will be validated by independent data sets. Practical applications of the model will also be discussed.

2. Materials and methods

2.1. Fruit

A total of about 7500 apple fruits (*Malus x domestica* Borkh.) from three cultivars (Jonagold, Braeburn, and Kanzi) and two seasons (2008 – 2009 and 2009 – 2010) were used in this study. Apples were picked at three different stages of maturity corresponding to early, optimally and late picked apples. The early picked apples were picked about 2 weeks before the optimal picking dates, the optimally picked apples were harvested at the optimal picking dates, while the late picked apples were harvested about 2 weeks after the optimal picking dates. The optimal picking dates for the three cultivars were determined by the Flanders Centre of Postharvest Technology (Belgium), using a combination of starch stage, firmness, soluble solid content, background color, and size. The apples were harvested at the experimental station Proefcentrum Fruitteelt, Experimental Garden for Pome and Stone fruit (Sint – Truiden, Belgium).

2.2. Storage experiments

Each batch of apples was stored at 1 °C (and also at 4°C for the Kanzi apple), under different CA conditions (Fig. 1). CA was applied after pre-cooling for two days, except for the Braeburn apples in which the application of CA was delayed for three weeks to prevent incidents of browning disorders (Elgar et al., 1998). Storage was done for either 4 or 6 months and at the end of storage, the apples were placed in shelf conditions (20.8 kPa O₂, 0.03 kPa CO₂ and a temperature of 18 °C) for 18 days. Shelf life evaluations were performed before storage as well. The data collected for the apples picked in the 2009 – 2010 season were used for model calibration, while validation was done on the data collected for the apples picked in the 2008 – 2009 season, except for the Kanzi apples, of which the

data set for the 2008 – 2009 season was more suitable for model calibration (more storage conditions were used).

(Insert Fig. 1)

2.3. Measurements

The initial flesh firmness and ethylene production of eight fruits randomly selected from each batch of apples (i.e., apples of the same cultivar, picked on the same date) were measured immediately after harvest. After 4 months and 6 months of storage, the firmness and ethylene production of eight fruits randomly selected from the fruits stored under the different CA conditions were measured, such that depending on the apple cultivar, between 48 to 80 fruits (eight fruits per storage conditions) were measured at each sampling time. Also, fruits that had been stored for 4 months or 6 months, for all the different storage conditions, were placed at shelf life conditions for 18 days, during which the firmness and ethylene production of eight randomly selected fruits were measured at 6 days intervals.

Firmness was measured using an LRX Universal Testing Machine (Lloyd Instruments, UK), equipped with a load cell of 500 N. A self-cutting cylindrical plunger with surface of 1 cm² (diameter = 11.3 mm) was attached to the load cell and allowed to move at a constant speed of 8 mm s⁻¹ towards the fruit. The firmness was taken as the maximum force (N) needed for the plunger to penetrate the fruit to a depth of 8 mm. Two measurements were taken on the equator, one at the blush side and one at the green side, and the average was taken as the firmness value.

Ethylene emission was measured following the protocol described by Bulens et al. (2011). To measure the ethylene emission, each apple was initially enclosed in a separate jar of 1.7 L and flushed with humidified gas with the same composition as the atmosphere and temperature under which the apple was stored. The flushing was done for a period of 24 hours, to allow steady state to be attained between the headspace and the internal atmosphere of the apple. The inlet and outlet of the jars were then closed and 3 mL samples were withdrawn from the jars and analyzed by injecting into a CompactGC (Interscience, Louvain-la-Neuve, Belgium). Calibration was done by ethylene standards ranging from 50 ppb to 50 ppm. The temperature, the free volume of the jar and the pressure inside

the jar before sampling, were used to convert ethylene concentration (ppm) to nmol by using the ideal gas law. The ethylene emission ($\text{nmol kg}^{-1} \text{ s}^{-1}$) was obtained by doing a second measurement after a period of about 18 h. For ethylene emission measurements done at higher temperatures, the time interval between the first and the second measurements was 3 h because of the much higher rate of ethylene production within the fruit.

3. Model development

3.1. Mathematical modeling of firmness breakdown

The model equations used in this study to describe the loss of firmness during storage as a function of storage time, temperature and gas compositions were based on the firmness model developed by Gwanpua et al. (2012b). The assumption made in that study was that firmness breakdown in apple is a result of the breakdown of pectin by pectin degrading enzymes (Tijskens et al., 1998; Van Dijk et al., 2006; Róth et al., 2008), and that the activities of these enzymes depend on the internal ethylene concentration. The firmness model is represented by the following set of differential equation: Eqs. (1) – (3).

$$\frac{d[P]}{dt} = -k_{\text{pect}} [P][E_{\text{pect}}], \text{ with } [P](t=0) = [P]_0 \quad (1)$$

$$\frac{d[E_{\text{pect}}]}{dt} = k_{E_{\text{pect}}} [C_2H_4] - k_{E_{\text{deg}}} [E_{\text{pect}}], \text{ with } [E_{\text{pect}}](t=0) = [E_{\text{pect}}]_0 \quad (2)$$

$$\frac{d[C_2H_4]}{dt} = \frac{V_{m,C_2H_4} p_{O_2}}{K_{m,O_2,C_2H_4} + p_{O_2} \left(1 + \frac{p_{CO_2}}{K_{mu,CO_2,C_2H_4}} \right)} - P_{C_2H_4} A([C_2H_4] - [C_2H_4]_{\infty}), \quad (3)$$

$$\text{with } [C_2H_4](t=0) = [C_2H_4]_0$$

where $[P]$ (mmol m^{-3}) is the amount of unhydrolysed pectin; $[E_{\text{pect}}]$ (mmol m^{-3}) is the concentration of the pectin degrading enzyme, E_{pect} ; $[C_2H_4]$ (mmol m^{-3}) is the internal ethylene concentration; k_{pect} ($\text{m}^3 \text{ mmol}^{-1} \text{ d}^{-1}$), $k_{E_{\text{pect}}}$ (d^{-1}), and $k_{E_{\text{deg}}}$ (d^{-1}) are the rate constants for pectin breakdown, the

synthesis and the turnover of E_{pect} respectively; V_{m,C_2H_4} ($\text{mmol m}^{-3} \text{d}^{-1}$) is the maximum rate of ethylene production; K_{m,O_2,C_2H_4} (kPa) and K_{mu,CO_2,C_2H_4} (kPa) are the Michaelis-Menten constants for ethylene production and uncompetitive inhibition of ethylene production by carbon dioxide respectively; p_{O_2} (kPa) and p_{CO_2} (kPa) are the external partial pressure of oxygen and carbon dioxide respectively; $P_{C_2H_4}$ (m d^{-1}) is the ethylene permeability of the apple skin; A (m^2) is the surface area of the apple; $[C_2H_4]_{\infty}$ (mmol m^{-3}) is the ethylene concentration in the surrounding atmosphere, and t (d) is time.

Eq. (3) was modified to include the autocatalytic effect of ethylene production similar to the approach used by Génard and Gouble (2005), by assuming that the ethylene production was proportional to the square root of the concentration of ethylene. This modification of the model resulted in a higher percentage of the total variation of the measured data explained by the model. Also, the gas permeability of the apple skin varies from cultivar to cultivar (Ho et al., 2010). Therefore, different values of $P_{C_2H_4}$ are needed for different cultivars. As these could not be found in the current literature, $P_{C_2H_4}$ and A were lumped to a new parameter, $k_{C_2H_4}$ (d^{-1}), related to the rate of diffusion of ethylene from the apple to its surrounding. This new lumped parameter was estimated for each apple cultivar using experimental data of ethylene production during storage. Based on these two modifications, and assuming that the ethylene concentration of the surrounding air is negligible when compared to the internal concentration, Eq. (3) can be re-written as follows:

$$\frac{d[C_2H_4]}{dt} = \frac{V_{max,C_2H_4} p_{O_2} \sqrt{\frac{[C_2H_4]}{[C_2H_4]_{\text{ref}}}}}{K_{m,O_2,C_2H_4} + p_{O_2} \left(1 + \frac{p_{CO_2}}{K_{mu,CO_2,C_2H_4}} \right)} - k_{C_2H_4} [C_2H_4] \quad (4)$$

where $[C_2H_4]_{\text{ref}}$ is a reference ethylene concentration (1 mmol m^{-3}), and V_{max,C_2H_4} ($\text{mmol m}^{-3} \text{d}^{-1}$) is the new maximum rate of ethylene production in relation to the new autocatalytic factor. The autocatalytic

effect of $[C_2H_4]$ was expressed with respect to a reference value to ensure the units of Eq. (4) are balanced. It should be noted that the model for ethylene production in Eq. (4) is only valid for as long as the climacteric maximum is not reached, since the model will continue to predict a rise in internal ethylene concentration. This should not be considered as a practical limitation, since the climacteric maximum in most apple cultivars is only reached when the flesh firmness is far below the critical value for consumer acceptance (Johnston et al., 2001). Therefore, the current model is valid for extended storage of apples, and subsequent exposure to shelf conditions of 18°C in normal atmosphere for up to 18 days.

In the current study, no data on the pectin characterization and internal ethylene concentration were collected, only the firmness, F (N), and the ethylene emission, $\phi_{C_2H_4}$ (nmol kg⁻¹ s⁻¹), were measured. The relation between pectin and texture (in this case firmness) is a very complex one, which depends on the overall pectin structure (the molar mass distribution of the pectin molecules, the degree of methyl esterification of the pectin chain, the amount and type of neutral sugars attached to the pectin molecule, the amount of the different fractions of the pectin, etc. (Brummell et al., 2001; Lo Scalzo et al., 2005; Billy et al., 2008; Houben et al., 2011)). However, for modeling purposes, a direct relation is assumed between the overall pectin breakdown reactions and decrease in firmness. Firmness was assumed to be related to $[P]$ by a linear relation:

$$F = F_c + \gamma[P] \quad (5)$$

where F_c (N) is a fixed part of firmness that is not affected by enzymatic breakdown and γ (N mmol⁻¹ m³) is a conversion factor that relates the amount of unhydrolyzed pectin to firmness. The ethylene emission is related to the internal ethylene concentration by the following expression:

$$\phi_{C_2H_4} = k_{C_2H_4} [C_2H_4] / \beta \quad (6)$$

where β is a constant to convert the measured ethylene emission data in $\text{nmol kg}^{-1} \text{s}^{-1}$ to $\text{mmol m}^{-3} \text{d}^{-1}$, taking into account the average density of the fruit. All rate constants were assumed to be related to temperature according to the Arrhenius model Eq. (7).

$$k_i = k_{i,\text{ref}} \exp\left(\frac{E_{a,i}}{R} \left(\frac{1}{T_{\text{ref}}} - \frac{1}{T}\right)\right) \quad (7)$$

where k_i is a rate constant, having $k_{i,\text{ref}}$ as a reference value at a certain reference temperature T_{ref} (283.15 K); $E_{a,i}$ (J mol^{-1}) is the activation energy for the respective reactions; R ($8.3144 \text{ J mol}^{-1} \text{K}^{-1}$) is the universal gas constant, and T (K) is the temperature.

3.2. Introducing biological variations

3.2.1. Identification of stochastic model parameters

Once the deterministic part of the model (Eqs. (4) to (7)) describing softening was developed, the first step in incorporating biological variation was to identify the main parameters responsible for the fruit-to-fruit variation in softening behavior. These parameters will be referred to as the stochastic model parameters. In principle all the model parameters and initial values could be treated as random. However, doing this would increase the complexity of the problem, and much more data would be required to get the distributions of all these stochastic parameters. Identification of the most important stochastic model parameters was done using assumptions based on knowledge of the kinetics involved in firmness breakdown. The sensitivity of the model with respect to changes in the values of the identified stochastic parameters was investigated by performing a one-parameter-at-a-time relative sensitivity analysis (Lammertyn et al., 2003b). This was done by changing each model parameter with 10 %, while keeping all other model parameters fixed, and subsequently calculating the normalized partial derivatives of the model output (predicted firmness) with respect to the stochastic model parameters. Only the parameters with high relative sensitivity were considered as the main stochastic parameters in the model.

3.2.2. Monte Carlo simulations

The Monte Carlo method is a numerical technique used to solve stochastic problems. It is based on repeated simulations of a model system with random model parameters, in which a new set of values for the random model parameters is used for each run of the simulations. In generating the sets of random parameter values used for each run, it is important that the correlations between the random variables are taken into account to avoid unrealistic simulation outputs (Hertog et al., 2009). Generating Gaussian correlated random variables can easily be done using the Cholesky decomposition of the covariance matrix (Rubinstein 1981). However, the model parameters for the firmness model used in this study were not normally distributed. An algorithm developed by Hertog et al. (2009) for randomly generating correlated non-Gaussian model parameters was used. In this algorithm, the non-Gaussian distributed parameters are first fitted to appropriately transformed normal density functions. Gaussian correlated random parameters are then generated using the Cholesky decomposition algorithm. Finally, the randomly generated Gaussian parameters are transformed into their corresponding values in the original non-Gaussian parameter space.

1000 random sets of correlated parameters were generated to carry out 1000 Monte Carlo runs, using using a public domain Matlab (The Mathworks Inc., Natick, USA) application, OptiPa (Hertog et al., 2007b).

3.2.3. Rescaling of distributions

The softening behavior could not be followed for individual fruits because firmness was measured destructively. As a consequence, mean data were used to estimate the fruit-to-fruit variability within a batch, such that the variations in the estimated values of the initial firmness were therefore generally smaller than those of the measured initial firmness. To correct for these differences, the distributions of the estimated random parameter, F_0 , were *rescaled* based on the measured initial firmness, using Eq. (8).

$$X_2 = \left(X_1 - \overline{X_1} \right) \frac{\sigma_{X,2}}{\sigma_{X,1}} + \overline{X_2} \quad (8)$$

where X_1 are the observations of the original distribution, with mean and standard deviations $\overline{X_1}$ and $\sigma_{X,1}$ respectively; X_2 are the observations of the *rescaled* distribution; $\overline{X_2}$ and $\sigma_{X,2}$ are the mean and standard deviations of the rescaling distributions.

Also, during model validation, the propagation of the probability density function of the firmness is predicted for the validation data set using the mean and standard deviations of the corresponding measured initial firmness to *rescale* F_0 using Eq. (8).

3.3. Model calibration

The model was calibrated using the data obtained from the 2009 – 2010 season for Jonagold and Braeburn apples and data obtained from the 2008 – 2009 season for Kanzi apples. $[P]_0$ and $[C_2H_4]_0$ were calculated from the measured initial firmness and ethylene production (Table 1) using Eqs. (5) and (6) respectively.

(insert Table 1)

$[E_{pect}]_0$ depends on the apple cultivar, and also the maturity at harvest. Therefore, different values of $[E_{pect}]_0$ are needed for Braeburn, Jonagold and Kanzi, and for the different picking dates. To simplify this, $[E_{pect}]$ was normalized to $[E_{pect}]'$ by dividing by $[E_{pect}]_0$, such that in all cases $[E_{pect}]'_0$ is equal to 1. In normalizing $[E_{pect}]$, new forms of Eqs. (1) and (2) were obtained and this is shown in Eqs. (9) and (10) respectively.

$$\frac{d[P]}{dt} = -k'_{pect} [P][E_{pect}]', \text{ with } [P](t=0) = [P]_0 \quad (9)$$

$$\frac{d[E_{pect}']}{dt} = k'_{E_{pect}} [C_2H_4] - k_{E_{deg}} [E_{pect}]', \text{ with } [E_{pect}'] (t=0) = [E_{pect}]'_0 = 1 \quad (10)$$

$$\text{where } [E_{\text{pect}}]' = \frac{[E_{\text{pect}}]}{[E_{\text{pect}}]_0}; k'_{\text{pect}} = k_{\text{pect}} [E_{\text{pect}}]_0; k'_{E_{\text{pect}}} = \frac{k_{E_{\text{pect}}}}{[E_{\text{pect}}]_0}$$

Because of the high correlation between the rate constant of a reaction and its activation energy – typical for such exponential relationships – $k_{i,\text{ref}}$ and $E_{a,i}$ could not be estimated together. Before model calibration, approximate values of $E_{a,i}$ were obtained based on initial model fitting, while γ was fixed at value of $1 \text{ N mmol}^{-1} \text{ m}^3$. It should be noted that the value of γ does not influence the estimated value of the other model parameters, since it is only a conversion factor relating firmness (N) to the amount of unhydrolyzed pectin (mmol m^{-3}). The values of β for the different apple cultivars were calculated using the measured average density of the fruit (883 kg m^{-3} , 833 kg m^{-3} and 914 kg m^{-3} for Braeburn, Jonagold and Kanzi respectively). $E_{a,i}$, β and γ were then kept fixed, while all other model parameters in Eqs. (4) – (10) were estimated by one step parameter estimation (per cultivar) using OptiPa (Hertog et al., 2007b).

In the initial model calibration, common values of the model parameters were estimated for each batch of fruits (i.e., apples of the same cultivar, harvested on the same date). From the assumption that generic model parameters are valid for any batch studied while the parameters identified as being random are fruit specific, a second model calibration was done in which the experimental data was re-fitted, estimating only the ‘fruit-specific’ values for the stochastic model parameters while keeping all other model parameters fixed at the values obtained during initial model calibration.

In this way, for each of the stochastic parameters a distribution is obtained characterizing the observed fruit-to-fruit variation of firmness within a batch of apples, including the covariance structure between them. This information was later used in generating the random model parameters used for the Monte Carlo simulations.

4. Results and discussion

4.1. Average behavior of firmness

The model could explain 89 %, 90 % and 77 % of the total variation in the average firmness and ethylene production in the Jonagold, Braeburn and Kanzi apples respectively. The estimates of the model parameters, together with their standard error, for all three apple cultivars are shown in Table 2. The mean values of the measured firmness, plotted together with the simulated values, and the mean values of the measured ethylene production, plotted together with the simulated values for some selected storage conditions for all three apple cultivars are shown in Fig. 2 and 3 respectively. Only the plots of selected conditions were shown in Fig. 2 and 3 because the complete data set was too large to be presented.

(insert Table 2)

(insert Fig. 2)

(insert Fig. 3)

Softening was generally inhibited during low temperature CA storage. The apples that were stored under an atmosphere with higher O₂ level showed more rapid softening. The high rate of firmness breakdown in Jonagold could be related to its high rate of ethylene emission (Fig. 3), while Braeburn apples with a lower rate of ethylene emission, when compared to Jonagold, still showed rapid softening during CA storage. This suggests that the synthesis of pectin degrading enzymes in Braeburn apples have a higher sensitivity to ethylene. Despite the rapid softening of Braeburn apples, it stayed firmer than the Jonagold apples at the end of CA storage because the firmness of Braeburn at harvest was much higher than that of Jonagold. Kanzi apples did not undergo much softening during storage and even when placed under shelf conditions, and also showed very low ethylene production rates (about 30 times lower compared to Jonagold and 10 times lower compared to Braeburn). When the apples were placed in shelf life conditions, there was a rapid increase in the rate of softening due to the high temperature, high O₂ concentration and low CO₂ concentrations in air.

The model for softening used in this study could not well explain the postharvest evolution of the mean firmness of the Kanzi apples, suggesting that other aspects not covered by the model could play a very important role in the softening of this particular apple cultivar. Also, looking at the mean data in

Fig. 2 for the Kanzi apples, the firmness appears to be increasing during shelf life. This observation is most likely not a consequence of any biological changes occurring within the fruit, but may be due to fruit-to-fruit biological variability or some sort of toughening of the tissues related to water loss (decrease in turgor pressure). Bajema (1995) reported that a larger deformation was needed to induce failure with decreasing turgor which might result in a higher force observed.

In Fig. 3, the initial increase in the simulated ethylene production for Braeburn apples is related to the fact that the introduction of CA during storage was delayed. Furthermore, increased rate of firmness loss was always associated with an increased ethylene production for all three apple cultivars. Similar observations were made by Johnston et al. (2001).

4.2. Biological variation in firmness

4.2.1 Sources of variation

The initial value of the firmness and ethylene production, which are related to the maturity at harvest, will influence the rate of softening during storage (Shafiq et al., 2011; Bulens et al., 2012; Gwanpua et al., 2012b). Since apples picked even on the same date have different initial firmness and ethylene production, and will consequently soften at different rates, F_0 and $\phi_{C_2H_4,0}$ were considered to be stochastic variables. Also, the activity of the cell wall degrading enzymes present at harvest should be treated as a random parameter when dealing with variability in softening. This is because depending on the maturity at harvest, different apples will have different amount/activity of these enzymes and will therefore show different rates of postharvest softening. However, since in the current model the enzyme concentration was normalized by dividing with the initial concentration of the enzyme (meaning the relative amount of enzyme present at harvest is always equal to 1), the effect becomes coupled to the rate constants (Eq. (9) and (10)). Therefore, the rate constants were treated as random parameters. Furthermore, it was assumed that for a particular cultivar, all apples will soften to the same final firmness, hence F_c was treated as a generic parameter. Since the Michaelis-Menten constants (K_{m,O_2,C_2H_4} and K_{mu,CO_2,C_2H_4}) are properties of the substrate, they were treated as non-

336 random parameters. Finally, all activations energies were kept at their fixed values. F_0 , $\phi_{C_2H_4,0}$, $k'_{\text{pect,ref}}$
337 and $V_{\text{max},C_2H_4,\text{ref}}$ were therefore identified as the main random parameters in the firmness model.

338 The maximum relative sensitivity of the model output (firmness) to the different random model
339 parameters is shown in Fig 4.

340 (insert Fig. 4)

341 Fig. 4 shows that $\phi_{C_2H_4,0}$ does not significantly influence the predicted firmness in all the three apple
342 cultivars. To further confirm if indeed the predicted firmness was not sensitive to this parameter,
343 $\phi_{C_2H_4,0}$ was changed by 100% and the change in firmness predicted. Again, the firmness did not
344 change significantly (result not shown). This means that even for very large variations in $\phi_{C_2H_4,0}$ (like
345 those shown in Table 1), the predicted firmness will almost stay the same. The random model
346 parameters used for the stochastic simulations were therefore limited to F_0 , $k'_{\text{pect,ref}}$, and $V_{\text{max},C_2H_4,\text{ref}}$.

347 Fig. 5 shows how the estimated values of the stochastic parameters varied with picking dates for all
348 three cultivars. To investigate if there was an effect of picking on the values of these parameters, a
349 one-way analysis of variance (ANOVA) was carried out and ranking was done using the Tukey's
350 Studentized Range test. The results are indicated on Fig. 5 by letters A, B, and C, in which mean
351 values with the same letter were not significantly different (at 5% significance level). From the results,
352 F_0 was picking date dependent for all three cultivars, with early picked apples generally having larger
353 initial firmness values than the late picked ones. There was no significance difference in the $k'_{\text{pect,ref}}$
354 value for the different picking dates for Braeburn. For Jonagold, the $k'_{\text{pect,ref}}$ value for the early picked
355 apples was different from that for the late picked apples, but there was no difference between the early
356 and optimally picked apples, and also between the optimally and late picked apples. For the Kanzi
357 apples, the $k'_{\text{pect,ref}}$ value was different between the early picked and the optimally picked apples,

between the early and the late picked apples, but not between the optimally and late picked apples. The $V_{max,C_2H_4,ref}$ value was not significantly different between picking dates for all three apple cultivars.

The fruit-dependent values of the random model parameters were used to obtain the frequency distributions and the covariance matrix of the random model parameters. Fig. 6 shows the covariance structure and frequency distribution plots of the random model parameters for the Jonagold apples, as an example. As can be seen from this figure, the model parameters are both non-Gaussian and also correlated with each other. This information was used in generating the random model parameters that were used for each run of the Monte Carlo simulations. Random model parameters generation and Monte Carlo simulations were done using OptiPa (Hertog et al., 2007b).

(insert Fig. 5)

(insert Fig. 6)

4.2.2. Propagation of biological variability in firmness

The propagation of the probability distribution was obtained from the output of the Monte Carlo simulation. From this, the mean firmness and different prediction intervals (PI) were calculated. In Fig. 7, the mean firmness, the 95 and 99% PI calculated for 1000 Monte Carlo simulations, for different storage conditions are plotted together with the measured firmness of the calibration data sets for optimally picked Jonagold, Braeburn and Kanzi apples respectively.

(Insert Fig. 7)

A general trend in Fig. 7 is that the initial fruit-to-fruit variability in firmness within a batch increased during storage. For Kanzi apples, which softened the least amongst the cultivars, the variability in the firmness stayed almost constant during storage. As a result of rapid breakdown in firmness, the variability of the firmness was generally highest during shelf life. Amongst the three cultivars, the variability in firmness during shelf life was highest for the Braeburn apples because they softened most rapidly when compared to Jonagold and Kanzi. Fig. 7 also shows that during extended shelf life

exposure, the distribution of firmness becomes skewed towards the final firmness (F_c), since all the apples softened to this final firmness value. In principle the firmness of some of the apples may get beyond this value, but this usually occurs when senescence has begun, an aspect that is not covered by the model. It should be noted, however, that the critical value of firmness for consumer acceptance is usually much higher than F_c . For example, the critical firmness for export quality apples in Belgium is 65 N for Jonagold, while the F_c value obtained in this study was about 39 N.

Most of the data points were found within the 95 % PI, as predicted by the Monte Carlo simulations. This suggests a good agreement between the predicted variability and the variability in the measured firmness data.

4.3. Model validation

The introduction of mathematical modeling in the field of postharvest sciences has been motivated in part by the need to provide a tool that can be used to predict what-if scenarios. This means that such models should be valid even when applied to conditions similar but different from those under which the models were developed. To further validate the model, it was used to predict the fruit-to-fruit variability in postharvest firmness for apples picked in a different season. The initial values of firmness for the validation data set were used to rescale the distribution of the initial values (using Eq. (8)), while the values of all the other model parameters were kept the same as determined during calibration. The validation results for the different cultivars are shown in Fig. 8 and Table 3.

(Insert Fig. 8)

Note that in Fig. 8 only simulations for storage at 1 °C, 2 % O₂ and 2 % CO₂ for late picked apples are shown. Similar results were obtained for storage under different conditions. A summary of the percentage of measured firmness data within the 95 and 99 % PI of 1000 Monte Carlo simulations for the validation data sets is presented Table 3. Looking at this table, most of the firmness data measured for individual fruits fell within the 99% PI of the model, suggesting that the model developed in the

current study can be used for predicting the fruit-to-fruit variability in firmness for a batch of apples stored under similar but different conditions from the ones under which the model was developed.

4.4. Practical applications

It is a common practice for apple growers to take out apples from storage only when prices are high. Care needs to be taken to ensure that the apples are not left too long in storage, otherwise some apples may soften below the critical value required for sales. The model developed in this study can be used to predict the propagation of the probability distribution of firmness within a batch of apple during storage and to perform hypothesis testing. To illustrate this, let us consider a situation in which the Jonagold apples picked in the 2009 – 2010 season were stored at three different temperatures (1, 2, or 3 °C), under the same CA conditions (1 % O₂ and 2.5 % CO₂), for a period of 9 months. If we assume that the critical firmness value in order to be sold as a high grade apple is 65 N for Jonagold, then the evolution of the probability that the apples in the storage have a firmness value higher than the critical firmness value is shown in Fig. 9A.

(Insert Fig. 9)

From Fig. 9A, there is almost a complete assurance that after four months of storage the firmness of apples stored at any of the three temperatures will be above the critical value of 65 N. This means that if apples will be sold within four months, storing at 3 °C wouldn't be that different as compared to storing at 1 °C. To see the effect of storing at higher temperatures on the shelf life, a critical firmness value for consumer acceptance was defined to be 45 N, and the evolution of probability that apples stored at the three different temperatures for four months does not soften below this critical value when subsequently placed in shelf life conditions (18°C and normal air) is shown in Fig. 9B. From Fig. 9B, about 97% of the apples that were stored at 3°C will still be firmer than 45N after 14 days of shelf life. This is not so different from the apples that were stored at 1°C, in which about 98.5% still remain firmer than this critical value for consumer acceptance. However, other aspects need to be considered such as the effect on other quality parameters, such as core browning, that was reported to have a higher incidence in Braeburn apples (Lau et al., 1998) and 'Conference' pears (Verlinden et al.,

2002) stored at high temperatures. Another strategy could be that apples that are intended to be sold first are stored at a higher temperature, and those that will stay in storage for an extended period, at the very low temperature. Such flexible chain management techniques can significantly reduce energy cost.

Conclusions

The fruit-to-fruit variability in firmness within a batch of apple present at harvest generally increases during storage. This is more significant for apple cultivars that show rapid softening during storage. Kanzi apples produce very limited amount of ethylene, and consequently this cultivar is able to maintain its firmness during extended CA storage. Also, the low rate of softening in Kanzi apples means the initial variation in firmness is almost maintained throughout CA storage. Variability in firmness along the apple cold chain is largest when the apples are placed in shelf life conditions, and this is related to the rapid breakdown occurring within the fruit during shelf life. Kanzi apples appear to toughen when placed in shelf life. The toughening might be related to a decrease in cell turgor pressure resulting from water loss when the fruits are exposed to shelf life conditions with high temperatures and low air humidity. This was not observed for other cultivars, probably because their rate of softening was so high that the effect of water loss is overshadowed.

The model developed in the current study is valid for a range of conditions, including seasonal differences. It provides a useful tool for proper and flexible management of the apple chain, and to explain the postharvest fruit-to-fruit biological variation of firmness in apples. Although the model was developed only for firmness loss in Jonagold, Braeburn and Kanzi apples, the same model can be extended to other apple cultivars and also for different quality parameters using the same approach.

Acknowledgements

This publication has been produced with the financial support of the European Union (grant agreement FP7/2007-2013 - Frisbee). The opinions expressed in this document do by no means reflect the official opinion of the European Union or its representatives.

References

- Bajema, R.W., 1995. Measurement and analysis of impact failure in potato tuber tissue. Ph.D. dissertation. College of Engineering and Architecture Program in Engineering Science, Washington State University, Pullman, WA, p. 99164.
- Billy, L., Mehinagic, E., Royer, G., Renard, C.M.G.C., Arvisenet, G., Prost, C., Jourjon, F., 2008. Relationship between texture and pectin composition of two apple cultivars during storage. *Postharvest Biol. Technol.* 47, 315 – 324.
- Brummell, D.A., Harpster, M.H., 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Mol. Biol.* 47, 311–340.
- Bulens, I., Poel, B.V. de, Hertog, M.L., Proft, M.P.D., Geeraerd, A.H., Nicolaï, B.M., 2011. Protocol: An updated integrated methodology for analysis of metabolites and enzyme activities of ethylene biosynthesis. *Plant Methods* 7, 17.
- Bulens, I., Van de Poel, B., Hertog, M.L.A.T.M., De Proft, M.P., Geeraerd, A.H., Nicolai, B.M., 2012. Influence of harvest time and 1-MCP application on postharvest ripening and ethylene biosynthesis of Jonagold apple. *Postharvest Biol. Technol.* 72, 11–19.
- Busschaert, P., Geeraerd, A., Uyttendaele, M., Van Impe, J., 2011. Sensitivity Analysis of a Two-Dimensional Quantitative Microbiological Risk Assessment: Keeping Variability and Uncertainty Separated. *Risk Analysis*. 31, 1295–1307.
- Carroll, R.J., 2003. Variances Are Not Always Nuisance Parameters. *Biometrics* 59, 211–220.
- De Ketelaere, B., Lammertyn, J., Molenberghs, G., Desmet, M., Nicolaï, B., De Baerdemaeker, J., 2004. Tomato cultivar grouping based on firmness change, shelf life and variance during postharvest storage. *Postharvest Biol. Technol.* 34, 187–201.

479 De Ketelaere, B., Stulens, J., Lammertyn, J., Cuong, N.V., De Baerdemaeker, J., 2006. A
 480 methodological approach for the identification and quantification of sources of biological variance in
 481 postharvest research. *Postharvest Biol. Technol.* 39, 1–9.

482 Elgar, H.J., Burmeister, Watkins, C.B., 1998. Storage and handling effects on a CO₂-related internal
 483 browning disorder of Braeburn apple. *HortScience* 33, 719–722.

484 Génard, M., Gouble, B., 2005. ETHY. A Theory of Fruit Climacteric Ethylene Emission. *Plant*
 485 *Physiol.* 139, 531–545.

486 Gwanpua, S.G, Verlinden, B., Hertog, M., Van Impe, J., Nicolai, B., Geeraerd, A., 2012a. A
 487 stochastic model for firmness loss in Braeburn apples during controlled atmosphere storage.
 488 International conference of agricultural engineering CIGR-AgEng 2012. Valencia Conference Centre,
 489 Valencia, Spain, 8-12 July 2012.

490 Gwanpua, S.G., Verlinden, B.E., Hertog, M.L.A.T.M., Bulens, I., Van de Poel, B., Van Impe, J.,
 491 Nicolaï, B.M., Geeraerd, A.H., 2012b. Kinetic modeling of firmness breakdown in “Braeburn” apples
 492 stored under different controlled atmosphere conditions. *Postharvest Biol. Technol.* 67, 68–74.

493 Hertog, M.L.A.T.M., 2002. The impact of biological variation on postharvest population dynamics.
 494 *Postharvest Biol. Technol.* 26, 253–263.

495 Hertog, M.L.A.T.M., Ben-Arie, R., Róth, E., Nicolaï, B.M., 2004a. Humidity and temperature effects
 496 on invasive and non-invasive firmness measures. *Postharvest Biol. Technol.* 33, 79–91.

497 Hertog, M.L.A.T.M., Nicholson, S.E., Banks, N.H., 2001. The effect of modified atmospheres on the
 498 rate of firmness change in “Braeburn” apples. *Postharvest Biol. Technol.* 23, 175–184.

499 Hertog, M.L.A.T.M., Lammertyn, J., Desmet, M., Scheerlinck, N., Nicolaï, B.M., 2004b. The impact
 500 of biological variation on postharvest behavior of tomato fruit. *Postharvest Biol. Technol.* 34, 271–
 501 284.

502 Hertog, M.L.A.T.M., Lammertyn, J., De Ketelaere, B., Scheerlinck, N., Nicolaï, B.M., 2007a.
 503 Managing quality variance in the postharvest food chain. *Trends Food Sci. Technol.* 18, 320–332.

504 Hertog, M.L.A.T.M., Scheerlinck, N., Nicolaï, B.M., 2009. Monte Carlo evaluation of biological
 505 variation: Random generation of correlated non-Gaussian model parameters. *J. Comput. Appl. Math.*
 506 223, 1–14.

507 Hertog, M.L.A.T.M., Verlinden, B.E., Lammertyn, J., Nicolaï, B.M., 2007b. OptiPa, an essential
 508 primer to develop models in the postharvest area. *Comput. Electron. Agric.* 57, 99–106.

509 Ho, Q.T., Verboven, P., Verlinden, B.E., Schenk, A., Delele, M.A., Rolletschek, H., Vercammen, J.,
 510 Nicolaï, B.M., 2010. Genotype effects on internal gas gradients in apple fruit. *J. Exp. Bot.* 61, 2745 –
 511 2755.

512 Hoang, M.H., Flick, D., Derens, E., Alvarez, G., Laguerre, O., 2012. Combined deterministic and
 513 stochastic approaches for modelling the evolution of food products along the cold chain. Part II: A
 514 Case Study. *Int. J. Refrig.* 35, 915–926.

515 Houben, K., Jolie, R. P., Fraeye, I., Van Loey, A. M., Hendrickx, M. E., 2011. Comparative study of
 516 the cell wall composition of broccoli, carrot, and tomato: structural characterization of the extractable
 517 pectins and hemicelluloses. *Carbohydr. Res.* 346, 1105–1111

518 Johnston, J.W., Hewett, E.W., Hertog, M.L.A.T.M., Roger Harker, F.R., 2001. Temperature induces
 519 differential softening responses in apple cultivars. *Postharvest Biol. Technol.* 23, 185–196.

520 Kingston, C.M. 1991. Maturity indices for apple and pear. *Hort. Rev.* 13, 407–432.

521 Lammertyn, J., De Ketelaere, B., Marquenie, D., Molenberghs, G., Nicolaï, B., 2003a. Mixed models
 522 for multicategorical repeated response: modelling the time effect of physical treatments on strawberry
 523 sepal quality. *Postharvest Biol. Technol.* 30, 195–207.

524 Lammertyn, J., Scheerlinck, N., Jancsó, P., Verlinden, B.E., Nicolaï, B.M., 2003b. A respiration -
525 diffusion model for 'Conference' pears II. Simulations and relations to core breakdown. Postharvest
526 Biol. Technol. 30, 43 - 55.

527 Lau, O.L., 1998. Effect of growing season, harvest maturity, waxing, low O₂, and elevated CO₂ on
528 flesh browning disorder in Braeburn apples. Postharvest Biol. Technol. 14, 131 – 141.

529 Mziou, S., Scheerlinck, N., Nicolaï, B.M., 2009. Study and modelling of two apple quality attributes:
530 the soluble solids content and the firmness. Math. Comput. Model. Dyn. Systems. 15, 317–336.

531 Nicolaï, B., Scheerlinck, N., Hertog, M.L.A.T.M., 2006. Probabilistic Modeling. In Handbook of Food
532 and Bioprocess Modeling Techniques, S. Sablani, A. Datta, M. Shafiur Rehman, and A. Mujumdar,
533 eds. (CRC Press), pp. 265–289.

534 Nicolaï, B.M., Verboven, P., Scheerlinck, N., De Baerdemaeker, J., 1998. Numerical analysis of the
535 propagation of random parameter fluctuations in time and space during thermal food process. J. Food
536 Eng., 38, 259 – 278.

537 Nicolaï, B.M., Verlinden, A., Beuselinck, A., Jancsó, P., Quenon, V., Scheerlinck, N., Verboven, P.,
538 Baerdemaeker, J.D., 1999. Propagation of stochastic temperature fluctuations in refrigerated fruits. Int.
539 J. Refrig. 22, 81–90.

540 Ochoa-Ascencio, S., Hertog, M., & Nicolaï, B., 2009. Modelling the transient effect of 1-MCP on
541 Hass avocado softening: a Mexican comparative study. Postharvest Biol. Technol. 51, 62–72.

542 Poschet, F., Geeraerd, A.H., Scheerlinck, N., Nicolaï, B.M., Van Impe, J.F., 2003. Monte Carlo
543 analysis as a tool to incorporate variation on experimental data in predictive microbiology. Food
544 Microbiol. 20, 285–295.

545 Pouillot, R., Delignette-Muller, M.L., 2010. Evaluating variability and uncertainty separately in
546 microbial quantitative risk assessment using two R packages. Int. J. Food Microbiol. 142, 330–340.

547 Róth, E., Hertog, M.L., Kovács, E., Nicolai, B., 2008. Modelling the enzymatic softening of apples in
 548 relation to cultivar, growing system, picking date and season. *Int. J. Food Sci. Technol.* 43 (4), 620 –
 549 628.

550 Rubinstein, R.Y., 1981. Simulation and the Monte Carlo method, in: *Wiley Series in Probability and*
 551 *Statistics*, John Wiley and Sons, p. 278

552 Lo Scalzo, R., Forni, E., Lupi, D., Giudetti, G., Testoni, A., 2005. Changes of pectic composition of
 553 ‘Annurca’ apple fruit after storage. *Food Chem.* 93, 521–530.

554 Scheerlinck, N., Peirs, A., Desmet, M., Schenk, A., Nicolaï, B.M., 2004. Modelling Fruit
 555 Characteristics During Apple Maturation: A Stochastic Approach. *Math. Comput. Model. Dyn.*
 556 *Systems.* 10, 149–168.

557 Schouten, R.E., Jongbloed, G., Tijskens, L.M.M., van Kooten, O., 2004. Batch variability and cultivar
 558 keeping quality of cucumber. *Postharvest Biol. Technol.* 32, 299–310.

559 Shafiq, M., Singh, Z., Khan, A.S., 2011. Delayed harvest and cold storage period influence ethylene
 560 production, fruit firmness and quality of “Cripps Pink” apple. *Int. J. Food Sci. Technol.* 46, 2520–
 561 2529.

562 Tijskens, L.M.M., Heuvelink, E., Schouten, R.E., Lana, M.M., van Kooten, O., 2005. The biological
 563 shift factor. Biological age as a tool for modelling in pre- and postharvest horticulture. *Acta Hort.* 687,
 564 39–46.

565 Tijskens, L.M.M., Konopacki, P.J., Schouten, R.E., Hribar, J., Simčič, M., 2008. Biological variance
 566 in the color of Granny Smith apples modeling the effect of senescence and chilling injury. *Postharvest*
 567 *Biol. Technol.* 50, 153–163.

568 Tijskens, L.M.M., Konopacki, P., Simčič, M., 2003. Biological variance, burden or benefit?
 569 *Postharvest Biol. Technol.* 27, 15–25.

570 Tijskens, L.M.M., Konopacki, P., Simčič, M., Hribar, J., 2000. Biological variance in agricultural
571 products. Colour of apples as an example. In: F. Artés, M. I. Gil, & M. A. Conesa (Eds.), Improving
572 Postharvest Technologies Of Fruits, Vegetables And Ornamentals, IIR conference: Vol. 1 (pp. 25–42).
573 France: IIF-IIR.

574 Tijskens, L.M.M., Rodis, P.S., Hertog, M.L.A.T.M., Kalantzi, U., van Dijk, C., 1998. Kinetics of
575 polygalacturonase activity and firmness of peaches during storage. *J. Food Eng.* 35, 111–126.

576 Van Dijk, C., Boeriu, C., Peter, F., Stolle-Smits, T., Tijskens, L.M.M., 2006. The firmness of stored
577 tomatoes (cv. Tradiro). 1. Kinetic and near infrared models to describe firmness and moisture loss. *J.*
578 *Food Eng.* 77, 575–584.

579 Verlinden, B.E., de Jager, A., Lammertyn, J., Schotsmans, W., Nicolai, B.M., 2002. Effect of harvest
580 and delayed controlled atmosphere storage conditions on core breakdown incidence in Conference
581 pears. *Biosyst. Eng.* 83, 339–347.

582

583

584 Table 1. The mean value of the measured initial values of firmness and ethylene emission, together
585 with the standard deviation for eight fruits. These values were used in model calibration.

Initial values	Braeburn	Jonagold	Kanzi
$F_{0,early}$ (N)	103.5 (8.8)	79.4 (1.9)	95.9 (4.2)
$F_{0,opt}$ (N)	97.0 (2.9)	77.1 (1.2)	90.0 (3.8)
$F_{0,late}$ (N)	94.2 (4.1)	69.2 (1.0)	87.4 (3.7)
$\phi_{C_2H_4}^{0,early}$ (nmol kg ⁻¹ s ⁻¹)	0.049 (0.086)	0.00041 (0.00075)	0.00025 (0.00019)
$\phi_{C_2H_4}^{0,opt}$ (nmol kg ⁻¹ s ⁻¹)	0.00027 (0.00019)	0.00035 (0.00019)	0.00071 (0.00083)
$\phi_{C_2H_4}^{0,late}$ (nmol kg ⁻¹ s ⁻¹)	0.004 (0.011)	0.00039 (0.00035)	0.0009 (0.0015)

586
587

588 Table 2. Model parameters estimate and the corresponding standard deviations (s.d.).

Parameter ^a	Estimate (s.d.)		
	Braeburn	Jonagold	Kanzi
$k'_{\text{pect,ref}}$ (mmol m ⁻³ d ⁻¹)	0.0072 (0.0008)	0.00027 (0.00014)	0.0036 (0.002)
$k'_{\text{E}_{\text{pect}},\text{ref}}$ (m ³ mmol ⁻¹ d ⁻¹)	0.00018	0.00029	0.000049
$k_{\text{E}_{\text{pect,deg}},\text{ref}}$ (d ⁻¹)	0.014 (0.003)	0.10 (0.08)	0.0024 (0.004)
$V_{\text{max,C}_2\text{H}_4,\text{ref}}$ (mmol m ⁻³ d ⁻¹)	5.0 (0.5)	14 (2)	0.31 (0.38)
$K_{\text{m,O}_2,\text{C}_2\text{H}_4}$ (kPa)	23 (3)	4.4 (1.1)	5 (22)
$K_{\text{mu,CO}_2,\text{C}_2\text{H}_4}$ (kPa)	4.2 (3.0)	0.76 (0.21)	12 (64)
$k_{\text{C}_2\text{H}_4}$ (d ⁻¹)	0.039 (0.004)	0.0014 (0.0005)	0.0024 (0.0043)
$E_{a,\text{pect}}$ (J mol ⁻¹)	82000	90021	102480
$E_{a,\text{E}_{\text{pect}}}$ (J mol ⁻¹)	110000	59798	162770
$E_{a,\text{E}_{\text{pect,deg}}}$ (J mol ⁻¹)	47000	65241	46936
$E_{a,\text{C}_2\text{H}_4}$ (J mol ⁻¹)	50600	71403	118770
F_c (N)	40.2	39 (2)	60 (11)
R^2	90.06	89.35	77.14

589 ^a $k'_{\text{pect,ref}}$, $k'_{\text{E}_{\text{pect}},\text{ref}}$, $k_{\text{E}_{\text{pect,deg}},\text{ref}}$ are the rate constants for pectin breakdown, the synthesis and the
590 turnover of the pectin degrading enzymes respectively; $V_{\text{max,C}_2\text{H}_4,\text{ref}}$ is the maximum rate of ethylene
591 production; $K_{\text{m,O}_2,\text{C}_2\text{H}_4}$ and $K_{\text{mu,CO}_2,\text{C}_2\text{H}_4}$ are the Michaelis-Menten constants for ethylene production
592 and uncompetitive inhibition of ethylene production by carbon dioxide respectively; $k_{\text{C}_2\text{H}_4}$ is a
593 parameter that relates the rate of diffusion of ethylene from the apple to its surrounding; $E_{a,\text{pect}}$,
594 $E_{a,\text{E}_{\text{pect}}}$, $E_{a,\text{E}_{\text{pect,deg}}}$, $E_{a,\text{C}_2\text{H}_4}$ are the activation energies for degradation of pectin, synthesis of pectin
595 degrading enzymes, turnover of pectin degrading enzymes and production of ethylene respectively and
596 F_c is the part of firmness not affected by pectin degradation.

597

598

599 Table 3. Summary of the validation results of the model for predicting biological variability in flesh
600 firmness of Braeburn, Jonagold and Kanzi apples.

cultivar	Storage conditions [T– O ₂ – CO ₂] ^b	Percentage of measured firmness within the 95 % and 99 % PI ^a					
		Early picked		Optimally picked		late picked	
		95 % PI	99 % PI	95 % PI	99 % PI	95 % PI	99 % PI
Jonagold	[1 – 1 – 5]	96	100	75	83	100	100
	[1 – 2 – 2]	100	100	96	100	92	96
	[4 – 1 – 5]	88	92	71	79	96	100
	[4 – 2 – 2]	96	100	96	96	96	100
Braeburn	[1 – 1 – 0.7]	83	96	92	96	96	96
	[1 – 2 – 2]	79	88	92	96	96	96
Kanzi	[1 – 2 – 2]	93	98	88	93	93	98

601 ^a PI: prediction interval

602 ^b T is the temperature in °C, O₂ and CO₂ are the oxygen and carbondioxide levels in kPa. After
603 storage, the apples were placed in shelf life conditions (1 °C, 20.8 % O₂ and 0.03 % CO₂) for about 18
604 days.

605

606

607

608

Figure 1

Figure 2

613

614

615 Figure 3

616

617 Figure 4

Figure 7

Figure 8

Figure 9

Figure caption

Fig. 1. Summary of storage experiments used for model calibration and validation.

Fig. 2. Examples for the evolution of the firmness of optimally picked apples during controlled atmosphere storage and subsequent exposure to shelf life conditions (18 °C, 20.08 % O₂ and 0.03 % CO₂). The lines are the simulated values, while the points are the mean of the measured values, with the error bars denoting the 95 % confidence interval of the mean of 8 measurements. Broken lines represent shelf life, solid lines represent storage, and bold solid lines (for Braeburn only) represent delayed CA.

Fig. 3. Selected examples for the evolution of the ethylene production in optimally picked apples during controlled atmosphere storage and subsequent exposure to shelf life conditions (18 °C, 20.08 % O₂ and 0.03 % CO₂). The lines are the simulated values, while the points are the mean of the measured values, with the error bars denoting the 95 % confidence interval of the mean of 8 measurements. Broken lines represent shelf life, solid lines represent storage, and bold solid lines (for Braeburn only) represent delayed CA.

Fig. 4. Maximum relative sensitivity of firmness with respect to the random model parameters, F_0 (the initial firmness), $\phi_{C_2H_4,0}$ (the initial endogenous concentration of ethylene), $k'_{pect,ref}$ (the rate constant for the breakdown of pectin), and $V_{max,C_2H_4,ref}$ (the rate constant for ethylene production).

Fig. 5. Estimates of experiment-specific values for the random model parameters, F_0 (N), $k'_{pect,ref}$ (mmol m⁻³ d⁻¹) and $V_{max,C_2H_4,ref}$ (mmol m⁻³ d⁻¹). The point shows the mean estimated value for the early, optimal, late picked apples, while the bars represent the range of estimated values. In each parameter cultivar combination, values with the same letter are not significantly different on a 95 % confidence level.

Fig. 6. Covariance structure and frequency distribution of the random model parameters in the firmness model for Jonagold apples. F_0 (N) is the initial firmness (2008 – 2009 season), $k'_{pect,ref}$

654 $(\text{mmol m}^{-3} \text{ d}^{-1})$ is the rate constant for the breakdown of pectin, and $V_{\text{max}, \text{C}_2\text{H}_4, \text{ref}}$ $(\text{mmol m}^{-3} \text{ d}^{-1})$ is the
655 rate constant for ethylene production.

656 Fig. 7. Evolution of firmness in optimally picked Jonagold, Braeburn and Kanzi apples stored at two
657 different conditions. The points are the measured firmness, while the solid line, the dashed lines and
658 the dotted lines represents mean, the 95 % and the 99 % prediction intervals respectively for 1000
659 Monte Carlo simulations. Storage and shelf life are respectively shown in the un-shaded and shaded
660 regions.

661 Fig. 8. Model validation: the model predicts the propagation of the variability in firmness for late
662 picked apples at 1 °C, 1 % O₂ and 5 % CO₂, followed by subsequent exposure to shelf life. The points
663 are the measured firmness, while the solid line, the dashed lines and the dotted lines represents mean,
664 the 95 % and the 99 % prediction intervals respectively for 1000 Monte Carlo simulations. Storage
665 and shelf life are respectively shown in the un-shaded and shaded regions.

666 Fig. 9. Evolution of the probability that the critical firmness (65N) is not reached during CA storage of
667 Jonagold apples at three different temperatures (A), and that after subsequent exposure to shelf
668 conditions (18°C and normal air) for 14 days, the apples do not soften below a critical firmness of 45N
669 for consumer acceptance.

670

671