Prediction of Firmness of Apples under Dynamic Chain Conditions

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Abstract

   We present a predictive model of apple firmness. The model was developed using data from storage experiments with cv. Elstar apples. The model was validated using data from the cultivars Cox Orange Pippin and Royal Gala. After having shown that the model was valid also for these cultivars, the model has been tested on experiments simulating dynamic chain conditions. Good predictions for these experiments have been obtained.

INTRODUCTION

For storage and transport of apples it is desirable to known how environmental factors like temperature and gas conditions impart the firmness of apples. This will enable advanced control of storage conditions, which maintains a minimum level of quality and keeps energy consumption at an acceptable level (van der Sman and Verdijck, 2003). With this application in mind, we have developed a model predicting firmness of apples.

It is commonly known that softening of climacteric fruits like apples is due to enzymatic degradation of cell walls, i.e. solubilization of pectin by PG possibly with help of the action of PME (Johnston et al., 2002). Softening of apples is regulated by ethylene, as in many other climacteric fruits. The studies by Hertog et al.(2001) and Johnston et al.(2001) give a good qualitative indication of this regulation. In the pre-climacteric stage the ripening is slow, and is proportional with the metabolic rate. During the onset of the climacteric rise of ethylene, the ripening is accelerated if the ethylene crosses some threshold. The level of ethylene does not influence the rate of the loss of firmness (Johnston et al., 2001). Our model will focus on the above two main processes: cell wall breakdown and triggering of softening via climacteric rise.

After the description of the model, we will validate the model on static storage experiments on Elstar, Cox Orange Pippin, and Royal Gala cultivars. After validation model predictions are compared with experiments using dynamic storage conditions. Without any parameter adjustments predictions are shown to be in agreement with these experimental data.

MODEL DESCRIPTION

The model consists of two main parts, a) enzymatic breakdown of cell wall material, and b) triggering of this breakdown process by the climacteric rise of ethylene. These two processes are loosely coupled, and are discussed in separate sections.

Kinetics of Enzymatic Breakdown of Pectin and Firmness Loss

We largely follow the model of Tijskens et al.(1998), describing the loss of firmness in peaches via solubilization of pectin by PG. Also in apple PG is mainly responsible for solubilization of pectin (Pe), but also other enzymes may take part (Johnston et al.,2002). Hence, in our model all activity of these enzymes is grouped in the action of a single anonymous pectolytic enzyme Enz. Following Tijskens et al., we assume the rate of solubilization ($d_t Pe$) to be proportional to the loss of firmness ($d_t F$), as described by the following equation:

$$d_t\, Pe = d_t\, F = -\, k_{Enz}\, Pe\, Enz \qquad (1.1)$$

The amount of the pectolytic enzyme *Enz* changes in time via its formation and its degradation. In the original model of Tijskens et al. the formation of *Enz* is thought to be proportional to the level of a pre-cursor, which we indicate with an activator *Act,* whose production is triggered by the climacteric rise of ethylene. Hence, the enzyme kinetics is as follows:

$$d_t \, Enz = k_f \, Act - k_d \, Enz \qquad (1.2)$$

In Tijskens model both reaction rates k_{Enz} and k_f, are temperature dependent, and follow Arrhenius. However, apple cultivars show only a slight temperature dependency on the rate of softening (Johnston et al.,2001). Hence, we assume only k_f to be temperature dependent:

$$k_f = k_{f,0} \, exp \, (\, - \, \Delta E_{a,f} \, / \, R.T \,) \qquad (1.3)$$

Here $\Delta E_{a,f}$ is the activation energy of the k_f reaction rate, R is the gas constant and T is the temperature in Kelvin.

Triggered Production of Activator

To our knowledge, there is yet not a detailed model on the regulation of softening by ethylene available in scientific literature. But, it is known that softening of apples occurs in two stages: before the climacteric rise the softening is slow, but is accelerated during the climacteric rise of ethylene if it rises above a certain threshold (Johnston et al.,2001). Seemingly, the climacteric rise of ethylene acts as a kind of biological switch (trigger) for the softening. For our model we use assume that the climacteric rise of ethylene somehow triggers the formation of the activator *Act* – inducing the action of the pectolytic enzyme *Enz*.

For this triggering of softening we take the 'biological switch' model of Meinhardt (1992) for describing the production of the activator, which is described with the general equation below:

$$d_t \, g = \gamma \, g^2 \, / \, (\, 1 \, + \, \kappa \, g^2 \,) - \mu \, g + m \qquad (2.1)$$

The compound *g* will show threshold behaviour. At low concentrations, the linear decay rate is dominating and *g* tends to small value (g=m/μ). If the source term rises above a certain limit the autocatalytic production will dominate, and *g* increases until a steady state at high concentration is reached due to saturation. The switching between low and high value is regulated via the source term *m*.

The biosynthesis of ethylene shares many of the 'biological switch' model. Before the climacteric rise the ethylene production via System I is low. At a certain threshold the autocatalytic production via System II is triggered (Mathooko,1996), leading to the climacteric rise of ethylene and increased softening (Johnston et al., 2001). For apples it is known that after the climacteric rise ethylene stays at a certain steady state value (Song and Bangerth, 1996), as in the biological switch model. Softening also occurs in the pre-climacteric apples (Johnston et al., 2001). This slow softening is also regulated by ethylene, which is produced in pre-climacteric fruits ethylene at a low rate by a non-autocatalytic process, called System-I (Mathooko, 1996). We assume that both autocatalytic and non-autocatalytic production of ethylene will induce the production of the activator. Both these process are thus captured by the biological switch model.

The rate limiting step in the synthesis of ethylene via System II, is the conversion of AdoMet to ACC by ACC-synthase (Brownleader et.al., 1999). To account for this, we extend the 'biological switch' model to the activator-depleted substrate scheme (Meinhardt, 1995). Here the autocatalytic production of the activator *Act* will also be proportional with the amount of substrate *Sub*. The change in substrate is described by its reduction via activator synthesis (either mediated via System I or System II), and by an autonomous production term.

For the conversion of ACC also oxygen is required (Mathooko, 1996). Furthermore, carbon dioxide is an inhibitor of the synthesis of ACC by ACC-synthase (Mathooko, 1996). These kinetics will be incorporated in the autocatalytic production of the activator *Act,* following the model of de Wild et al.(1999), describing the action of

respiratory gases on the ethylene production in pears.). In this model Michaelis-Menten kinetics for O_2 is used with uncompetitive inhibition by CO_2.

For the formation of the substrate of ethylene, Adomet, ATP is required (McKeon, 1995). Hence, we assume that the production of the substrate *Sub* is proportional with the metabolic rate, or rather the respiration rate, similar to Hertog et al. (2001) who modelled the effect of respiratory gasses on softening of pre-climacteric Braeburn apples stored under controlled atmosphere (CA) conditions. As our model is validated against experiments with Elstar apples, we take the respiration model of Peppelenbos (1996).

The complete activator-depleted substrate scheme is as follows:

$$d_t\, Act\ =\ +\, S_{autocat} - S_{decay} + S_{system,I} \qquad (2.2)$$

$$d_t\, Sub\ =\ -\, S_{autocat} - S_{system,I} + S_{prod} \qquad (2.3)$$

$$S_{autocat} = k_{au}.Sub.O_2.Act^2 / [(1+k_{as}.Act^2)(1+K_{me,co2}.CO_2).(K_{me,O2} + O_2)] \qquad (2.4)$$

$$S_{system,I} = k_I.Sub \qquad (2.5)$$

$$S_{decay}\ = k_{diff}.Act \qquad (2.6)$$

$$S_{prod}\ = k_{prod}.O_2 / [\ (\ 1+ CO_2/K_{mo,CO2}\ ).(K_{mo,O2} + O_2)\ +\ \eta/(\ 1+O_2/K_{mf,O2}\ )\ ] \qquad (2.7)$$

S_{prod} follows the respiration model of Peppelenbos (1996), who uses Michaelis Menten kinetics with uncompetitive inhibition of O_2 and CO_2. η is the ratio of ATP production between aerobic and anaerobic respiration, which is $\eta=1/7$. We assume that reaction rates k_{au}, k_I and k_{prod} is temperature dependent and follows Arrhenius, with activation energy E_a equal to the activation energy of respiration.

Parameter Values

Values for respiration related parameters are taken from the data given for Elstar apples by Peppelenbos (1996): $K_{mo,CO2} = 91$ kPa, $K_{mo,O2} = 4.6$ kPa, $K_{mf,O2} = 0.2$ kPa. The value of the activation energy, $E_a/R=6380$ K, is taken from Hertog et al. (1998). The decay rate of enzyme k_d is estimated from data in the study of Zhou et al.(2000b), where it is said that PG activity decays in 9 days from 100% to 10% at 0^oC. Hence, we estimate that $k_d=0.25$ day^{-1} (Note it is assumed temperature independent). For the amounts of the various compounds (*Act, Sub, Enz, Pe*) we use dimensionless numbers, with *Sub* and *Pe* are normalized with respect to their initial value for Elstar apples. Hence, initial values for the various compounds are $Sub_0=1$, $Act_0=0$, $Enz_0=0$, and $Pe_0=1$. Parameters which have to be estimated yet are: $E_{a,f}$, $K_{me,O2}$, k_{au}, k_{as}, k_I, k_f, k_{Enz} and for cultivars other than Elstars the initial amount of substrate Sub_0.

EXPERIMENTS UNDER STATIC STORAGE CONDITIONS

Elstar

Elstar apples are stored for about 30 days in atmospheric conditions, at several temperatures. At intervals of about 1 week, firmness and ethylene release are measured. The experiments are performed with 4 different batches either shortly after harvest or after CA-storage. The experimental results will be discussed in more detail in a subsequent full paper. However, we like to note here that all batches have shown a transition in softening rate, which occurred before the peak in the climacteric rise of ethylene is reached. This observation confirms the hypothesis of Johnston et al.(2001), who state that the transition occurs at early stages of the climacteric rise of ethylene, if the concentration of ethylene crosses a threshold. After the transition apples soften at a comparable rate, which is largely independent of temperature and ethylene level.

The transitions in softening rates occurs at 10, 20 and 30 days for apples stored at respectively 14, 9 and 5^oC. In batches stored at same temperature, transition in softening rate occurred at comparable times. Hence, experimental data on firmness is rescaled with respect to initial value, and averaged over batches with corresponding storage temperature. Data is shown in figure 1b.

In next experiments Elstar apples are stored at 10^oC, two levels of oxygen (3% and 21%) and various CO_2 levels. After 21 days of storage the loss of firmness (relatively to

the initial firmness of 54 N) is measured. The experimental results are shown in figure 2b. The experimental error is in the order of 5%.

Parameter estimation of the model is based on the experimental data on Elstar apples. Via non-linear regression we have obtained (at reference temperature 14^0C) k_{as}=4.0, k_{prod}=3.2day^{-1}, k_{au}=0.5 day^{-1}, k_I= 0.008 day^{-1}, k_f =k_{Enz}=0.14 day^{-1}, $\Delta E_{a,f}/R$ = 1000 K, k_{decay}=2.0 day^{-1}, $K_{me,CO2}$ = 12 kPa, and $K_{me,O2}$ = $K_{mo,O2}$. Comparison of model predictions and experimental data is presented in figures 1 and 2.

Royal Gala and Cox Orange Pippin

The general applicability of the model is tested by comparing model predictions with experimental data on other apple cultivars, namely Royal Gala and Cox Orange Pippin by Johnston et al. (2001). For the model predictions we have taken the same parameter values as for Elstar, except for the initial amount of the substrate (Sub_0). Via non-linear regression we found for Royal Gala Sub_0=40, and for Cox Orange Pippin Sub_0=57. In figures 3 and 4 model predictions are compared with experimental data.

EXPERIMENTS UNDER DYNAMIC CA-CONDITIONS

Experiments are performed with Elstar apples, where the gas conditions are changed from atmospheric conditions to CA-conditions (1.2 % O2 + 2.5 % CO2). These switching experiments are performed at 3 temperatures, namely 2, 5 en 9 °C. The switching of gas conditions have occurred after 7, 11, 14, 19, 21 days or not at all. After 33 days the firmness of the treated apples is determined. Part of the apples are further stored under shelf life conditions (atmospheric gas conditions and 18^0C). After the shelf life experiments (at day 40) also the firmness is determined.

The effect of the above described treatments on apple firmness has been predicted by the model, using identical parameter values as listed above. The model predictions of the firmness at day 33 (after the gas switching experiment) and at day 40 (after the shelf life experiment) are compared to experimental data in figures 5a and b. As one can observe the model gives reasonable good predictions for the firmness of the Elstar apples.

DISCUSSION

In general one can conclude that the model predictions are in quite good agreement with experiments. Many estimated parameter values are shared by different apple cultivars, namely Elstar, Royal Gala, Cox Orange Pippin. Differences between their behaviour can be explained by a single parameter: initial amount of substrate Sub_0. This implies that the model might applicable for many different cultivars or even other kinds of fruits.

Model prediction substantiate the hypothesis by Johnston et al.(2001), that softening in apples have an initial slow, and subsequently fast decay rate. The change in decay rate linked to climacteric rise of ethylene (or climacteric rise), acting as a 'biological switch'. Our experiments and model studies show that this switching is strongly influenced by 1) temperature, 2) carbon dioxide, and 3) oxygen (to a lesser extend). The initial slow rate of decay is proportional to the metabolic rate, which can be derived from respiration models as studied by Peppelenbos (1996).

Having knowledge on the softening of apples, one can use that for controlling it in distribution chains, as shown in the experiments with dynamic CA-conditions. If during transport one notices the incidence of the onset of climacteric rise, one can decide to transport the fruits further transport under different CA/MA conditions or even lower temperature, which will slow down the softening rate.

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Figures
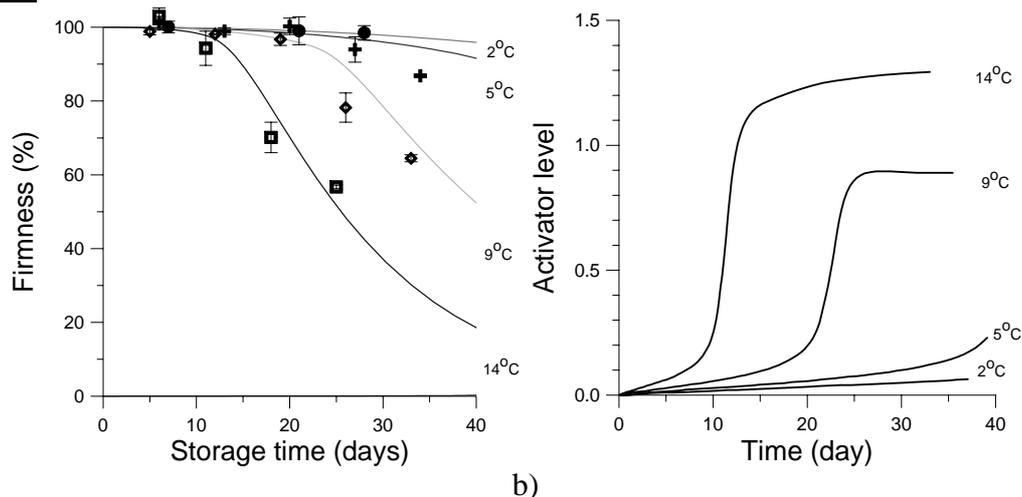
a)   b)

Fig. 1. a) Comparison model predictions (lines) and averaged experimental data (symbols) for Elstar apples stored at atmospheric conditions and various temperatures. Initial amount of substrate $Sub_0=20$. b) Change in activator level in model simulations as shown in figure 1a.
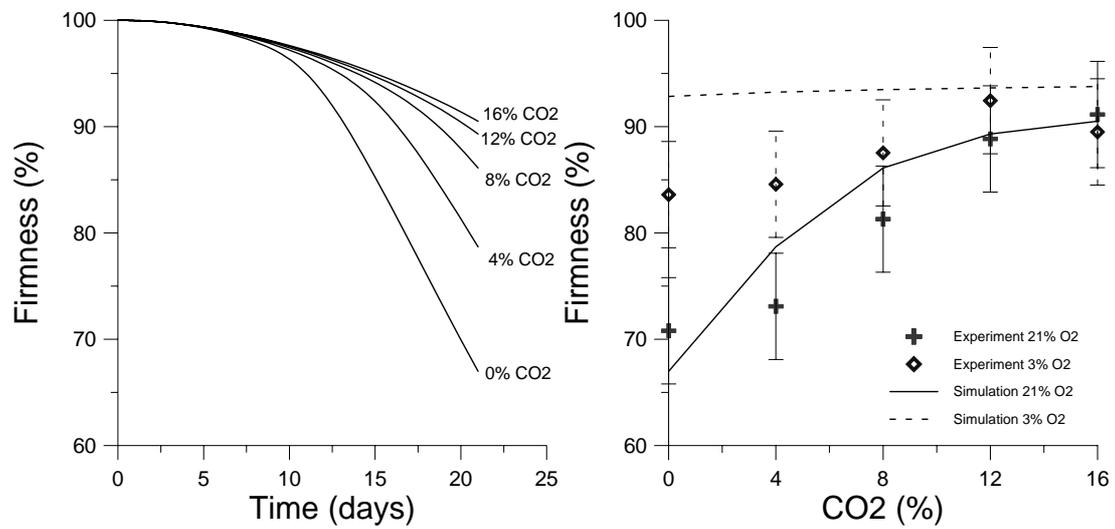
Fig. 2. a) Model predictions of change in firmness during experiments at $10^{o}C$, 21% O_2 and various CO_2 levels. b) Model predictions of final firmness (lines) versus experimental data (symbols). In the experiments apples are stored for 21 days at $10^{o}C$, 21% or 3% O_2, and various levels of CO_2. Firmness is expressed relatively to the initial firmness. Error bars indicate experimental error of 5%.
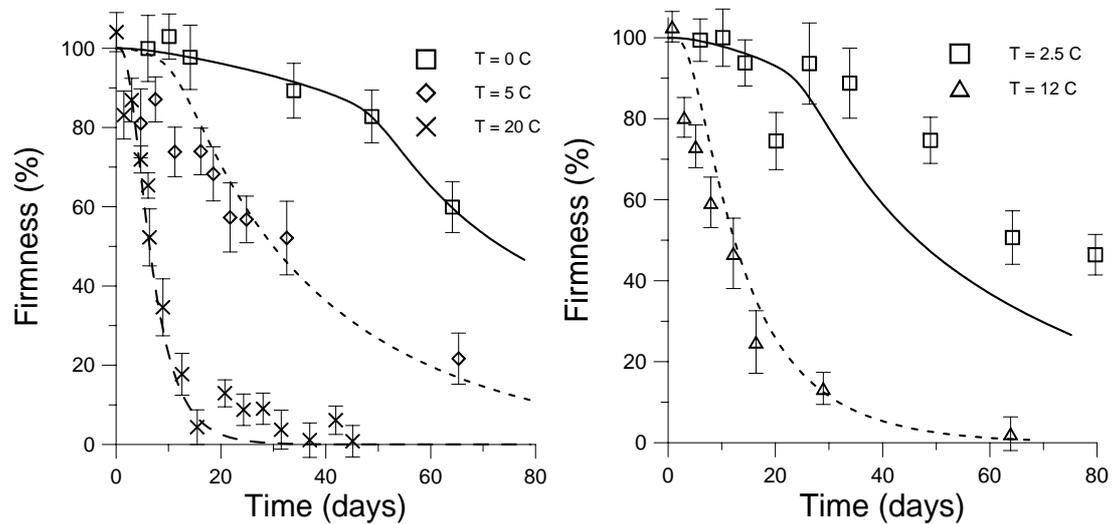

Fig. 3. Model predictions (lines) versus experimental data (symbols) of the change in firmness of Royal Gala apples stored at various temperatures. Temperatures are indicated in the legends.
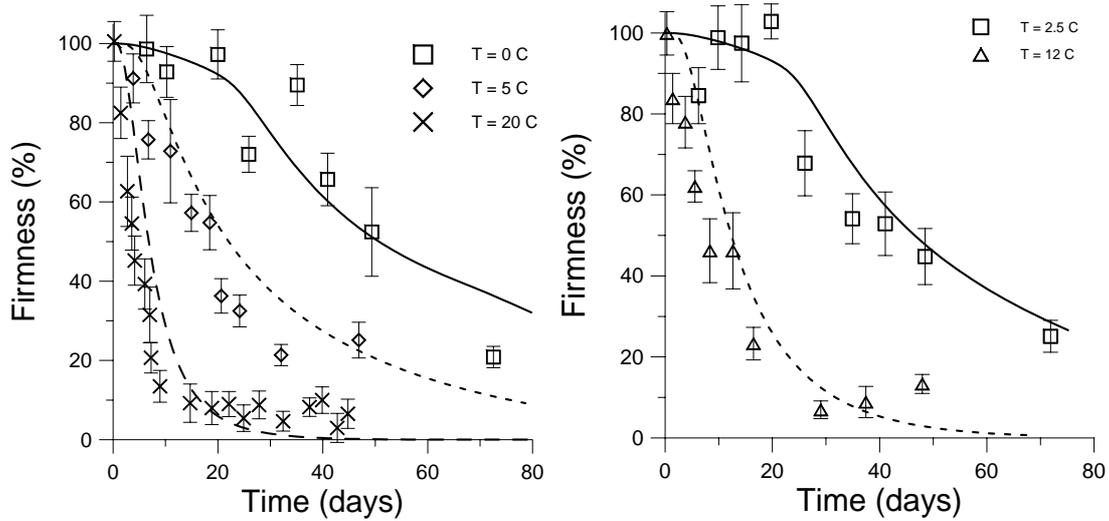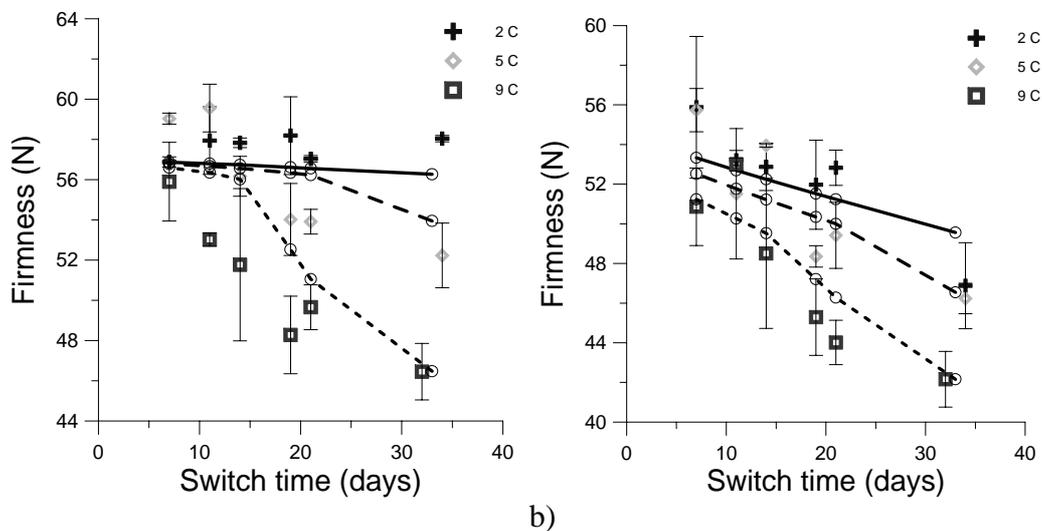
Fig. 4. Model predictions (lines) versus experimental data (symbols) of the change in firmness of Cox Orange Pippin apples stored at various temperatures. Temperatures are indicated in the legends.

a)   b)

Fig. 5. Model predictions of firmness (lines and open circles) directly after gas switching experiment versus experimental data (symbols) for different temperatures at 2,5 and 9°C, after a) pretreatment (day 33), and b) end of shelf life (day 44).