Modelling Mango Postharvest Firmness Change at Different Temperatures by Using Acoustic Measurements in Combination with Nonlinear Mixed Effects Models.

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Abstract

A storage experiment was conducted in Can Tho, Vietnam at different temperatures (12°C, 18°C and room temperature) on 270 mango fruits (Cat Chu species). Mango firmness was assessed repeatedly on each single mango using a commercial firmness sensor. The resulting repeated measures data are characterised by the large biological variance among mangoes and the non-constant variance over storage time. A nonlinear sigmoidal firmness change was assumed. The data were modelled using the concepts of nonlinear mixed effects models. It was found that the sigmoidal firmness change assumption described the data well. Regarding the storage temperatures, it was found that at 12°C, chilling injury was induced, resulting in a significant lower shelf life of the fruits.

INTRODUCTION

Mango crop is grown commercially in 87 countries. Its global production in 2003 was estimated to be 26 million metric tons, of which 10 million in India (FAOSTAT data, 2004). In many development countries, mango is the most important fresh eaten fruit, containing fibres and different important vitamins.

Mango fruits have a short shelf life at ambient temperatures, but the use of low temperatures can extend the shelf life up to four weeks. As a tropical fruit, mangoes suffer from chilling injury if stored at low temperature for longer periods. Some authors report successful storage at 7°C, but in general 10°C is considered the minimum temperature to prevent chilling injury (Kalra et al. 1995). Recently, storage under controlled atmosphere conditions has been used to further extend the shelf life of mangoes. Sing et al. (1989) tested the effect of altering nitrogen and oxygen concentration on the respiratory system of mango.

The colour of mango fruit is a very important quality factor for consumers. During ripening, the green colour changes to red or yellow, depending on cultivar. In research, colour of peal or tissue can be measured with commercially available colorimeters. Because mango fruit changes from very hard to very soft during ripening, firmness is an accurate

parameter to follow the ripening process. Firmness is also an important quality factor for customers and handling. Destructive measurement of firmness can be done with different types of penetrometers (operated by hand or mechanical). These tests only measure the local firmness of the tissue. Shmulevich et al (1995) developed a fruit bed to measure firmness non destructive by exciting the mango with a rubber hammer. They showed that the equator is the best place to measure vibrations. In this work, a related nondestructive method that is commercialised by AWETA B.V. (Nootdorp, The Netherlands) is used in a storage experiment to estimate fruit firmness change from the vibrational characteristics, and to investigate the temperature effect.

The data resulting from such an experiment have some very specific characteristics. One of the major issues is caused by the natural heterogeneity of a batch of biological products: no two biological objects are identical resulting in a substantial biological variance.

A powerful approach to include experimental variation into an analysis is by using the concept of mixed effects models, where the term 'mixed' points to the presence of a mixture of fixed and random effects (Verbeke and Molenberghs, 2000; Diggle *et al.*, 2002). This type of models has been successfully used by several authors to model biological data (De Ketelaere *et al.*, 2003 & 2004; Lammertyn *et al.*, 2003). It offers a well founded framework to test which of the sources of variance are significantly present which is desperately needed in order to make accurate predictive models, or to test treatment or cultivar effects.

The *objective* of this study is to apply the concepts of nonlinear mixed effects models to model repeated measures data obtained from a storage experiment at different temperatures. The effect of temperature on firmness decay is studied.

MATERIALS AND METHODS

Experimental data

The mango fruit (Cat Chu species) were bought directly from the farmer in Dong Thap province, South-Vietnam. Mangoes were harvested under commercial conditions. When delivered in the laboratory, 270 mangoes were stored in small plastic boxes and stacked in the refrigerator at 12°C, at 18°C and at room temperature, 30°C on average (90 fruits for each temperature). Fruits were measured on a regular basis (2-3 days) until complete softening. The total measurement span was 20 days. For the acoustic firmness measurements, a commercial desktop unit was used (AFS, AWETA, Nootdorp, The Netherlands). Firmness measurements were performed along the equator of the fruits at 2 equidistant places.

Firmness decay: parametric model formulation

In order to describe the firmness change of the mangoes during storage, a sigmoidal function was assumed, and defined as follows:

$$F_i(t) = F_{\max} - \frac{F_{\max} - F_{\min}}{1 + \exp(C - kt)}, \quad (1)$$

where $F_i(t)$ is the firmness of subject (mango) i at time t, F_{max} is the upper asymptote of the sigmoidal function, F_{min} the lower firmness asymptote, C a location (shift) parameter and k the decay rate. Straightforward calculus reveals that the inflection point of the curve is given by C/k.

Introducing biological variance

Equation (1) provides the deterministic function describing the average firmness decay. We now introduce biological variance into Eq. (1). We restrict ourselves to two different sources of experimental variance – we make the distinction between biological variance and uncertainty in view of the arguments by Nauta (2000). By uncertainty, we address the fact that the instrument used (here the AFS) has an inevitable measurement error. The biological variance denotes the different components of true heterogeneity of the population. In the presented example, we will restrict ourselves to only 1 component of biological variance, namely on the biological age of the different products (dt_i) in the population. Here, the biological age of an individual product is defined as the age relative to the batch average. Introducing these concepts into Eq. 1 gives

$$F_i(t) = F_{\max} - \frac{F_{\max} - F_{\min}}{1 + \exp\left(C - (k + dk_i)(t + dt_i)\right)} + \varepsilon_i, \tag{2}$$

with the parameters defined as above. The biological variance is introduced by dt_i being a normally distributed random term with 0 mean and variance of σ^2_{dt}. This term thus denotes the subject specific deviations from the mean value t. The term $\varepsilon_i \sim N(0, \sigma^2)$ denotes the uncertainty (measurement error). The subscript i clearly indicates that the value of these random components depends on subject (mango). The inflection point of this function is now given by $C/k+dt_i$. which differs dt_i from the inflection point of Eq. 1. In case $C = 0$, the inflection point is given by dt_i.

Temperature dependence

Since the three groups were stored at three different temperatures, a temperature effect needs to be incorporated into Eq. (2). The most straightforward way to achieve this is by assuming an Arrhenius type of temperature dependency of the decay rate k.

$$k = k_{ref} \exp\left(\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \tag{3}$$

with k_{ref} the decay rate at the reference temperature T_{ref}, R the universal gas constant and E_a the activation energy. Another way of introducing temperature dependency is by taking a different decay rate for each temperature. This can be achieved, for instance, by inserting two dummy variables to denote temperature regime.

The concept of nonlinear mixed effects models

Nonlinear mixed effects models are a natural extension to the linear mixed effects models (Verbeke and Molenberghs, 2000) in order to allow the fixed and random effects to enter in a non linear way in the model function. Although the estimation of the model

parameters are obtained from one single optimisation routine, it can best be thought of as a hierarchical model, in which the first level models the *i*-th subject as

$$\boldsymbol{y}_i = \boldsymbol{f}_i(\phi_i, v_i) + \boldsymbol{\varepsilon}_i, \tag{4}$$

with y_i presenting the vector of n_i repeated measurements on subject $i = 1 \ldots M$, f is a general, real-valued, differentiable function of the subject specific parameter vector ϕ_i and a covariate vector v_i and $\boldsymbol{\varepsilon}_i$ the normally distributed error vector. The function f is nonlinear in at least one component of the subject specific parameter vector ϕ_i which is modelled as

$$\phi_i = \boldsymbol{A}_i\beta + \boldsymbol{B}_i b_i, \tag{5}$$

where β is a p-dimensional vector of fixed effects and b_i is a q-dimensional random effects vector which is assumed to be normally distributed with a zero vector as mean and variance covariance matrix ψ. Through the random effects and associated distribution, this type of models allows for heteroscedasticity of the data resulting in correct estimation of the standard errors of the parameters under such conditions. The matrices $\boldsymbol{A}_i$ and $\boldsymbol{B}_i$ are of appropriate dimensions and depend on the subject and possibly on the values of the covariates used in the model (Pinheiro and Bates, 2000). Different methods have been proposed in literature to estimate the parameters in the nonlinear mixed effects model, most of them based on the likelihood function. It falls beyond the scope of this paper to go into more detail on this topic. Nevertheless, it is of utmost importance to have the appropriate test statistics to test which fixed and random (the different components of biological variance) components are significantly present, and to accurately quantify them. For the random part, having the correct sources of biological variance is much defining the future behaviour of the considered batch of subjects. The most widely used test statistic is the so-called Likelihood Ratio Test (LRT) given by

$$G^2 = -2\log\left[\frac{ML\left(\hat{\theta}_0\right)}{ML\left(\hat{\theta}\right)}\right], \tag{6}$$

where ML denotes the marginal likelihood function and $\hat{\theta}_0$ the parameters estimated under maximum likelihood for a reference (null) model, which is a subset of the parameters in $\hat{\theta}$. In the results section, the log likelihood is denoted by the symbol ℓ. When testing for *fixed* effects in the model, G^2 follows asymptotically, under the null model, a χ^2 distribution with degrees of freedom equal to the difference between the dimensions of the two parameter spaces. To test whether *random* effects are needed in the model, the likelihood ratio test defined above was used but follows asymptotically a null distribution that is a mixture of chi–squared distributions, rather than the classical single chi–squared distribution that was used to test fixed effects (Verbeke and Molenberghs, 2000). For the case of testing no random effect versus one random effect, the null distribution is a mixture of χ^2_1 and χ^2_0 with equal weights 0.5, denoted by $\chi^2_{0:1}$. In case of testing one versus two random effects, the null distribution is a mixture of χ^2_2 and χ^2_1 distributions with equal weights 0.5, denoted by $\chi^2_{1:2}$. It is common practice to start with an elaborate model, and sequentially to test which of the parameters can be discarded, using the LRT. The SAS software (The SAS Institute, Inc., NC, USA) was used throughout all analyses.

RESULTS AND DISCUSSION

In Figure 1, an overview of the average firmness decay of the 270 mangoes is given, for the three temperature groups separately. It is clear that at harvest, the mangoes are showing already an exponential-type of decay, being at or around the inflection point of the logistic curve. The figure also suggests that mangoes stored at 18°C have the lowest firmness decay rate, being in contradiction with the expectation of a lower decay rate for lower temperatures. As will be shown later, this will be due to chilling injury.

Figure 2 shows the heteroscedastic nature of the data, with experimental variance being large at harvest and lowering during storage (only the 12°C group is shown). Although heteroscedasticity does not (asymptotically) affect the point estimates of the model parameters when applying a classical fixed effects nonlinear regression model, it has its important repercussion on the estimation of their variance, and, thus, on inferences drawn from these estimates.

The nonlinear mixed effects model based on Eq. 2 was build (model 0), with one source of biological variance (dt_i), and 6 fixed effects (batch) parameters (F_{max}, F_{min}, C and k_j). The -2ℓ of the full model is 8868. The model without parameter C (model 1) has a -2ℓ of 8868.5, resulting in a G^2 of 0.5 on 1 degree of freedom favouring the model without C (P = 0.48). This means that mango fruits are harvested while they are at the inflection point of the sigmoidal firmness curve. Introducing the Arrhenius temperature dependency instead of a separate decay rate for each temperature regime did not result in a better model ((-2ℓ = 9496.5, G^2 = 628, $P < 0.0001$). As a logic result, a common decay rate k cannot be used (-2ℓ = 9813.0, G^2 = 944.5, $P < 0.0001$). Removing the biological age dt_i from model 1 results in a -2ℓ of 9223.4, or a G^2 of 354.9 or strongly favouring model 1 ($P < 0.0001$). Table 1 lists the different parameters of the preferred model 1 and their standard errors. It is remarkable that the decay rate k_{12} is significantly higher than that of the 18°C group (0.321 vs. 0.201, $P <$ 0.0001). This is against the expectations in view of Arrhenius law, as was already indicated higher. The reason why k_{12} is higher that k_{18} can be explained by chilling injury. Although several references state that temperatures above 10°C can be regarded as 'safe' (see f.i. Kalra et al., 1995), the opposite is encountered here. A picture of the effect of chilling injury on the visual appearance of the fruits is given in figure 3. One could conclude at this point that the critical temperature to prevent chilling injury might depend on the mango cultivar under study.

Figure 4 shows the measured and predicted firmness values as a function of time for the three different temperature regimes. The model fits the data adequately and explains 94 % of the data variance. The inflection point of the logistic curve occurred at harvest, on average, but exhibits a substantial variance due to the inclusion of the random effect dt_i . When inspecting dt_i using Table 1, it is seen that the 95% confidence interval of the biological age is ~2 days wide. The uncertainty is constant over time and has a variance σ^2 of 9.273.

CONCLUSIONS

A nonlinear mixed effects model was fitted to a longitudinal study of mango firmness change during postharvest storage under different temperature regimes. The model revealed that on average, the firmness decay is of a sigmoidal nature. It also shows that individual mango

fruits exhibit a large variance around the group average. This biological variance is mainly due to the different biological ages of the fruits. The research showed also that for this cultivar of mango fruits (Cat Chu species), chilling injury occurs at 12°C.

Literature Cited

De Ketelaere, B., Lammertyn, J., Molenberghs, G., Nicolaï, B. and De Baerdemaeker, J. 2003. Statistical models for analysing repeated quality measures of horticultural products. Model evaluations and practical example Math. Biosci. 185(2):169-189.

De Ketelaere, B., Lammertyn, J., Molenberghs, G., Desmet. M., Nicolaï, B. and De Baerdemaeker, J. 2004. Tomato cultivar grouping based on firmness change, shelf–life and variability during postharvest storage. Postharvest Biol. Technol. 34:187-201.

Diggle, P.J., Heagerty, P., Liang, K.Y. and Zeger, S.L. 2002. The analysis of longitudinal data. Oxford university press. Oxford, England.

Kalra, S.K., Tandon, D.K. & Singh, B.P (1995). Mango. In: Handbook of Fruit Science and Technology. Salunkhe, D.K. & Kadam, S.S. (eds.). Marcel Decker, New York. 123-169.

Lammertyn J., De Ketelaere, B., Marquenie, D., Molenberghs, G. and Nicolaï B.M. 2003. Mixed models for multicategorical response: modelling the time effect of physical treatments on strawberry sepal quality. Postharvest Biol. Technol. 30(2):195-207.

Nauta, M.J. 2000. Separation of uncertainty and variability in quantitative microbial risk assessment models. Int. J. Food Microbiol &124(3):365-373.

Pinheiro, J. and Bates, D. 2000. Mixed-effects models in S and S-PLUS. Springer, New York

Shmulevich I., Galili N. & Benichou N. (1995). Development of non-destructive method for measuring the shelf-life of mango fruit. Proceedings of the FPAC IV conference. 275-287.

Singh, B.N., Seshagiri P.V.V. & Gupta, S.S. (1989). Ontogenic drifts in the physiology and chemistry of tropical fruits under orchard condition. Indian J. Agr. Sci. 7:176.

Verbeke, G. and Molenberghs, G. 2000. Linear mixed models for longitudinal data. Springer, New York.

Tables

Table 1: Parameter estimates of the final nonlinear mixed effects model (model 1), their standard error, and significance.

parameter	estimate	s.e.	*P*
F_{max}	79.690	0.8922	< 0.0001
F_{min}	3.977	0.1616	< 0.0001
k_{12}	0.321	0.0078	< 0.0001
k_{18}	0.201	0.0073	< 0.0001
k_{30}	0.458	0.0111	< 0.0001
σ^2	9.273	0.3566	< 0.0001
σ^2_{dt}	0.936	0.1165	< 0.0001

Figures

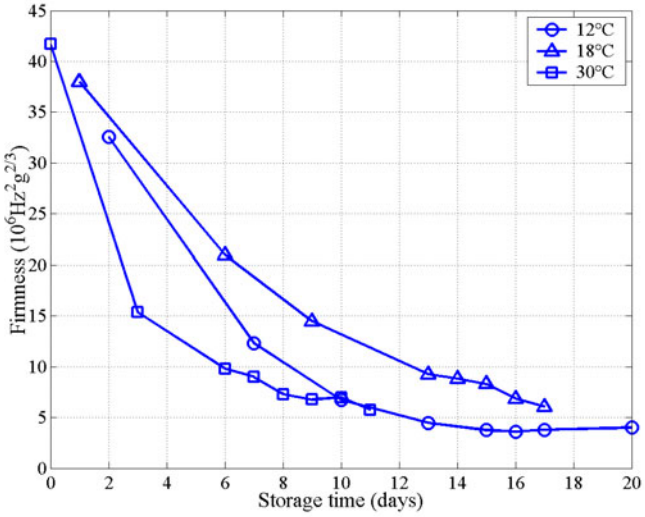

Figure 1: Firmness decay for the three different treatment groups.

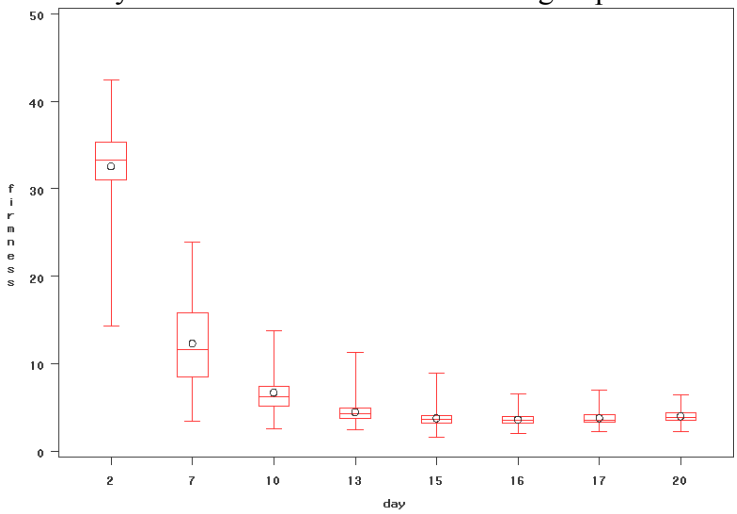

Figure 2: Box plot of the mango data (12°C group) showing the firmness decay as a function of time. Heteroscedasticity is evident. X-axis: time (days); Y-axis: firmness (10^6Hz^{2}g$^{2/3}$)

Figure 3: The effect of chilling injury on the visual appearance of the mango fruits.

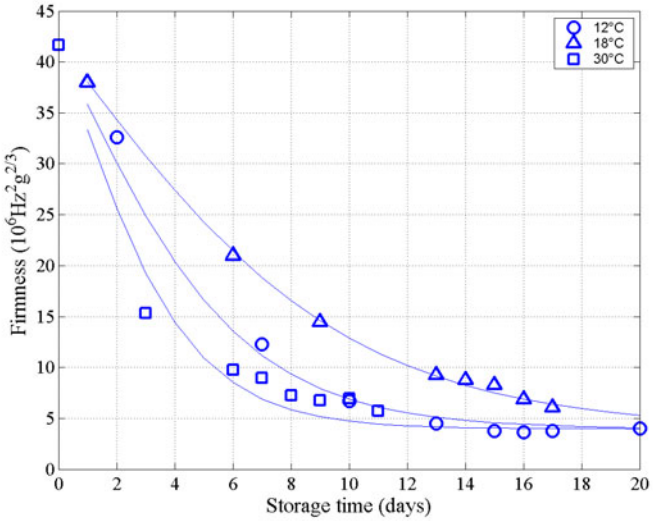

Figure 4: Observed (symbols) and fitted (full line) firmness decay of the three different treatment groups. For the fitted values, the nonlinear mixed effects model 1 was used.

Modélisation du changement de fermeté des mangues après récolte à différentes températures par des mesures acoustiques en combinaison avec des modèles à effets non linéaires.

Résumé

Une expérience de conservation a été conduite à Can Tho, Vietnam, à différentes températures (12°C et température ambiante) sur 270 mangues (variété Cat Chu). La fermeté des mangues a été mesurée sur chaque mangue en utilisant un capteur de fermeté commercial. Ces mesures répétées sont caractérisées par la forte variance biologique des mangues et par une variance hétérogène qui apparaît lors du stockage. Une variation sigmoïde de fermeté est attendue. Les données ont été modélisées en utilisant des modèles à effets non linéaires. Nous avons trouvé que l'hypothèse de variation sigmoide de la fermeté décrivait correctement les données. Par rapport aux températures de stockage, il a été trouvé des défauts induits par le froid à 12°C qui se sont traduits par des durées de vie significativement réduites pour les fruits.