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BIOLOGICAL VARIATION IN RIPENING OF NECTARINES

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Summary

The optimal harvest date of nectarines can no longer be based on the colour since newer cultivars have an intensive blush, even in the unripe stage. Measuring the absorption of the fruit flesh by Time Resolved Spectroscopy (TRS) at 670 nm, provide information on the ripening stage and the variation in ripening stages of batches of fruit. Since each individual fruit is harvested and measured at some arbitrary stage of development, the analysis of the data gathered was based on the system of the biological shift factor (Tijskens *et al*. 2005) applying non-linear mixed effects regression analysis. The results show that μ_a, and therefore also the fruit flesh colour and chlorophyll content, change according a sigmoidal pattern, which was approximated with a symmetrical logistic function. The explained parts obtained (R^2_{adj}) were about 0.97 for storage at 20ºC and about 0.86 at 10ºC. The rather large variation on apparent behaviour between individual fruit, each with its own stage of development, was nicely taken care of by the system of the biological shift factor. During ripening, the distribution of the biological shift factor was constant and normal, while the distribution of μ_a changed. Also that change in distribution over time is nicely described by the system and models used.

key words: harvest maturity, flesh colour, non-destructive measurement technique, biological variation, modelling

INTRODUCTION

The optimal harvest time and the maturity of peaches and nectarines are usually determined based on the background colour of the fruit. The newer cultivars however, are so much more colourful and blushing that the background colour can hardly be distinguished. A new technique to assess harvest time and the maturity has to be developed.

A recently developed technique, Time Resolved Spectroscopy (TRS) uses laser light at 670 nm, and allows to assess the flesh colour of intact fruit. In previous studies (e.g. Cubeddu *et al*. 2001, Eccher Zerbini *et al*. 2006) it was

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already indicated that the TRS absorption coefficient μ_a can be used to determine the stage of development (maturity status) of peaches and nectarines. In this study attention is devoted to unravel the postharvest behaviour of the TRS absorption coefficient μ_a during storage, and the occurrence and dynamics of the biological variation between individual fruit. The results of these experiments and analyses allow a better understanding and a more successful application to determine and optimise the maturity at harvest of peaches and nectarines.

MATERIALS AND METHODS

Fruit of 'Spring Bright' nectarine cultivar were harvested during the season 2005, at the second commercial harvest in Faenza (Ravenna, Italy). At the moment of harvest fruit were graded in two sizes (A=large, B=medium), individually weighed, measured on two sides by TRS (Cubeddu *et al*. 2001) and ranked by decreasing absorption coefficient at 670 nm (μ_a) (Eccher Zerbini *et al*. 2006). The ranked fruit were grouped with a total of 30 groups corresponding to 30 levels of μ_a. Fruit from each group was randomly assigned to storage at 10°C or 20°C. The absorption coefficient μ_a was measured during storage individually in each fruit.

Model development was conducted using Maple 10 (Waterloo Maple Inc, Waterloo, Canada). The data gathered were analysed using mixed effects non linear regression analysis (nlme procedure in R, R Development Core Team, 2005) based on Eq. 2, estimating the rate constant k_m in common for all fruit and Δt separate for each individual fruit. The estimated time shift factors were checked on normality using the Kolmogorov-Smirnov test in R.

The change in μ_a during storage is reported in Tijskens *et al*. (2006). The behaviour of μ_a, the absorption by the fruit flesh of laser light at 670 nm, clearly shows a sigmoidal behaviour. This was modelled by a standard logistic function (Eq.1):

$$\mu_a = \frac{\mu_{a,\max}}{1 + \left(\frac{\mu_{a,\max}}{\mu_{a,0}} - 1 \right) \cdot e^{k_m \cdot t}} \qquad \text{Eq. 1}$$

Where: μ_a = the absorption coefficient at 670 nm measured by TRS
$\mu_{a,max}$ = the absorption coefficient at minus infinite time (maximum absorption ever possible)
$\mu_{a,0}$ = initial absorption coefficient at time zero
k_m = rate constant of μ_a decay in time
t = storage time

In the pre-exponential factor, $\mu_{a,0}$ represents the condition of the fruit at the moment of harvest. This is of course different for each individual fruit. This pre-exponential factor can be expressed as a time shift, relative to the behaviour

of some "mean" fruit. After some algebraic manipulations this results in the final equation:

$$\mu_a = \frac{\mu_{a,\max}}{1 + e^{k_m \cdot (t + \Delta t)}} \qquad \text{Eq. 2}$$

Where Δt = biological shift factor, relative to the midpoint of the logistic curve. Δt now contains all the information on the initial conditions of each individual fruit. The biological variation present in batches of harvested fruits is contained in the shape and type of distribution over this biological shift factor.

Applying some algebraic calculus, the biological shift factor Δt (at zero time) can be represented as Eq. 2a.

$$\Delta t = \frac{\log\left(\frac{\mu_{a,\max}}{\mu_{a,0}} - 1\right)}{k_m} \qquad \text{Eq. 2a}$$

On many occasions, the estimated biological shift factor was normally distributed (Tijskens *et al*. 2005, Hertog *et al*. 2004). Based on this assumption, and on the appropriate model for the quality property under study, the distribution and the dynamics of the distribution of μ_a in time can be deduced (Hertog *et al*. 2004, Schouten 2004). In this case, using the model in Eq. 2 this conversion results in Eq. 3:

$$p = \frac{1}{2} \cdot \frac{\sqrt{2} \cdot e^{\left(-\frac{1}{2} \cdot \frac{\left(t - \frac{\log\left(\frac{\mu_{a,\max}}{\mu_a} - 1\right)}{k_m} + \mu \right)^2}{\sigma^2} \right)} \cdot \mu_{a,\max}}{\sqrt{\pi} \cdot \sigma \cdot \mu_a \cdot \left(\mu_{a,\max} - \mu_a\right) \cdot k_m} \qquad \text{Eq. 3}$$

Where p is the density function of the distribution of μ_a, μ and σ are the mean value and the standard deviation respectively for Δt in a normal distribution.

RESULTS AND DISCUSSION

The results of the non linear mixed effects regression analysis is briefly indicated in Table 1 and Fig. 1. The explained parts are all very high: above 0.96 at 20ºC and around 0.85 at 10ºC. The rate constants (per temperature) are about the same, irrespective of the size of the fruit. Also the standard deviations of the

biological shift factor are the same, irrespective of size and temperature. The only real difference is to be found in the mean values of the biological shift factor Δt. Smaller fruit (B) are less ripe (lower value) then are the larger fruit (A). In that sense it is correct what is normally assumed that smaller fruit are less ripe than larger fruit, with a difference of about 1.5 day at 20ºC. As already pointed out, the standard deviation of Δt is the same for both temperatures. That is logical since all variation in ripeness and biological shift factor is built up during the growth and ripening at the tree, at the conditions in the orchard. And that is of course the same for both fruit sizes.

Table 1. Results of the non-linear mixed effects regression analysis based on Eq. 2 for both temperatures and both sizes individual and combined

Temp	20 ºC			10 ºC		
Size	A	B	Both	A	B	Both
k_m	0.213	0.236	0.226	0.148	0.123	0.134
Δt_mean (=μ)	3.191	1.491	2.272	3.275	2.970	3.186
Δt_stdev (=σ)	1.618	1.437	1.6250	1.357	1.644	1.664
$\mu_{a,max}$ [b]	0.6	0.6	0.6	0.6	0.6	0.6
N_{obs}	150	150	300	180	180	360
N_{fruit}	30	30	60	30	30	60
R^2_{adj}	0.968	0.976	0.972	0.855	0.844	0.850
[b]	Fixed					

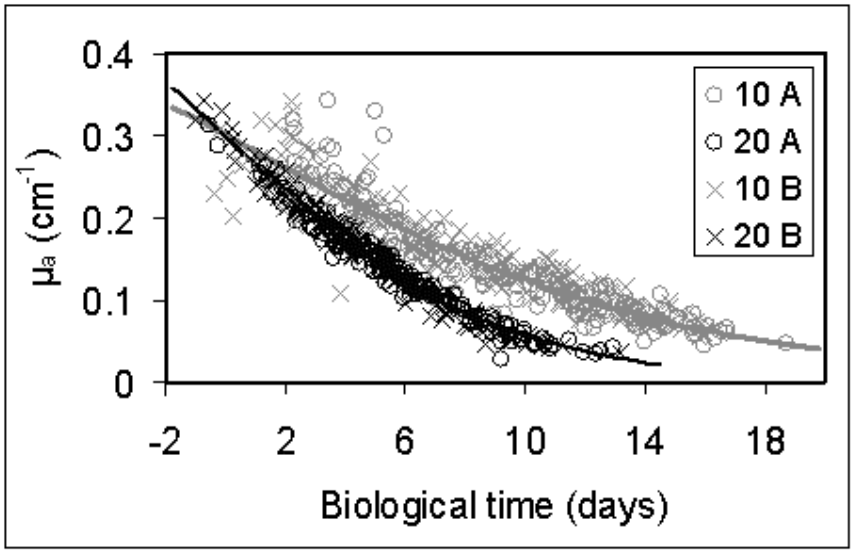


Fig. 1. Behaviour of μ_a as a function of time, expressed as biological time (t+ Δt) for both temperatures (10 and 20ºC) and both sizes (A and B)

To check the similarity in Δt distribution for both temperatures, the Δt values were expressed as difference to their respective mean:

$$\Delta t_{dif} = \Delta t - \Delta t_{mean} \qquad \text{Eq. 4}$$

In this way the differences in distribution for each time of storage, temperature and size can be taken care of and the distributions "standardised". The distributions of Δt_{dif} were checked on normality using the one-sample Kolmogorov-Smirnov test. All p values obtained were well above 0.88 indicating normality. Moreover, the two distributions per temperature were found the same in a two-sample-test with a p value of 0.93. This means that the two distributions expressed as standardised biological shift factor (Δt_{dif}) can be treated as equal. In Fig. 2 the distribution of all Δt_{dif} values (both temperatures, both sizes) are shown.

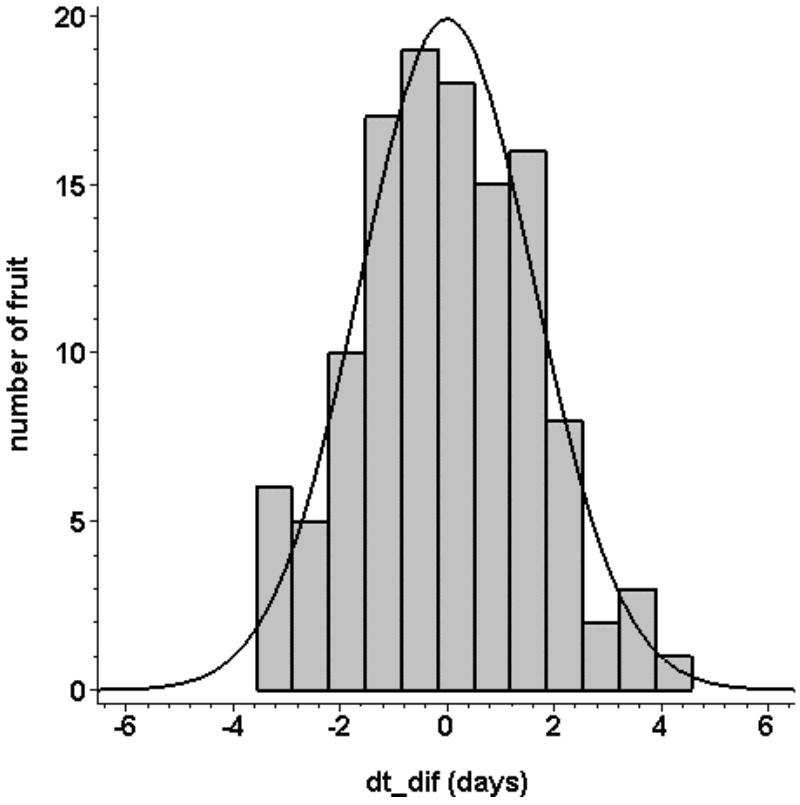


Fig. 2. Distribution of Δt_{dif}. Bars= estimated, lines= theoretical.

The fact that the distributions of the standardised biological shift factor (Δt_{dif}) can be pooled for all times during storage, does mean that the biological shift factor does only change in mean value (μ), not in the standard deviation (σ). Ripening hence represents a moving of the distribution over time: for each

day at a certain storage temperature, the mean value for Δt will change with the value of k_m (at that temperature). The standard deviation remains however the same.

On the measured property however, here the TRS absorption at 670 nm, time will have a marked effect, as described by the derived density distribution function (Eq. 3). In Fig. 3 the behaviour of μ_a (all sizes at 20ºC) is shown as function of the storage time. Clearly can be seen that the mean value shift during storage towards more ripe fruit (low μ_a values), while at the same time, the distribution becomes more and more skew, pushing up against the physical limit of zero absorption.

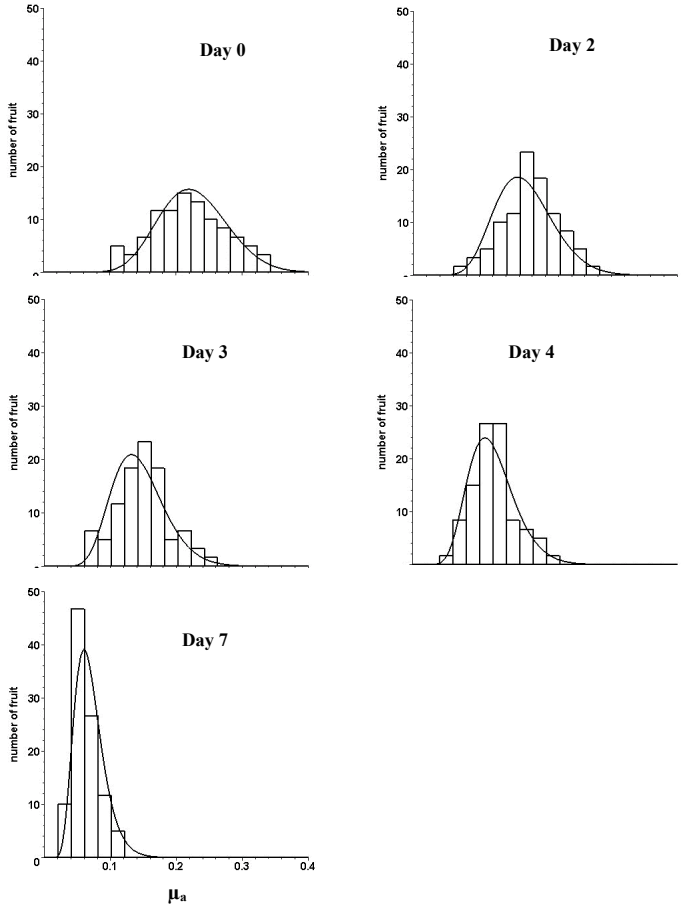


Fig. 3. Distribution in μ_a during storage (20ºC both sizes)

CONCLUSIONS

Ripening of fruit only affects the mean value of biological shift factors. The variation present remains unchanged during postharvest storage. The same conclusion can be drawn for different sizes of fruit: only the mean value is af-

fected, not the variation. The effect on the property itself (here TRS absorption), that is what we and consumer do observe is much more pronounced. The theoretically deduced distribution function does describe properly the changes in observed variation as a consequence of storage and ripening.

Non linear mixed effect analysis of data on individual fruit is a very powerful statistical technique to extract information on the variation and its dynamics in batches of fruit. That again stresses the importance of developing sensitive, reliable but above all non destructive measuring techniques, like TRS, for optimising harvest date, chain management and product quality to consumers.

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ZMIENNOŚĆ BIOLOGICZNA W DOJRZEWANIU NEKTARYN

Streszczenie

Optymalizacja terminu zbioru nektaryn nie może być dłużej opierana na kolorze skórki, ponieważ nowe odmiany charakteryzują się intensywnym rumieńcem, nawet gdy są jeszcze niedojrzałe. Pomiar absorpcji miąższu owoców metodą spektroskopii opóźnionej (Time Resolved Spectroscopy - TRS) przy długości fali 670 nm, dostarcza informacji na temat dojrzałości owoców jak również zróżnicowania stanu dojrzałości poszczególnych partii owoców. Ponieważ poszczególne owoce są zbierane i mierzone w różnych stanach dojrzałości analiza zebranych danych oparta została na systemie przesuniętego wieku biologicznego (Tijskens i in. 2005)

Wyniki wskazują, że μ_a, a co za tym idzie również barwa miąższu i zawartość chlorofilu zmieniają się sigmoidalnie i zostały przybliżone symetryczną funkcją logistyczną. Udział wariancji wyjaśnionej (R^2_{adj}) wyniósł około 0,97 dla owoców przechowywanych w 20°C i około 0,86 dla owoców przechowywanych w 10°C. Te wysokie wartości wariancji wyjaśnionej wskazują, że raczej duża zmienność barwy obserwowana pomiędzy poszczególnymi owocami (z których każdy był na swoim własnym etapie rozwoju) została skutecznie wyjaśniona systemem przesuniętego wieku biologicznego.
W czasie dojrzewania rozkład przesunięć w wieku biologicznym był normalny i nie zmieniał się, podczas gdy rozkład μ_a ulegał zmianom.
Również zmiany rozkładu w czasie są dobrze opisane przez użyty system i modele.