Influence of Cold Storage Time on the Softening Prediction in 'Spring Bright' Nectarines

A. Rizzolo, M. Vanoli and P.E. Zerbini
CRA-IAA Agric. Research Council Food
Technology Research Unit
Milano
Italy

L. Spinelli
CNR-IFN
Istituto di Fotonica e Nanotecnologie
Milano
Italy

A. Torricelli
Politecnico di Milano
Department of Physics
Milano
Italy

Keywords: *Prunus persica* L. Batsch, firmness decay model, softening rate constant, prediction ability

Abstract

With Time-resolved Reflectance Spectroscopy (TRS) the maturity of nectarines at harvest can be assessed by measuring the absorption coefficient at 670 nm (μ_a 670) in the fruit flesh. A kinetic model has been developed linking the optical properties as measured by TRS with the models of μ_a 670 and firmness decay in shelf-life at 20°C, making the prediction of the softening time for individual fruit possible. In order to study the influence of cold storage time prior to shelf life on the softening prediction, 540 (year 2003) and 870 (year 2004) 'Spring Bright' nectarines were measured at harvest with TRS; then fruit were put in shelf life after various periods of cold storage at 0°C (4 and 10 d, year 2003; 6, 13 and 20 days, year 2004). During the 5-day period of shelf life at 20°C, fruit were analysed for firmness by pressure test after 30, 48, 54, 72, 78, 96, 102 and 120h in 2003 and after 36, 43, 62, 87, 108 and 135h in 2004. For each year and cold storage time, the parameters of the logistic model of softening as a function of μ_a 670 at harvest were computed. The cold storage up to 13 days did not significantly influence the estimates of the softening rate constant (k_f), of the maximum firmness at minus infinite time (F_{max}) and of parameter alpha (α) in both years, whereas parameter beta (β) in 2003 significantly decreased from -1.867 at day 4 to -2.237 at day 10. The further 7 days of cold storage in 2004 significantly affected k_f, which decreased from 0.00084 at days 6 and 13 to 0.00069 at day 20, and β which increased from -2.395 at day 6 to -2.053 at day 20. Our results indicate that the cold storage time significantly influences the softening prediction of nectarines as the longer the cold storage, the lower the softening rate.

INTRODUCTION

The maturity at harvest of nectarines can be assessed by measuring the absorption coefficient at 670 nm (μ_a) with the non-destructive technique of Time-resolved Reflectance Spectroscopy (TRS) (Eccher Zerbini et al., 2006). A kinetic model links μ_a, expressed as the biological shift factor (Δt*), to firmness decrease during ripening; in this way the model includes the variations in maturity at harvest of individual fruit (Tijskens et al., 2006). The μ_a decrease in nectarines is synchronized with softening, hence the shelf life for individual fruit can be predicted (Tijskens et al., 2007). In 2006 this methodology was successfully applied in an export trial from Italy to the Netherlands, showing the application of a prototype under commercial transport conditions and simulating on a small scale the fruit supply chain from the packing-house to the consumers (Eccher Zerbini et al., 2009). The relationship between μ_a and firmness has been found not to be affected by fruit size/mass (Eccher Zerbini et al., 2006) or by cold storage, as Eccher Zerbini et al. (2006) found that nectarine fruit soften as much in 100h at 0°C as in 1h at

20°C.

The softening process in peaches has been found to be caused by depolimerization of pectins and hemicellulose, decrease in pectin methyl esterification and decrease in the neutral sugar side chains of rhamnogalacturan I (Lurie et al., 1994; Zhou et al., 2000). During this ripening process pectin esterase (PE) activity decreases and polygalacturonase (PG) activity increases (Beuscher and Furmanski, 1978). However, in cold stored peaches an increase in PE activity and an inhibition of PG activity have been found when compared to their activities in normal fruit ripening (Ben-Arie and Sonego, 1980). Furthermore, Lurie et al. (2003) found that storage at low temperatures for a few weeks can cause in peach fruit an imbalance in cell wall metabolism: during a 30 day storage at 0°C there was de-methylation due to higher PE activity, whereas in fruit ripened after storage there was only little changes in pectin methylation or pectin content in cell walls as de-esterification of pectins was not accompanied by depolimerization, as occurs in fruit ripened at harvest. The fact that the balance between PE and PG activities changes with cold storage could influence the kinetic model of softening based on μ_a as measured by TRS, which was based on the enzymatic pattern at harvest. So, the aim of this study was to evaluate whether storage time at 0°C affects the parameters of firmness decay model and its prediction ability in 'Spring Bright' nectarines, by testing it for misclassification.

MATERIALS AND METHODS

'Spring Bright' nectarines, harvested on 16 July 2003 (540 fruit) and 19 July 2004 (870 fruit) in the same commercial orchard in Faenza (Forlì, Italy), were selected by size (A=73-79.9 mm; B=67-72.9 mm), measured on two sides by TRS at 670 nm using a prototype built at Politecnico di Milano (Torricelli et al., 2008) and ranked separately for each size according to decreasing μ_a value (average of the two sides), that is from less to more mature fruit. In both years ranked fruit of each size were grouped into 30 sets, corresponding to 30 levels of μ_a. Each fruit from each set was randomly assigned to a different time of analysis in order to have fruit from the whole range of μ_a at every sampling time. Then nectarines were put in shelf life after cold storage at 0°C for 4 and 10 days (year 2003) and 6, 13 and 20 days (year 2004). During the 5-day period of shelf life at 20°C, fruit were analyzed for firmness (Texture Analyzer TA.Xtplus, Stable Micro Systems, England, 8 mm diameter plunger, crosshead speed 200 mm/min) after 30, 48, 54, 72, 78, 96, 102 and 120h in 2003 and after 36, 43, 62, 87, 108 and 135h in 2004.

For each year and cold storage time, the μ_a values were converted into the TRS biological shift factor ($\Delta t_{\mu a}*$) according to Equation (1) (Tijskens et al., 2006):

$$\Delta t_{\mu a}* = \log\left(\frac{\mu_{a,\max}}{\mu_a} - 1\right) \tag{1}$$

where $\mu_{a,\max}$ is the maximum μ_a value possible fixed at 0.65 cm^{-1} (Tijskens et al., 2006). Then firmness (F) data were analysed by non-linear regression analysis (PROC NLIN, SAS/STAT, SAS Institute Inc., Cary, NC, 2002) according to the logistic Equation (2) (Tijskens et al., 2007) which describes the sigmoidal decay of firmness:

$$F = \frac{F_{\max} - F_{\min}}{1 + e^{k_f \cdot (F_{\max} - F_{\min}) \cdot t + \Delta t_F^*}} + F_{\min} \tag{2}$$

where: F_{max} is the maximum firmness at minus infinite time; F_{min} is the minimum firmness achieved at infinite time; k_f is the softening rate constant at a given temperature; t is time; Δt_F* is the biological shift factor for firmness, which, in nectarines, is linearly related with the biological shift factor for μ_a ($\Delta t_{\mu a}*$) according to Equation (3):

$$\Delta t_F * = \alpha\left(\Delta t_{\mu a} * + \beta\right) \tag{3}$$

where α and β are parameters to be estimated.

The prediction ability of firmness decay models for each year and cold storage time was evaluated by comparing the predicted firmness (F_{pred}) of every fruit to its measured firmness (F_{meas}) by using linear regression analysis and the standard error of the estimate of the regression and the mean absolute error (MAE) were chosen to measure the performance of models. MAE is the average of the absolute errors between the measured and predicted values and it is computed according to Equation (4):

$$MAE = \frac{1}{n}\sum_{i=n}^{n}\left|F_{meas\,i} - F_{pred_i}\right| \tag{4}$$

In addition, in order to test models for misclassification, fruit of each year and cold storage time were categorized into six principal μ_a classes of predicted firmness potential for handling and eating according to the upper and lower limits of μ_a reported by Eccher Zerbini et al. (2009), and considering the intervals between classes as additional classes (Table 1). Then in each class based on the μ_a value at harvest (class M_i where *i* is the class number in Table 1) the F_{meas} value after shelf life was compared to the firmness predicted for the limits of the class according to the firmness decay model. The prediction was considered correct when the F_{meas} value fell within the firmness interval predicted by the model for the specific M_i class. Furthermore, also F_{meas} values which fell within the limits of the immediately adjacent M classes (firmer, F_{meas} belonging to class M_{i-1}, softer, class F_{meas} belonging to class M_{i+1}) were considered. Classification results for these three types of prediction for each usability class and model were expressed as percent to total number of fruit categorized in each class M_i.

The models studied in this research, hereafter referred to by the subscript "cool", were also compared with the models computed using all data of each year, independently of the time spent by fruit at 0°C (model's parameters in Tijskens et al., 2007, Table 2), hereafter referred to by the subscript "T".

RESULTS AND DISCUSSION

The models' parameters estimated by non-linear regression analysis of firmness data in shelf life separately for different length of storage are reported in Table 2. The percentage variance accounted for (R^2_{adj}) was high for all the models, well above 80%. The values of the estimates of parameters α, F_{max} and F_{min} were not different among the models within the same season. The time at 0°C prior to shelf life did not significantly affect the value of k_{f} till 10-13 d in both seasons, whereas the extension of the period at 0°C to 20 d (year 2004) resulted in a significant decrease in k_{f} value. In addition, time at 0°C significantly influenced the estimate of β parameter in both years: in 2003 it decreased, while in 2004 it increased. These differences significantly affected the average biological shift factor for firmness (Δt_F*) computed with parameters of models of Table 2 (BSF_{cool}): in 2003 BSF_{cool} was more negative with the increase of time at 0°C, independently of class, while in 2004 the longer the time at 0°C, the less negative the BSF_{cool}, whatever the class (Table 3). Comparing BSF_{cool} to the average Δt_F* computed with parameters reported in Table 2 by Tijskens et al. (2007) (BSF_T) for each class M_i of usability (Table 3) it can be seen that in 2003, BSF_{cool} after 4 d at 0°C had significantly higher values than BSF_T, whatever the usability class taken into consideration, whilst after 10 d at 0°C BSF_{cool} showed significantly lower values than BSF_T, independently of the usability class. In season 2004, after 6 d and 13 d at 0°C BSF_{cool} had significantly lower values than BSF_T, for all the classes of usability, except for O class after 13 d, for which BSF_{cool} was not different form BSF_T. After 20 d at 0°C, BSF_{cool}, if compared to BSF_T, had lower values for the classes from T to RF, it was not different for RS class, while it had higher values for ORS and O classes. These differences in BSF can actually influence the performance of firmness models in predicting softening in shelf life and, hence, the goodness of fruit classification at harvest for different market destinations. The BSF for firmness indicated that, if the effect of cold time were not considered in analyzing data sets for the estimate of firmness decay model, the estimate of the time necessary to reach the midpoint of the firmness decay curve could be uncorrected, that is either postponed (4 d-2003) or advanced (10 d-2003, 6 d-2004, 13 d-2004). In the case of 20 d-2004 the comparison between BSF_{cool} and BSF_{T} stressed the difference in k_f, with fruit characterized at harvest by $\mu_a > 0.18\ \mathrm{cm}^{-1}$ (classes T, RFT and RF) in a less advanced point of the decay curve according to BSF_{cool} when compared to BSF_T, and fruit with $\mu_a < 0.1\ \mathrm{cm}^{-1}$ at harvest (classes ORS and O) in a more advanced point. This fact significantly influenced the percentage of correctly predicted fruit for the classes ORS and O. So, our data indicate that the cold storage time significantly influenced the softening prediction of nectarines, thereby it has to be considered in developing the firmness decay model in order to correctly predict the market destination of fruit.

The prediction ability of the firmness decay model was evaluated firstly by comparing predicted firmness (F_pred) to actual firmness (F_meas) values by using linear regression analysis, and, then, by comparing the predicted class based on firmness model to the actual firmness. Considering the results of regression analyses for models of year 2003 (Table 4), all the regressions showed $r>0.92$ and R^2_adj higher than 85%, even if the regressions calculated with F_pred from models 4 d_{cool} and 10 d_{cool} were characterized by slightly lower SEE and MAE than those computed with F_pred from model *T* for the same sets of fruit (columns 4 d_T and 10 d_T in Table 4). Quite different was the scenario for year 2004 (Table 5). Except for the set of fruit kept at 0°C for 6 d, for which model *T* had higher performances than model 6 d_{cool}, with the increasing of time at 0°C the performance of model *T* decreased. In fact, regressions calculated with F_pred from models 13 d_{cool} and 20 d_{cool} showed $\mathrm{r}>0.91$ and $R^2_\mathrm{adj} >83\%$, whereas regression 13 d_T had r=0.84 and $R^2_\mathrm{adj}<71\%$, and regression 20 d_T showed the worst performance, having r=0.76 and $R^2_\mathrm{adj}<60\%$. In addition, regressions calculated with F_pred from models 6 d_{cool} and 13 d_{cool} were characterized by lower SEE and MAE than those computed with F_pred from model *T* for the same sets of fruit (Table 5).

Comparing the classification results obtained with *cool* and *T* models in both years (Fig. 1A and B), when the effect of time at 0°C is taken into consideration there was on average a higher percentage of correctly predicted fruit, especially for O and ORS classes in both years, and RF and RFT classes in 2004.

CONCLUSIONS

The softening kinetics decreased significantly after 20 days of storage at 0°C, confirming the hypothesis of a change in the activity of the enzymes involved in the softening process. Also the β parameter changed with cold storage, but in a different way in the two years. As a consequence, when the cold storage period exceeded 13 days, the models estimated by segregating fruit according to cold storage length gave a better prediction than the general models neglecting the cold storage period. This fact improved the percentage of fruit correctly classified.

In conclusion, the cold storage period should be considered when developing firmness decay models in order to correctly predict the shelf life of fruit.

Literature Cited

Ben-Arie, R. and Sonego, L. 1980. Pectolytic enzyme activity involved in woolly breakdown of stored peaches. Phytochemistry 19:2553-2555.

Buescher, R.W. and Furmanski, R.J. 1978. Role of pectinesterase and polygalacturonase in the formation of woolliness in peaches. J. Food Sci. 43:264-266.

Eccher Zerbini, P., Vanoli, M., Grassi, M., Rizzolo, A., Fibiani, M., Cubeddu, R., Pifferi, A., Spinelli, L. and Torricelli, A. 2006. A model for the softening of nectarines based on sorting fruit at harvest by time-resolved reflectance spectroscopy. Postharvest Biol. Technol. 39:223-232.

Eccher Zerbini, P., Vanoli, M., Rizzolo, A., Jacob, S., Torricelli, A., Spinelli, L. and Schouten, R.E. 2009. Time-resolved Reflectance Spectroscopy as a management tool in the fruit supply chain: an export trial with nectarines. Biosystems Engineering 102(3):360-363.

Lurie, S., Levin, A., Greve, L.C. and Labavitch, J.M. 1994. Pectic polymer changes in nectarines during normal and abnormal ripening. Phytochemistry 36:11-17.

Lurie, S., Zhou, H.W., Lers, A., Sonego, L., Alexandrov, S. and Shomer, I. 2003. Study of pectin esterase and changes in pectin methylation during normal and abnormal peach ripening. Physiologia Plantarum 119:287-294.

Tijskens, L.M.M., Eccher Zerbini, P., Vanoli, M., Jacob, S., Grassi, M., Cubeddu, R., Spinelli, L. and Torricelli, A. 2006. Effects of maturity on chlorophyll related absorption in nectarines, measured by non-destructive time-resolved reflectance spectroscopy. Int. J. Postharvest Technol. Innov. 1:178-188.

Tijskens, L.M.M., Eccher Zerbini, P., Schouten, R.E., Vanoli, M., Jacob, S., Grassi, M., Cubeddu, R., Spinelli, L. and Torricelli, A. 2007. Assessing harvest maturity in nectarines. Postharvest Biol. Technol. 45:204-213.

Torricelli, A., Spinelli, L., Contini, D., Vanoli, M., Rizzolo, A. and Eccher Zerbini, P. 2008. Time-resolved reflectance spectroscopy for non-destructive assessment of food quality. Sensing and Instrumentation for Food Quality and Safety 2:82-89.

Zhou, H.W., Lurie, S., Lers, A., Khatchitski, A., Sonego, L. and Ben-Arie, R. 2000. Delayed storage and controlled atmosphere storage of nectarines: two strategies to prevent woolliness. Postharvest Biol. Technol. 18:133-141.

Tables

Table 1. Lower limit of μ_a of each class M_i of predicted firmness potential for handling and eating for 'Spring Bright' nectarines of seasons 2003 and 2004.

i	Class	Code	μ_a (cm^{-1})	i	Class	Code	μ_a (cm^{-1})
1	will never soften	N	0.42	6		RFT	0.18
2		NH	0.39	7	ready to eat-firm	RF	0.14
3	dangerously hard	H	0.30	8	ready to eat-soft	RS	0.10
4		TH	0.27	9		ORS	0.09
5	transportable	T	0.20	10	overripe	O	<0.089

Table 2. Parameters of firmness model estimated by non-linear regression analysis of firmness data in shelf life after different periods of storage at 0°C according to Equation (2) combined with Equation (3). In each year and row, means followed by different letters are significantly different.

Parameter value	Year 2003		Year 2004		
	Model				
	3 d_{cool}	10 d_{cool}	6 d_{cool}	10 d_{cool}	17 d_{cool}
	Nobs				
	270	270	310	280	280
k_f	0.00120 ^{a}	0.00152 ^{a}	0.000845 ^{b}	0.000840 ^{b}	0.000693 ^{a}
α	3.80 ^{a}	3.56 ^{a}	2.79 ^{a}	3.15 ^{a}	2.80 ^{a}
β	-1.88 ^{b}	-2.25 ^{a}	-2.40 ^{a}	-2.15 ^{ab}	-2.05 ^{b}
$F_{\max}$	71.05 ^{a}	69.70 ^{a}	79.64 ^{a}	81.34 ^{a}	78.27 ^{a}
$F_{\min}$	5.19 ^{a}	5.21 ^{a}	5.53 ^{a}	5.70 ^{a}	4.84 ^{a}
R^2_{adj}	0.86	0.87	0.87	0.85	0.84
Standard error					
k_f	0.000173	0.000215	0.000127	0.000151	0.000153
α	0.38	0.36	0.28	0.33	0.35
β	0.058	0.072	0.069	0.079	0.094
$F_{\max}$	3.14	3.04	4.31	6.39	7.83
$F_{\min}$	0.85	0.78	0.87	0.68	0.81

Table 3. Comparison between the average biological shift factor for firmness (Δt_F*) computed with parameters of models of Table 2 (BSF_{cool}) and the average Δt_F* computed with parameters reported in Table 2 by Tjiskens et al. (2007) (BSF_T) for each class. SE is the standard error of the mean.

2003	4 d at 0°C				10 d at 0°C			
	BSF_{cool}		BSF_T		BSF_{cool}		BSF_T	
class	mean	SE	mean	SE	mean	SE	mean	SE
H	-5.88	0.067	-6.20	0.064				
TH	-5.37	0.025	-5.71	0.024				
T	-4.27	0.045	-4.65	0.043	-5.12	0.056	-4.60	0.058
RFT	-3.30	0.015	-3.72	0.015	-4.28	0.027	-3.75	0.027
RF	-2.25	0.036	-2.99	0.035	-3.52	0.035	-2.96	0.034
RS	-1.23	0.054	-1.73	0.052	-2.36	0.041	-1.77	0.042
ORS	-0.43	0.020	-0.96	0.019	-1.34	0.024	-0.73	0.025
O	0.37	0.072	-0.19	0.069	-0.96	0.093	-0.35	0.095

2004	6 d at 0°C				13 d at 0°C				20 d at 0°C			
	BSF_{cool}		BSF_T		BSF_{cool}		BSF_T		BSF_{cool}		BSF_T	
class	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
TH	-6.05	0.083	-4.52	0.069								
T	-4.99	0.139	-3.63	0.116	-4.71	0.084	-3.50	0.061	-3.72	0.051	-3.36	0.042
RFT	-4.25	0.031	-3.02	0.026	-4.00	0.025	-2.98	0.018	-3.31	0.027	-3.02	0.023
RF	-3.50	0.036	-2.39	0.030	-3.18	0.036	-2.38	0.027	-2.58	0.031	-2.41	0.026
RS	-2.53	0.028	-1.59	0.023	-2.06	0.034	-1.55	0.025	*-1.56*	0.032	*-1.57*	0.027
ORS	-1.77	0.016	-0.96	0.013	-1.20	0.019	-0.92	0.014	-0.78	0.027	-0.92	0.022
O	-0.86	0.071	-0.20	0.059	*-0.36*	0.063	*-0.30*	0.047	-0.06	0.053	-0.32	0.044

Table 4. Results of linear regression analyses between F_{meas} after shelf life at 20°C and F_{pred} from μ_a at harvest according to firmness decay models of year 2003 with F_{pred} values from models of Table 2 (subscript "cool") and F_{pred} values for model 2003 by Tijskens et al. (2007, data Table 2) (subscript "T").

	4 d_{cool}	10 d_{cool}	4 d_T	10 d_T
*N*obs	270	270	270	270
intercept[§]	2.86 (0.618)	2.68 (0.604)	1.93 (0.656)	2.07 (0.617)
slope[§]	0.86 (0.021)	0.87 (0.021)	0.89 (0.022)	0.86 (0.021)
r	0.927	0.932	0.924	0.929
R^2_{adj}	85.94	86.91	85.41	86.26
SEE[¥]	7.227	7.082	7.675	7.228
MAE[¥]	4.833	4.529	5.350	4.862

[§] estimate and standard error between brackets.

[¥] SEE is standard error of the estimate of the model; MAE is mean absolute error.

Table 5. Results of linear regressions analysis between F_{meas} after shelf life at 20°C and F_{pred} from μ_a at harvest according to firmness decay models of year 2004 with F_{pred} values from models of Table 2 (subscript *cool*) and F_{pred} values for model 2004 by Tijskens et al. (2007, data Table 2) (subscript *T*).

	6 d_{cool}	13 d_{cool}	20 d_{cool}	6 d_T	13 d_T	20 d_T
*N*obs	250	279	280	250	279	280
intercept[§]	2.75 (0.640)	2.46 (0.512)	2.67 (0.520)	2.44 (0.729)	3.15 (0.690)	2.97 (0.448)
slope[§]	0.91 (0.028)	0.85 (0.021)	0.84 (0.022)	0.86 (0.020)	0.80 (0.031)	0.63 (0.032)
r	0.898	0.922	0.915	0.938	0.839	0.762
R^2_{adj}	80.59	85.01	83.63	87.89	70.35	57.92
SEE[¥]	7.101	6.221	6.226	7.441	7.668	5.134
MAE[¥]	4.644	3.829	3.905	5.194	5.555	3.758

[§] estimate and standard error between brackets;

[¥] SEE is standard error of the estimate of the model; MAE is mean absolute error.

Figures

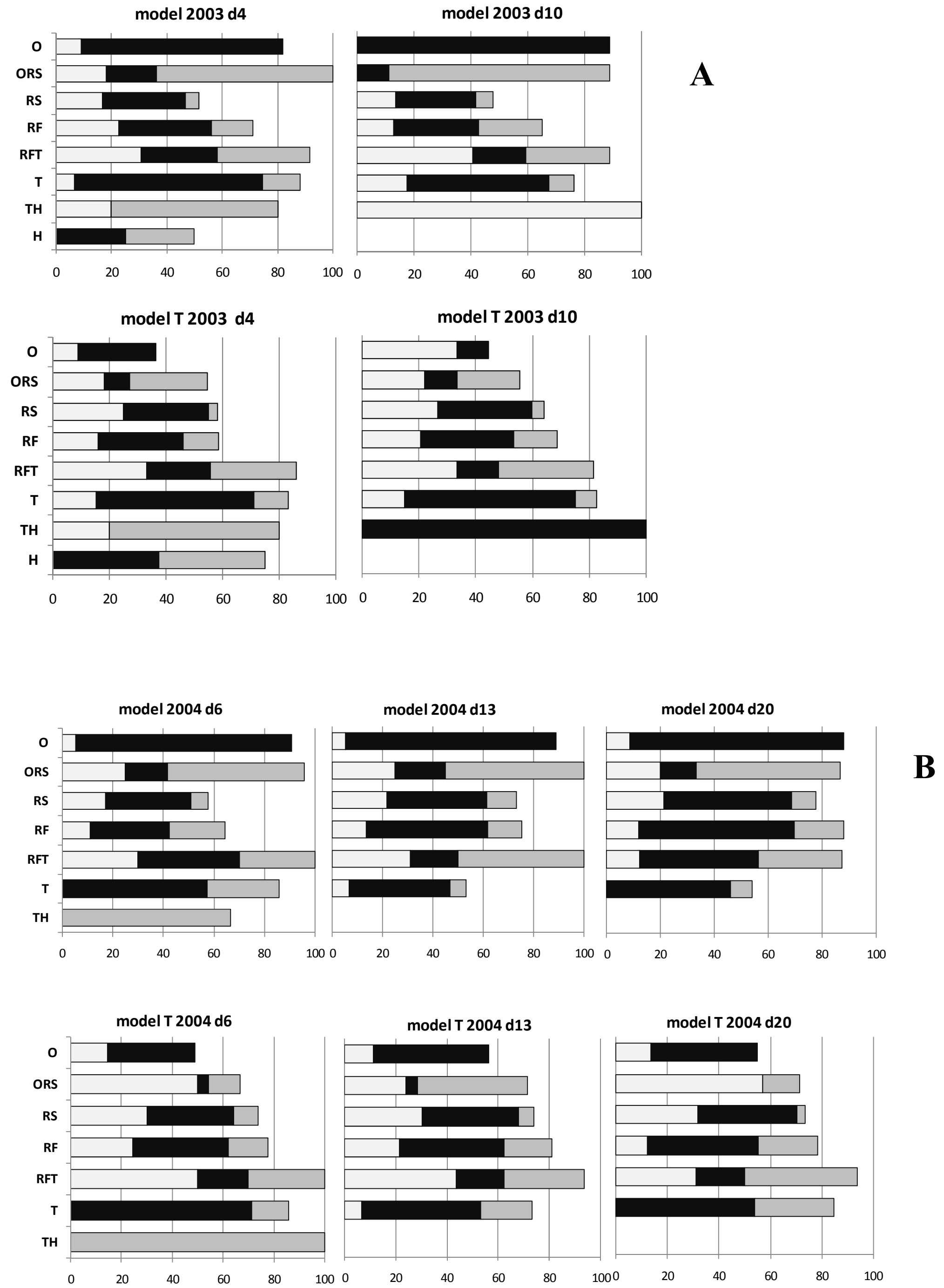

Fig. 1. Percentages of fruit of each class which had been correctly predicted (F_{meas} = class M_i, black) and with F_{meas} included within the limits of the immediately adjacent class (firmer fruit, F_{meas} =class M_{i-1}, white; softer fruit, F_{meas} =class M_{i+1}, grey). The fruit which fell outside these limits constitute the difference to 100%. Comparison of classification results for the year 2003 (A) and year 2004 (B) obtained with firmness models *cool* and firmness model *T*.