Environmentally Friendly and Safe Technologies for Quality of Fruits and Vegetables

UNIVERSIDADE DO ALGARVE | 2010

The papers contained in this book report some of the peer reviewed Proceedings of the International Conference "Environmentally friendly and safe technologies for quality of fruit and vegetables", but also other papers related with the subject were included. The manuscripts were reviewed by the Editor and Editorial Board, and only those papers judged suitable for publication were accepted. The Editor wish to thank to all the reviewers and authors for their contribution.

Proceedings of the International Conference "Environmentally friendly and safe technologies for quality of fruit and vegetables", held in Universidade do Algarve, Faro, Portugal, on January 14-16, 2009. This Conference was a join activity with COST Action 924.

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SECTION 2. PRE-HARVEST FACTORS AFFECTING POSTHARVEST QUALITY AND SAFETY

11. VARIATION IN APPLE COLOUR AND MATURITY. CAUSES AND SIMILARITIES OVER ORCHARDS, MANAGEMENT, CULTIVARS AND STORAGE

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Abstract

The colour of apples (flesh colour or skin colour) was assessed using the same individual apples repeatedly in time at three different locations, in several seasons for five different cultivars. Two experiments were conducted in the orchard, one experiment during postharvest storage. The same logistic model was applied to analyse the data, separate for each location and cultivar. Non linear mixed effects regression analysis allows to extract not only information on the kinetic parameters like reaction rate constant and potential greenness, but also on the variation present in the data. The rate constant of the decolouration process was found to be largely the same for all combinations (with one exception). The variation in biological shift factor, as an expression for maturity, seems to be independent of orchard location and only slightly dependent on orchard management procedures. The main differences observed are in the potential greenness of the apples (col_{min}) that vary considerably between successive seasons and between cultivars. The applied technology provides the necessary tools to analyse the effects of season and orchard management, for all locations in the study. It opens wide alleys to investigate more dedicated the effects of weather, season, management and orchard location in growing apples with a constant quality (colour) over the seasons, locations and management procedures.

Introduction

Non destructive measurement of apple colour, skin colour or flesh colour using continuous wave or laser light, allows the assessment of colour aspects, in terms of colouring compounds, of the same individual apples both during growth as during storage. These so-called longitudinal data can be analysed using mixed effect non linear regression analysis. The technology and the benefit of this type of analysis has been reported in quite a number of recently published papers. One of the advantages of mixed effects analysis is that information is obtained on the variation in colour status or maturity in a batch of individuals.

In this paper an overview will be presented of the magnitude of variation in colour aspects of apples, both during growth (where does variation come from?) and during storage (how does variation develop further?). Apples from three different regions and seasons of growth, of different cultivars and grown with different orchard management were separately analysed using the mixed effect technique. The results with respect to kinetics of change in colour, as well as to the magnitude and dynamics of change of the variation will be compared.

The main conclusion seems to be that the orchard (type of planting, soil) and its management (fertilisation, crop load) were the main causes of variation in maturity within a season, while the weather conditions seem to determine the variation over the seasons.

Materials & Methods

Three completely independent projects were carried out on apples of different cultivars.

1. 'Granny Smith' apples were harvest in Slovenia (1997), harvested at 2 stages of maturity. The apples

were individually labelled and the skin colour regularly measured (Minolta CR-200, Minolta Co., Japan) during storage at 1, 4 and 10 °C. The full details of setup and analysis are described in Tijskens *et al.* (2008). Results were expressed in the $L^*a^*b^*$ system.

2. Apples (cvs 'Elstar', 'Pinova' and 'Topaz') were grown in the same orchard in Germany in seasons 2004, 2005 and 2006. The orchard was situated on a hill slope. Six trees were selected uphill, six downhill. Excluding the margin rows, ten fruits from each tree were selected arbitrarily and individually labelled for identification. Flesh colour was measured regularly using a portable miniaturised spectrophotometer (Zude & Herold 2002; Truppel 2003; Herold *et al.* 2005) during development at the tree. Flesh colour was expressed as Red-edge, the wavelength with the steepest slope near the chlorophyll absorption peak around 690 nm. Details of setup and analysis are described in Herold *et al.* (2005) and Tijskens *et al.* (2006). More detailed information on the measuring technique can be taken from Zude (2003, 2006).

3. In two consecutive seasons (2001 and 2002) apple trees (cv. 'Golden Delicious') were fertilised at two levels of N-fertilisation, allowing a high, medium or low crop load. Allowing only fruit with a diameter of 15 mm, fruits were thinned by hand to the chosen crop load of 70, 50 or 28 fruits per tree. 4 fruit per tree were chosen and labelled. At regular times during the development of the apples at the tree, skin colour was measured using a Minolta CR-200 Chroma Meter. Fruit chromaticity was expressed in $L^*a^*b^*$ system. Colour measurement started approximately 1 month before the predicted technological maturity of fruits until harvest when the intensive chlorophyll degradation is expected to begin.

The colour model

The behaviour of colour, whether expressed as a*-value or red-edge wavelength, can be described by a logistic function. Expressing this function in the notation for biological shift factor gives Eq1.

$$col = \frac{col_{max} - col_{min}}{1 + e^{-k_c \cdot (col_{max} - col_{min}) \cdot (t + \Delta t)}} + col_{min} \qquad \textbf{Eq 1}$$

In case of application of a*-value (experiments 1 and 3), it is an increasing sigmoidal, in case of red-edge (experiment 2) a decreasing. The only difference is the sign of the rate constant k_c (positive respectively negative). The biological shift factor Δt is expressed relative to the midpoint of the logistic function. Details on the applied mechanism and the deduction of the model can be found in Tijskens *et al*. (2008).

Results & Discussion

The quite different experimental design of three independent experiments makes it difficult to compare results on kinetics and biological variation. These differences in setup have to taken into account at every comparison. Nevertheless, comparing the results of analyses and the behaviour of apples over season, regions and growing conditions may indicate where which effects are important.

Kinetic Behaviour

The applied model (Eq 1) is derived from a reaction mechanism of chlorophyll degradation (Tijskens *et al*. 2008). That ensures that the equation reflects a fundamental process related to chlorophyll decay and that the model is generic in nature. In Fig 1 some examples are shown for the colour development versus biological time (t+Δt). As a consequence, the rate constants should be comparable. Of course, the different temperatures during the experiments have to be taken into account, and also the possibility of a different level of chlorophyll degrading enzymes, affecting the rate of the process, in the produce from different cultivars, seasons and regions. The rate constants for location Potsdam, for location Maribor (2002) and for location South Slovenia are quite comparable, considering that the reference temperature for the latter is set at 10 °C.

The rate constant for location Maribor (2001) however is about 10 times higher. The climatic conditions were very different in these seasons: the season 2001 was very wet, while the early season 2002 was very dry, followed by an intense wet period. Possibly, the weather conditions induced this large discrepancy. Nevertheless, a rate constant 10 times higher is very unusual.

Another strange point in these data is that the mean biological shift factors (Δt in Table 1) are so different: in the orchard (location Potsdam and Maribor) is around -20 days, while in the storage experiments the mean biological shift factor is -190 days.

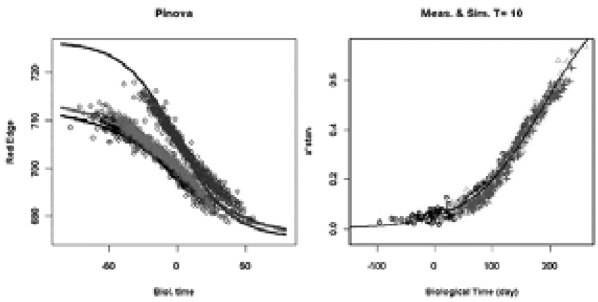

Fig 1. Colour development versus biological time. Left: Postdam data for cv. 'Pinova' in three seasons, right: South Slovenia data cv. 'Granny Smith' from all three orchards stored at 10 °C for the two moments of harvest. Symbols measured, lines simulated.

Even considering the lower temperatures affecting the biological shift factor, reflected in the dimensionless biological shift factor (Δt*=kt·Δt see Table 3), this difference is huge. This could indicate that the colour decay mechanism at the orchard is different than at storage.

Table 1. Overview of the separate result.

Location	Year	Cultivar	col_{min}	Δt	k_c	σ (Δt) *Fruit*	σ (Δt) *Tree*	N_{obs}	N_{gr}	R^2_{adj}
Slovenia South	1997	Granny Smith	-21.498	-189.69	0.00060[a]	41.007	na	3211	540	0.97
Potsdam	2006	Elstar	717.41	-13.53	0.00213	5.540	2.130	661	120	0.97
Potsdam	2005	Elstar	715.78	-22.51	0.00140	6.245	6.889	720	120	0.98
Potsdam	2004	Elstar	711.10	-37.68	0.00223	7.296	5.426	752	120	0.98
Potsdam	2006	Pinova	726.45	-12.00	0.00096	4.968	6.728	711	120	0.97
Potsdam	2005	Pinova	713.96	-34.66	0.00140	7.353	1.629	721	120	0.97
Potsdam	2004	Pinova	711.47	-44.94	0.00181	9.887	2.122	776	120	0.97
Potsdam	2006	Topaz	730.29	-15.06	0.00078	6.398	3.011	723	120	0.98
Potsdam	2005	Topaz	721.13	-7.70	0.00096	6.714	3.184	721	120	0.97
Maribor	2001	G. Delicious	-16.82	-38.74	0.01069	15.266	na	945	190	0.95
Maribor[b]	2002	G. Delicious	-24.00[c]	-33.69	0.00224	10.156	na	1774	299	0.94
a						at 10 °C				
b						applying an exponential model, rate constant corrected				
c						fixed				

Apparently the harvesting is more traumatic than usually expected. It is apparently not related to the measuring technique: at the Potsdam orchard, fruit flesh colour was measured as red-edge and at the South Slovenia and Maribor orchards, skin colour was measured as a*-value.

The initial conditions of col_{min} are of course different for each cultivar and measuring system.

Biological Shift Factor

The estimated values for the biological shift factor were analysed per appropriate series, on mean value (Δt_{mean}) and standard deviation (σ). Also the normality of the distributions was tested using the Shapiro Wilk test (pvalue). The results are shown in Table 2. In case of the 'Granny Smith' experiments (South Slovenia), the distribution could not be proven to be normal: all pvalues except for the orchard Blanca (BL) at commercial harvest (CM) where below the usually applied limit value of 0.05. The mean values nicely show the difference in harvest date: not exactly 10 days between early harvest (EH) and commercial harvest (CM), but quite close. That indicates that the harvest date was not completely correctly estimated for each orchard. It also indicates that this technology, when equipment and procedures are made practical, can provide a better estimate of the optimal or commercial harvest date. The standard deviations (σ) are high compared to the other two experiments. That is most probably related to the different storage temperatures applied in this experiment.

Table 2. Results of the analysis of the biological shift factor Δt relative to their own mean.

Region	Season	L	H	pvalue	Δt_{mean}	σ	N_{obs}
S. Slovenia	1997	AS	CM	0.0015	-22.28	28.42	90
S. Slovenia	1997	AS	EH	0.0002	-30.73	32.08	90
S. Slovenia	1997	BL	CM	0.1941	14.66	39.02	90
S. Slovenia	1997	BL	EH	0.0000	2.98	39.95	90
S. Slovenia	1997	KK	CM	0.0069	20.42	36.15	90
S. Slovenia	1997	KK	EH	0.0003	14.95	40.88	90
Region	Season	Cultivar		pvalue	Δt_{mean}	σ	Nobs
Potsdam	2004	Elstar		0.0979	-37.68	8.94	120
Potsdam	2005	Elstar		0.6973	-22.51	9.19	120
Potsdam	2006	Elstar		0.1033	-13.53	5.76	120
Potsdam	2004	Pinova		0.0250	-44.93	9.77	120
Potsdam	2005	Pinova		0.1951	-34.66	7.25	120
Potsdam	2006	Pinova		0.2175	-11.99	8.26	120
Potsdam	2005	Topaz		0.0011	-7.70	7.26	120
Potsdam	2006	Topaz		0.0000	-15.05	6.97	120
Region	Season	Nitrogen level	crop load	pvalue	Δt_{mean}	σ	N_{obs}
Maribor	2001	60	Lo	0.0013	-32.62	7.34	32
Maribor	2001	60	Med	0.0042	-32.43	7.71	33
Maribor	2001	60	Hi	0.0708	-31.89	7.01	31
Maribor	2001	105	Lo	0.0232	-33.73	6.44	29
Maribor	2001	105	Med	0.0003	-36.16	5.73	32
Maribor	2001	105	Hi	0.0008	-35.08	5.19	33
Maribor	2002	60	Lo	0.0499	-31.35	11.52	50
Maribor	2002	60	Med	0.8289	-32.33	11.93	51
Maribor	2002	60	Hi	0.7832	-35.07	8.56	50
Maribor	2002	105	Lo	0.5354	-34.06	9.02	49
Maribor	2002	105	Med	0.3574	-33.63	10.05	50
Maribor	2002	105	Hi	0.0996	-35.77	9.12	49

Table 3. Overview of the dimensionless biological shift factor.

Location	Year	CV	Δt	k_c	Δt^*
S. Slovenia	1997	Granny Smith	-189.69	0.0006	-0.1138
Potsdam	2006	Elstar	-13.53	0.0021	-0.0288
Potsdam	2005	Elstar	-22.51	0.0014	-0.0315
Potsdam	2004	Elstar	-37.68	0.0022	-0.0840
Potsdam	2006	Pinova	-12	0.0010	-0.0115
Potsdam	2005	Pinova	-34.66	0.0014	-0.0485
Potsdam	2004	Pinova	-44.94	0.0018	-0.0813
Potsdam	2006	Topaz	-15.06	0.0008	-0.0117
Potsdam	2005	Topaz	-7.7	0.0010	-0.0074
Maribor	2001	G. Delicious	-38.74	0.0107	-0.4141
Maribor	2002	G. Delicious	-33.69	0.0022	-0.0755

A lower temperature not only decreases the rate of the colouration process, but also increases the observed variation. That is reflected in the dimensionless biological shift factor ($\Delta t^* = kt \cdot \Delta t$).

In case of the Potsdam experiments, only the distribution of the biological shift of three series could not be proven to be normal: both series of cultivar 'Topaz', and 'Pinova' in 2004.

The standard deviation of the biological shift factor (σ) is low and almost the same for all 8 series.

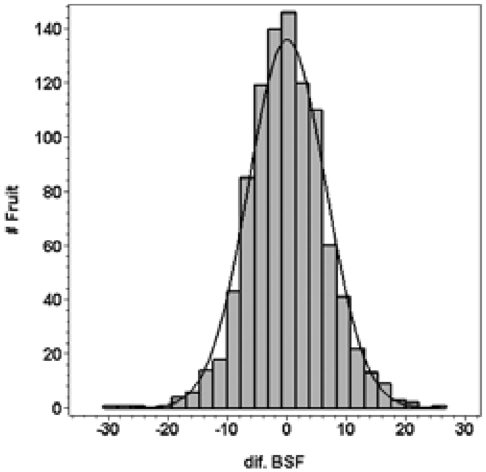

Fig 2. Estimated Biological Shift Factor Δtdif excluding the effects of tree, combined for all seasons and cultivars of the Potsdam experiments.

Moreover when only considering the variation over individual apples, leaving out the variation observed for the trees, the combined biological shift factors were highly normal distributed with a p-value of 0.75. In Fig 2 the distribution is shown.

For the Maribor experiments, there is a clear difference in season: in 2001 the standard deviation of the biological shift factor is of the same magnitude as the experiments in Potsdam. In 2002, the standard deviations are much higher. Clear effects of crop load can not be discerned, while a higher level nitrogen fertilisation seems to induce a lower standard deviation. However, more experiments at various levels and combinations are needed to validate these indications.

Variation in Colour Range

The data on the three cultivars location Potsdam were reanalysed, now applying the biological time (estimated values for the biological shift factor added to the calendar time) as independent variable. The rate constant was estimated in common (fixed effects) while the higher asymptote (Col_{min}) was estimated at random for the three seasons. In Table 4 the results are shown. The explained parts are again very high, but somewhat lower than in the separate analysis. That indicates that the data can indeed be pooled over season, at least for a single location and that the mechanism of colour change is indeed generic. The rate constants are similar but slightly different. The main difference is found in the mean value for col_{min} and especially in the variation over col_{min}. The number of seasons is, however, too low (only 3 seasons) to draw reliable conclusions.

Also in the experiments at the orchards in South Slovenia and Maribor 2001, a variation in the value of col_{min} had to be included to achieve a reliable analysis. The location of the apples at the tree determines the (continuous) effect of light and weather intensity. These differences are reflected in the potential greenness of the apples.

Table 4. Results of the analysis versus biological time for location Potsdam per season.

Cultivar	k_c	col_{min}	σ (col_{min})	R^2_{adj}	N_{obs}
Elstar	0.0019	714.52	2.647	0.956	2133
Pinova	0.0013	717.56	6.333	0.967	2208
Topaz	0.0009	725.74	4.556	0.981	1444

Conclusions

The mechanism of colour change is generic for all three independent experiments. With some exceptions, the rate constant of colour change seem also to be generic in nature, with a very similar value for all cultivars, orchards, and management procedures. Where the exceptions relate to is not known. The data sets are too limited (sic!) to extract that information. The variation in biological shift factor seems to be independent of orchard location and only slightly dependent on orchard management procedures. The main differences observed are in the potential greenness of the apples (col_{min}) that vary considerably between successive seasons and between cultivars.

As the main conclusions, the applied technology provides the necessary tools to analyse the effects of season (weather, temperature, rainfall etc) and orchard management quite well, for all locations in the study. It opens wide alleys to investigate more dedicated the effects of weather, season, management and orchard location in growing apples with a constant quality (colour) over the seasons, locations and management procedures.

Acknowledgements

The financial support of EU COST 924 action by means of Short Term Scientific Missions is gratefully acknowledged.

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