Modelling Biological Variation in the Skin Background Colour of 'Jonagold' Apples during Controlled Atmosphere Storage

S.G. Gwanpua, V. Vicent, M.L.A.T.M. Hertog, B.M. Nicolaï and A.H. Geeraerd
MeBioS (Mechatronica, Biostatistics and Sensors), Department of Biosystems
KU Leuven
W. de Croylaan 42, 3001 Leuven
Belgium

B.E. Verlinden
Flanders Centre of Postharvest Technology
W. de Croylaan 42, 3001 Leuven
Belgium

J. Van Impe
CIT-BioTeC, KU Leuven
W. de Croylaan 46, 3001 Leuven
Belgium

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Abstract

Skin background colour is a very important quality aspect in the grading of 'Jonagold' apples, with consumers usually preferring fruit with a green background colour. However, apple handlers are usually faced with large fruit-to-fruit variability of background colour within a population of fruit. In this study, a stochastic modelling approach is used to describe how the initial fruit-to-fruit variability in the background colour of 'Jonagold' apples present at harvest, propagates throughout the postharvest chain. Two hundred fruit were harvested and stored at 1 or 4°C, under different controlled atmosphere (CA) conditions for six months. The fruit were taken out of storage every two months, and the background colour and the ethylene production of individual fruit were measured. At the end of the six months storage, the fruit were placed in shelf life conditions for 15 days, during which skin background colour and ethylene production were measured every five days. A mathematical model was developed to describe the postharvest loss of the skin greenness of apples during CA storage, by assuming that the loss is principally due to chlorophyll breakdown, the rate of which is dependent on the endogenous ethylene concentration within the fruit. The stochastic model parameters in the model were identified, and by treating these parameters as fruit-specific parameters, the model could describe more than 93% of the data for the individual fruit. By considering these fruit-specific parameters as stochastic parameters, the Monte Carlo method was used to describe the propagation of the fruit-to-fruit variability of the background colour of 'Jonagold' apples within a population. The model developed in this study can be used to predict how the initial fruit-to-fruit variability within a batch of apple will propagate throughout the postharvest chain.

INTRODUCTION

Once harvested, fresh fruits and vegetables undergo continual degradation in quality due to various oxidative breakdown processes occurring within the cells. Postharvest handlers attempt to limit these breakdown reactions by storing fresh fruits and vegetables at low temperature in an atmosphere with reduced O_2 concentration and elevated CO_2 concentrations, hence prolonging their storage life (Kader, 1986). Kinetic modeling offers a tool through which the mechanisms underlying quality breakdown can be better understood, and by so doing, it provides a means for optimizing cold storage. The green background color is a very important quality indicator for grading of 'Jonagold' apples, with apples having a very green background classified under high grade apples with high commercial values. During storage, the green skin background color of 'Jonagold' apples turns yellow as the chlorophyll pigments within the skin are broken down. Ethylene is thought to play an important role in this process (Hershkovitz et

al., 2005). A model was developed by Gwanpua et al. (2012a) to explain the effect of ethylene on the loss in green background color of apples. However, that model did not yet take into account the fruit-to-fruit variability that is associated with any batch of fruit. The objective of the current study is to model the postharvest evolution of the fruit-to-fruit variability of the green background color of 'Jonagold' apples.

MATERIALS AND METHODS

Fruit

'Jonagold' apples (*Malus domestica* Borkh.) were harvested from Van der Velpen Company located at Opvelpsestraat, Bierbeek, Belgium. Apples were picked on 22 September 2012, corresponding to a commercial optimal picking date, and transported immediately to the Flanders Centre of Postharvest Technology (VCBT), Belgium, where the storage experiments were done. The optimal picking date was determined by the VCBT, using a combination of firmness, starch index, soluble solids content, color and respiration rates. 200 fruit were selected and numbered, and a spot with a diameter of about 2 cm was marked on the green region for each fruit.

Storage Experiments

The apples were divided into groups of 20 and each group was stored in one of the 10 storage conditions used in this study. These conditions were 1 and 4°C, with four different CA conditions (in addition to normal air) for each temperature (1 kPa O_2 and 3 kPa CO_2; 1 kPa O_2 and 10 kPa CO_2; 3 kPa O_2 and 3 kPa CO_2; 3 kPa O_2 and 10 kPa CO_2). Storage was performed for six months. The green background color of the apple skin and ethylene emission were measured at about every two months during storage. At the end of the storage, apples were put in shelf conditions (20.8 kPa O_2, 0.03 kPa CO_2 and 18°C) for 15 days, during which the same measurements were done every 5 days.

Measurements

Color was measured as a*-value of the CIE color system using a hand-held spectrophotometer (Model CM-2600D, Minolta, Kontich, Belgium) calibrated to a white tile. Ethylene emission was measured in the respective storage conditions following the protocol described in Bulens et al. (2011). The initial value of the green background color was taken on the marked spot for all 200 fruit and during each sampling time, the color measurements were done on these same spots. Also the ethylene production for all 200 fruit were measured immediately after harvest and during each of the sampling time.

Kinetic Model for Color Loss

Loss in green background color was assumed to be a consequence of the loss in the skin chlorophyll pigments. A simplified reaction to represent this breakdown process is written in Equation 1, in which chlorophyll, Chl, is broken down via the action of some chlorophyll degrading enzymes, E_{Chl}, into colorless non-fluorescent chlorophyll catabolites, $NCCs$. It was also assumed that the chlorophyll degrading enzymes were produced via the regulatory activities of the endogenous ethylene, C_2H_4, and that the enzymes were themselves subjected to normal protein turnover.

$$\begin{aligned} Chl + E_{Chl} &\xrightarrow{k_{Chl}} NCCs + E_{Chl} \\ E_{Chl,pre} + C_2H_4 &\xrightarrow{k_{Echl}} E_{Chl} \\ E_{Chl} &\xrightarrow{k_{Echl,deg}} E_{chl,deg} \end{aligned} \quad (1)$$

From Equation 1, the following sets of ordinary differential equations were written.

$$\frac{\mathrm{d}[\mathrm{Chl}]}{\mathrm{d}t} = -k_{\mathrm{Chl}}[\mathrm{Chl}][\mathrm{E}_{\mathrm{Chl}}],\ \text{with}[\mathrm{Chl}](t=0) = [\mathrm{Chl}]_0$$
$$\frac{\mathrm{d}[\mathrm{E}_{\mathrm{Chl}}]}{\mathrm{d}t} = k_{\mathrm{E_{chl}}}[\mathrm{C_2H_4}] - k_{\mathrm{E_{chl},deg}}[\mathrm{E}_{\mathrm{Chl}}],\ \text{with}[\mathrm{E}_{\mathrm{Chl}}](t=0) = [\mathrm{E}_{\mathrm{Chl}}]_0 \quad (2)$$

Ethylene production was modeled using the Michaelis Menten type equation, similar to the approach used by Gwanpua et al. (2012b), with the addition of a climacteric effect (Eq. 3) similar to the work of Bulens (2012).

$$\frac{d[\mathrm{C_2H_4}]}{dt} = \frac{V_{\mathrm{max,C_2H_4}}\mathrm{p_{O_2}}\dfrac{df_{clim}}{dt}}{K_{\mathrm{m,O_2,C_2H_4}} + \mathrm{p_{O_2}}\left(1 + \dfrac{\mathrm{p_{CO_2}}}{K_{\mathrm{mu,CO_2,C_2H_4}}}\right)} - k_{diff}[\mathrm{C_2H_4}],\ \text{with}[\mathrm{C_2H_4}](t=0) = [\mathrm{C_2H_4}]_0 \quad (3)$$
$$\frac{df_{clim}}{dt} = k_{clim} f_{clim}(1 - f_{clim}),\ \text{with} f_{clim}(t=0) = f_{clim,0}$$

where $V_{\mathrm{max,C_2H_4}}$ (mmol m^{-3} d^{-1}) is the maximum rate of ethylene production; $K_{m,\ \mathrm{O_2},\ \mathrm{C_2H_4}}$ (kPa) and $K_{mu,\ \mathrm{CO_2},\ \mathrm{C_2H_4}}$ (kPa) are the Michaelis-Menten constants for ethylene production and uncompetitive inhibition of ethylene production by carbon dioxide respectively; $\mathrm{p_{O_2}}$ (kPa) and $\mathrm{p_{CO_2}}$ (kPa) are the external partial pressure of oxygen and carbon dioxide respectively; k_{diff} (d^{-1}) is the rate of ethylene diffusing from the apple tissue to its surrounding; f_{clim} is the transition from the preclimacteric stage ($f_{clim}\approx 0$) to the climacteric stage ($f_{clim}\approx 1$).

The model outputs were the measured a^*-value of the CIE color system and the ethylene emission. The a^*-value was assumed to be linearly correlated to the chlorophyll content of the skin (Eq. 4), while ethylene emission was related to the endogenous ethylene concentration by the diffusion term of Equation 3:

$$a^* = a_c^* - [Chl], \qquad a_0^* = a_c^* - [Chl]_0 \quad (4)$$

where a_c^* is a fixed part, related to the background yellow pigments that are present in the skin but are not subjected to postharvest degradation.

All rate constants were assumed to be dependent on temperature according to the Arrhenius equation.

Model Calibration

To estimate the model parameters, the measured data on the green background color and ethylene production were fitted to the models using OptiPa (Hertog et al., 2007), a dedicated optimization tool which was developed for use with Matlab (The Mathworks, Inc., Natick, MA, USA).

RESULTS AND DISCUSSION

During storage and shelf life, 'Jonagold' apples lose their green background skin color. This is a result of the loss in the skin chlorophyll pigments, which make the skin to become yellow as the yellow pigments, original present in the skin, are unmasked (Matile et al., 1996). The rate of loss in the skin greenness was closely correlated with the rate of ethylene production. Fruit that were stored in normal air rapidly became yellow, and this

was related to the very high rates of ethylene production in normal air. Also, for all atmospheric conditions, fruit stored at 4°C showed faster rates in loss of the skin greenness compared to those stored at 1°C. Furthermore, during shelf life exposure, the rate at which the fruit lose their skin greenness was much higher compared to during storage. This was because the rate constants for the various reactions involved in chlorophyll breakdown increased with increasing temperatures.

During initial model calibration, some parameters were kept fixed at some reasonable values, while all other model parameters were treated as generic (Table 1). To identify the stochastic model parameters (i.e., those parameters responsible for the observed fruit-to-fruit variability in the green color), the model was refitted while treating certain parameters as fruit-specific. The root mean sum of square error (RMSE) during each model refitting is plotted in Figure 1. From this figure, it can be observed that the ideal case will be to treat $k_{\mathrm{Chl},ref}$, a_0^*, a_c^*, $V_{\max,C_2H_4}$ and $k_{\mathrm{clim},ref}$ as being stochastic. However, treating all five parameters as stochastic will require more data to obtain their distributions. A compromise solution was therefore adopted, in which we attempt to reduce the number of stochastic parameters, while ensuring the fruit-to-fruit variability is sufficiently explained. Therefore, $k_{\mathrm{Chl},ref}$, a_0^*, and $V_{\max,C_2H_4}$ were selected as the stochastic model parameters. The results of refitting the model to the data, treating these three random model parameters as fruit specific, is shown in Figure 2. More than 93% of the total variation in the measured green background color for all 200 fruit could be explained by the model.

The distribution and the correlation matrix of the random model parameters is shown in Figure 3. This information was used to generate 1000 correlated random model parameters that were used for a Monte Carlo simulation to predict the propagation of the fruit-to-fruit variability of the skin background color, similar to the approach used by Gwanpua et al. (2013). Initially, the distribution of the background color was skewed to the left, since most of the apples had a very green background color, with only few fruit already appearing yellow. During CA storage, the distribution changes slightly, as the fruit turn yellow, with the distribution appearing almost normal at the end of CA storage. During shelf life, the distribution becomes skewed to the right, as most of the fruit turned yellow. Similar observations were made by Hertog et al. (2004) for the hue angle in tomatoes.

Understanding how biological variability propagates throughout storage is very important in managing uniformity in quality. For example, it can enable postharvest handlers to predict the probability of having apples with a certain greenness after storage for some known storage conditions and duration. To illustrate this, based on the current study there was a 64% probability that fruit stored at 1°C under the optimal CA conditions (1% O_2 and 3% CO_2) will have an a^* value of less than -3 for the skin greenness. Such information is very important in grading of 'Jonagold' apples, as fruit is graded based on the greenness of the skin background color.

CONCLUSIONS

Biological variability in quality for postharvest produces can be better managed if postharvest handlers are able to predict how the variability present at harvest propagates throughout the chain. Stochastic modeling offers a unique way for describing, predicting and managing biological variability.

The model developed in this study covers most of the important mechanisms associated with loss in the green background color in apples, and can easily be extended to conditions different, but similar, to those under which the model was developed. It provides a useful tool that can be used in proper management of uniformity in quality within a batch of apples.

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Tables

Table 1. Mean model parameter estimates, together with the standard error.

	Parameter	Estimate (S.D.)[1]	Units
Green background color	a_0^*	-8.4 (2.5)	-
	$k_{\mathrm{Chl},ref}$	0.0041 (0.0020)	$d^{-1}\ m^{-3}\ mmol$
	$k_{\mathrm{Echl},ref}$	0.0000039	$d^{-1}\ m^{3}\ mmol^{-1}$
	$k_{\mathrm{Echl,deg},ref}$	0.000057 (0.017)	d^{-1}
	$E_{a,\mathrm{Chl}}$	70000	J
	$E_{a,\mathrm{E_{chl}}}$	100000	J
	$E_{a,\mathrm{E_{chl,den}}}$	80000	J
	a_c^*	12 (2.2)	-
Ethylene production	$\phi_{\mathrm{C_2H_4},0}$	0.00083 (0.00067)	$nmol\ kg^{-1}\ s^{-1}$
	$V_{\mathrm{max,C_2H_4}}$	92604 (30248)	$mmol\ m^{-3}\ d^{-1}$
	$E_{a,\mathrm{C_2H_4}}$	71403[2]	J
	$f_{clim,0}$	0.01[3]	-
	$k_{\mathrm{clim},ref}$	0.073 (0.001)	-
	$E_{a,\mathrm{clim}}$	100000[3]	J
	$K_{\mathrm{m,O_2,C_2H_4}}$	4.44[2]	kPa
	$K_{\mathrm{mu,CO_2,C_2H_4}}$	0.76[2]	kPa
	k_{diff}	0.0014299[2]	d^{-1}

[1] Parameters with no standard deviations were not estimated from the data.
[2] Parameter values taken from Gwanpua et al. (2013).
[3] Parameter values taken from Bulens (2012).

Figures

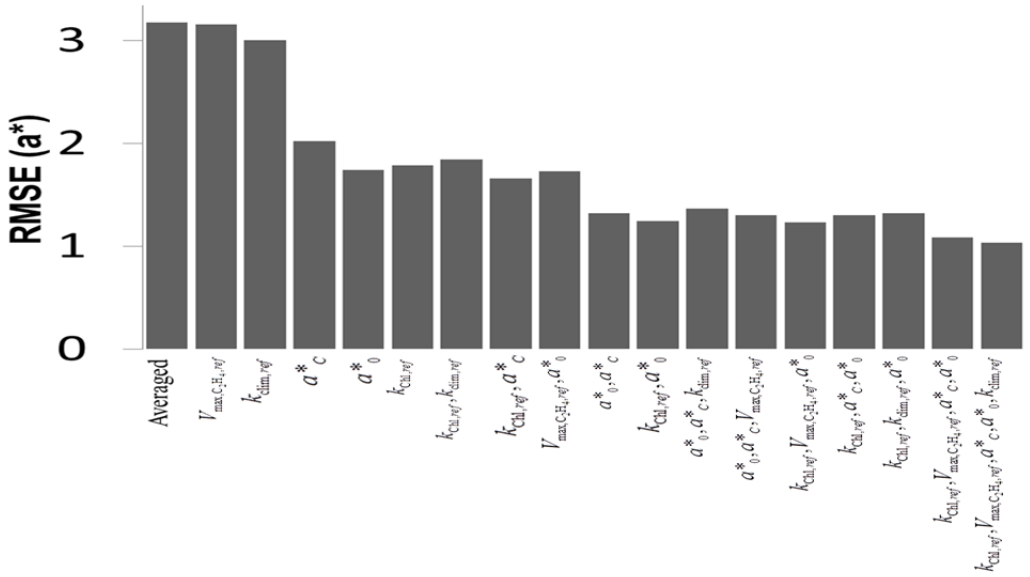

Fig. 1. The root mean square error (RMSE (a^*)) of different model fits as compared to the model describing the averaged batch behavior. The labels on the horizontal axes indicate which model parameters were estimated for each of the individual fruit.

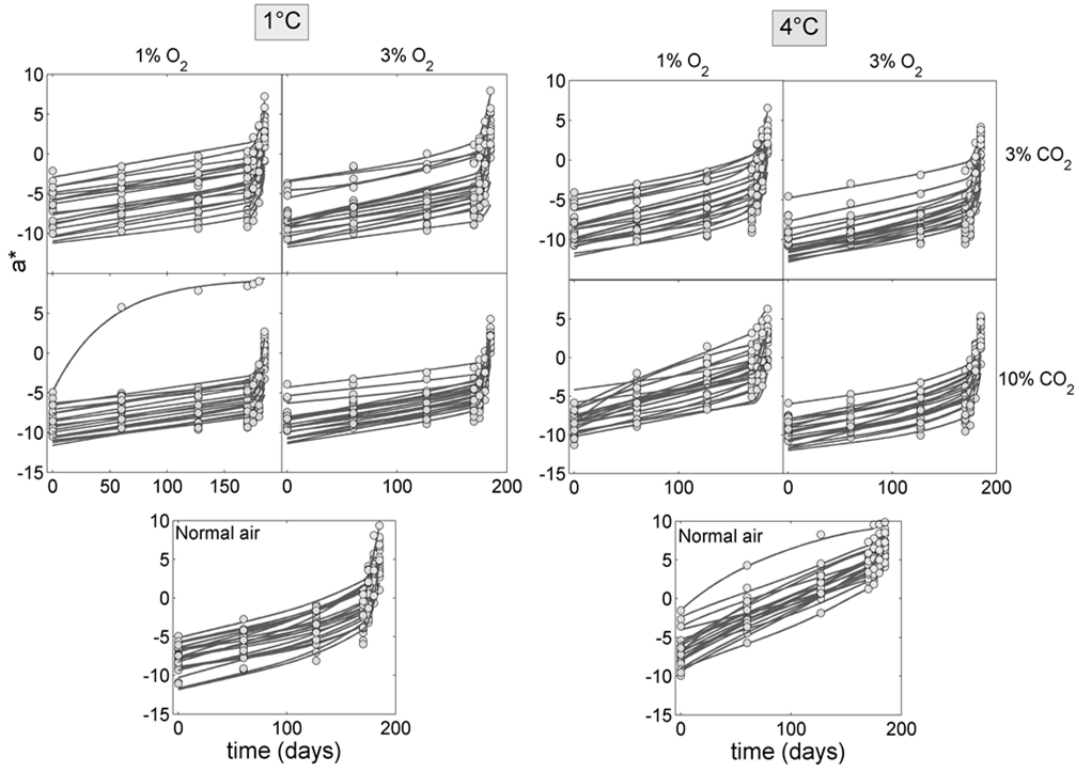

Fig. 2. Model fit treating $k_{\mathrm{Chl},ref}$, a_0^*, and V_{max,C_2H_4} as fruit-specific model parameters. The lines are the modeled values, while the points are measured values.

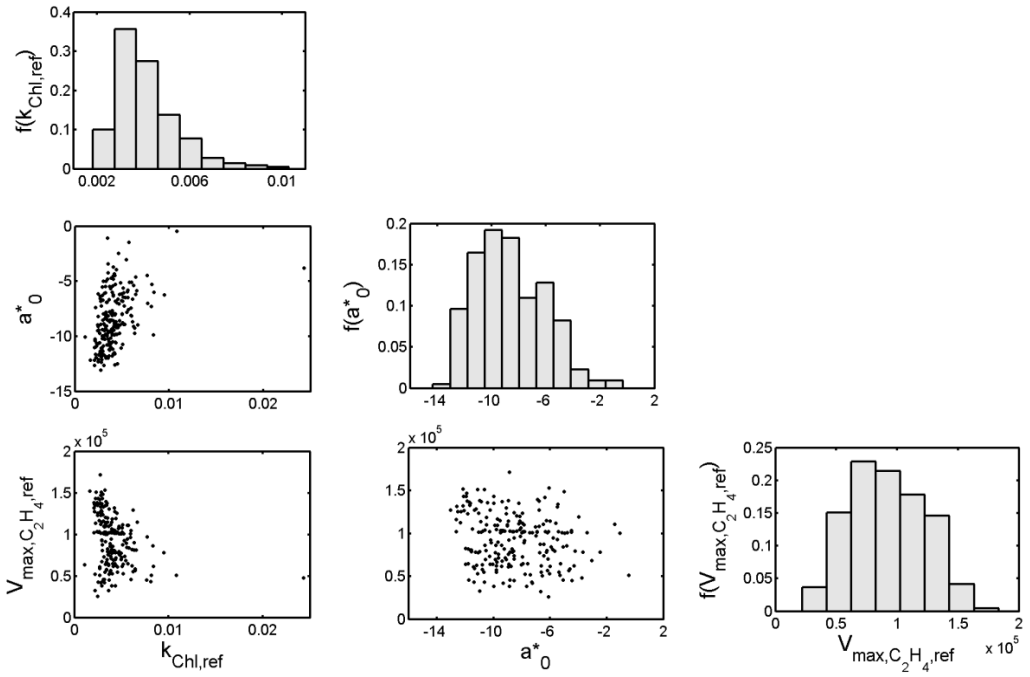

Fig. 3. Frequency distribution and the correlation matrix of the random model parameters for all 200 fruit.

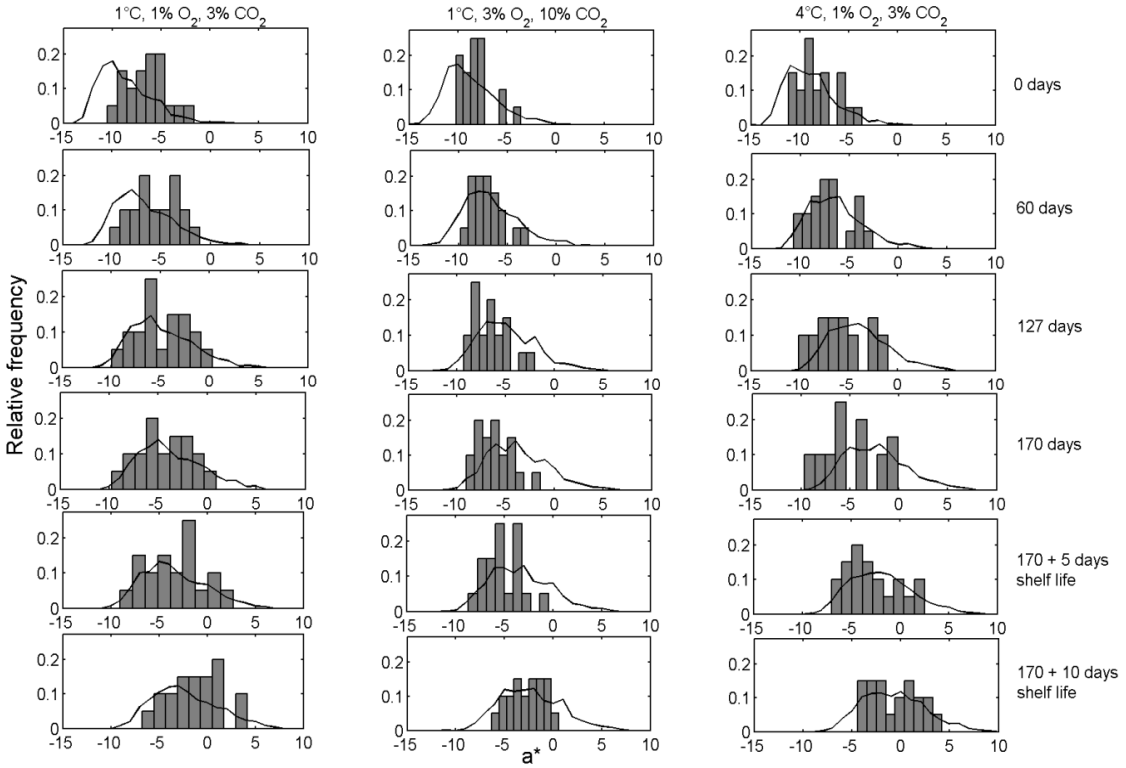

Fig. 4. Distribution of *a**-value of background color for apples stored under different conditions. The lines are based on the Monte Carlo simulations, while the bars are the relative frequency of the measured values.