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Applying prior knowledge to model batch keeping-quality of cucumber batches

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Abstract

Keeping-quality of individual cucumbers is limited by the green colour; the keeping-quality of batches of cucumbers is limited by the time it takes before 5% of the cucumbers in the batch reach a predefined colour limit. From literature concerning the synthesis and degradation of chlorophyll and a published colour model (Schouten, Tijskens and Van Kooten 2002) it is known that colour behaviour of individual cucumbers depends on the concentration of protochlorophyllide (Pchl), at harvest. Here a model for the synthesis and degradation of Pchl is proposed for individual cucumbers, assuming that light conditions during growth are a major factor for Pchl synthesis. Subsequently, this individual Pchl model is expanded to a batch model that describes the variation in the Pchl concentration by assuming that differences between cucumbers of the same batch are primarily caused by differences in light conditions during growth.

Pchl data were obtained from colour data from six batches from three cultivars ('Volcan', 'Beluga' and 'Borja') over two growing seasons. Pchl data were gathered per batch and compared with the proposed batch model. The variation in the Pchl concentration per batch could be described satisfactorily in terms of the parameters of the proposed Pchl batch model: batch maturity, batch variation (due to differences in light conditions during growth on a batch level) and a cultivar-dependent factor. Estimating this cultivar-dependent factor for new cultivars could be a tool for cucumber-breeding companies to obtain cultivars with an increased keeping-quality.
Keywords: Biological variation; postharvest; preharvest; precursor

Introduction

The limiting quality attribute for cucumbers is colour (Schouten et al. 1997). The keeping-quality can be defined as the time it takes for an individual cucumber to reach a predefined colour limit. Long shelf life of cucumbers has been associated with high chlorophyll content in the peel (Lin and Ehret 1991). However, it is known that cucumbers of the same colour can exhibit large differences in colour upon reaching the customer. So, for a high keeping-quality not only the initial colour is of interest, but also specifically the ability to stay green. Predicting this ability for individual cucumbers was not possible, because of the unknown stage of maturity (Schouten et al. 1997). However, information on the stage of maturity might be revealed when

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batches (one grower, one harvest and of the same cultivar) of cucumbers are considered. On a batch level extra information is available due to the shared harvest date, cultivar and grower. Batch keeping-quality can be defined as the time it takes before 5% of the cucumbers in a batch have reached the predefined colour limit (Schouten and Van Kooten 1998).

This paper focuses on the characterization of cultivar effects with respect to batch keeping-quality from a modelling point of view. Models will be presented on two aggregation levels. The first level is the level of the individual cucumber, the second is the batch level which describes the batch itself, but also a cultivar-dependent parameter. All models are based on non-destructive, repeated measurements of colour. The models will be applied to six batches of cucumbers from three cultivars over two growing seasons.

Material and methods

Cucumbers

Cucumbers consisted of six batches of 80-100 cucumbers each, belonging to either cultivar 'Volcan', 'Borja' or 'Beluga', obtained from the Almeria region in Spain. Three batches were harvested in September 1998 (autumn season) and three batches in June 1999 (spring season). All batches were grown under equal, commercial growing conditions and were of marketable size and colour. After harvest, batches were placed in boxes with air-filled polystyrene and transported to the measuring facility in the Netherlands within 24 hours. Upon arrival cucumbers were individually tagged on the lightest side and stored in the dark at 20°C and 100% relative humidity.

Colour measurements

Image analysis was used for colour measurements using a JVC KY-F30 3CCD colour video camera, with the same set-up as described in Schouten et al. (1997). Colour measurements per cucumber took place twice a week and were expressed as the ratios of the blue/red (B/R) values from the separate intensities of the blue and red values of the RGB colour scale. After a measurement the light intensities for the red and blue colours were separately averaged over all pixels that belonged to the cucumber image. Colour measurements began one day after arrival at the measuring facility and ended when yellowing was complete or decay of the cucumber was imminent.

Colour behaviour of individual cucumbers

The colour model is based on knowledge obtained from literature regarding the processes of synthesis and degradation of chlorophyll in terms of colour compounds. POR (protochlorophyllide oxidoreductase) is a photo-enzyme which catalyses the reduction from protochlorophyllide (Pchl) to chlorophyllide (chl), the direct precursor of chlorophyll (CHL) (Lebedev and Timko 1998). A ternary complex of POR:NADPH:Pchl has been observed, which may be assumed to be a safe form of Pchl storage as to prevent phototoxic events when illuminated during the initial greening of young tissues (Porra 1997). Here the assumption is made that the concentration of the complex POR:NADPH:Pchl formed during the preharvest stage is restrictive for the concentration of chl and CHL formed during the postharvest period. During senescence in fruits and vegetables chlorophyll can be cleaved by chlorophyllase resulting in the formation of chl, which will be turned into colourless compounds (Heaton and Marangoni 1996).

From a modelling point of view, only compounds with colour and their precursors are of interest. Next to CHL itself (blue-green), these are chl (blue-green) and the colourless precursor Pchl. Colour is defined as the sum of CHL and chl. Figure 1 shows the proposed mechanism for these compounds during synthesis and breakdown of CHL. A special position is held by chl as it is an intermediate in both synthesis and breakdown. The initial concentration of Pchl, as part of the ternary complex POR:NADPH:PChl, is depicted as crucial and governing the colour behaviour. The mechanism shown in Figure 1 can be expressed in mathematical form by coupled differential equations, one for each process (Schouten, Tijskens and Van Kooten 2002). These equations were solved analytically, which resulted in a model formulation describing colour behaviour from harvest time till complete yellowing of an individual cucumber in terms of $Pchl_0$ and CHL_0, with $Pchl_0$ and CHL_0 being the concentration of Pchl and CHL present at harvest time (Schouten, Tijskens and Van Kooten 2002). An indication of the colour behaviour in time decay for three hypothetical cucumbers, differing only in $Pchl_0$, is depicted in Figure 2. The colour model does not contain or need cultivar factors.

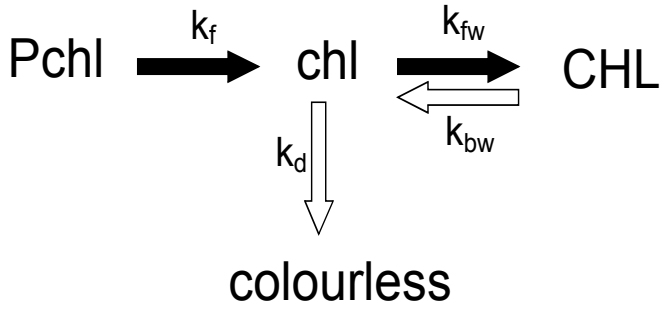


Figure 1. Kinetic model representation of the last part of the chlorophyll pathway for cucumbers stored in the dark. Closed arrows indicate chlorophyll synthesis and open arrows catabolism. Indicated are the reaction rate constants (k_f, k_{fw} and k_d). Protochlorophyllide, chlorophyllide and chlorophyll are represented by Pchl, chl and CHL, respectively

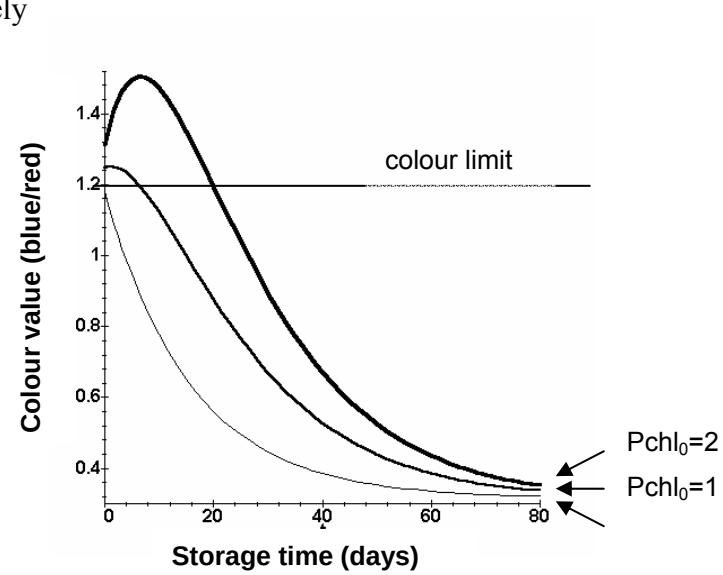


Figure 2. Colour behaviour of three cucumbers differing in $Pchl_0$. Indicated is the colour limit. The cucumber with the lowest concentration of $Pchl_0$ has an intercept with the colour limit at 0, the medium concentration at 8 and the highest concentration at 20 days. This intercept is defined as the keeping-quality for individual cucumbers

Keeping-quality of batches of cucumbers

Batch keeping-quality was determined for the six batches of cucumbers. First the colour data per cucumber, obtained by following the colour development in time by repeatedly measuring the same cucumbers with image analysis, were analysed with the colour model to estimate the values of $Pchl_0$ and CHL_0 per cucumber. Then, the time it took for each cucumber to reach the colour limit was determined using the estimates of $Pchl_0$ and CHL_0. To obtain the batch keeping-quality the keeping-qualities of all cucumbers in a batch were sorted on time. When necessary, a simple linear-interpolation procedure was used to estimate the time at which 5% of individuals in that batch crossed the colour limit (Schouten, Tijskens and Van Kooten 2002). Batch keeping-quality depended on season and cultivar, 'Borja' having the highest and 'Volcan' having the lowest keeping-quality (third column of Table 1). As expected, the *average* $Pchl_0$ per batch was closely related to the batch keeping-quality (compare the third and the eighth column of Table 1).

Table 1. Overview of parameters belonging to the six batches. $Pchl_{minvar}$ is estimated in common for batches of the same cultivar. t^b_m (batch maturity) and σ (batch variation) are estimated per batch

cultivar	season	batch keeping quality (days)	t^b_m		σ		$Pchl_{minvar}$	
			estimate	s.e.	estimate	s.e.	estimate	s.e.
'Volcan'	autumn	3.8	0.666	0.025	0.519	0.059	1.906	0.137
'Volcan'	spring	6.8	-0.131	0.105	0.336	0.061		
'Borja'	autumn	8.4	0.175	0.034	0.226	0.032	2.946	0.242
'Borja'	spring	13.1	-0.170	0.092	0.157	0.036		
'Beluga'	autumn	4.3	0.638	0.015	0.364	0.015	2.025	0.106
'Beluga'	spring	7.2	-0.022	0.067	0.267	0.011		
R^2_{adj} (%)	92.3							
N	282							

Behaviour of Pchl during pre- and postharvest stages for individual cucumbers

During the postharvest period Pchl is broken down into chl according to the mechanism shown in Figure 1. During the preharvest stage Pchl is thought to be stored in the ternary complex POR:NADPH:Pchl and subsequently released when exposed to light (Porra 1997). The behaviour of Pchl may be described as a consecutive reaction where Pchl as part of the ternary complex (TPchl) is transformed into Pchl, and the subsequent decay of Pchl. The proposed mechanism is shown in Eq. 1-2:

$$\text{TPchl} \xrightarrow{k_f \cdot \text{light}} \text{Pchl} \qquad (1)$$

$$\text{Pchl} \xrightarrow{k_d \cdot \text{light}} \text{colour components} \qquad (2)$$

where k_f is the reaction-rate constant for the formation of Pchl from TPchl and k_d the reaction-rate constant for the decay of Pchl. Light conditions affect the transformation from TPchl to Pchl and from Pchl to colour components directly by increasing the apparent reaction rate. Based on equations 1 and 2, differential equations can be set up, and these can be solved analytically for constant external conditions using the

fundamental rules of chemical kinetics. Given that no Pchl was present at the beginning of the cucumber growth, the Pchl concentration at time t after the start of cucumber growth can be expressed as follows (Eq. 3):
where $TPchl_s$ is the initial concentration of TPchl present at the beginning of

$$Pchl(t) = \frac{k_f \cdot TPchl_s (e^{-k_d \cdot light \cdot t} - e^{-k_f \cdot light \cdot t})}{k_f - k_d} \quad (3)$$

cucumber growth.

The batch-model formulation

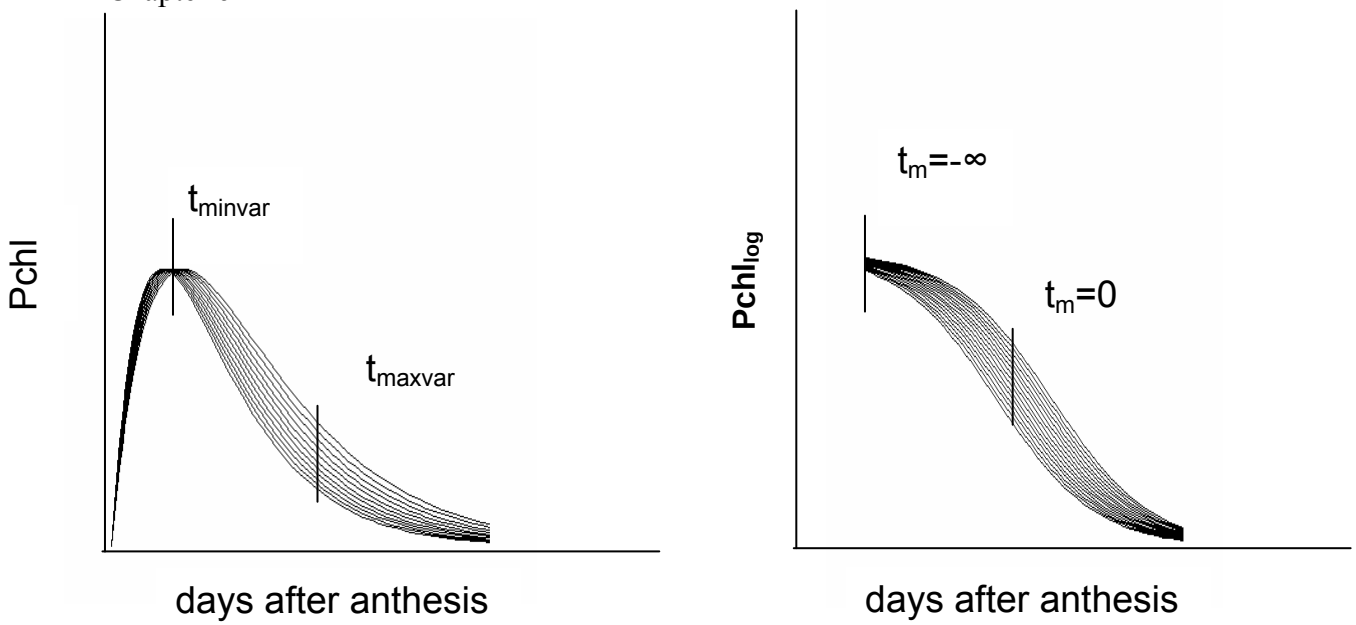
Assuming that differences between individual cucumbers of the same batch are primarily caused by differences in light conditions and not by differences in $TPchl_s$, the Pchl concentration for cucumbers belonging to the same batch can be simulated at constant external conditions. The upper left-hand-side plot of Figure 3 shows the simulated Pchl concentration after the beginning of cucumber growth assuming that light conditions vary maximally by a factor two between the lowest and highest amounts of light. Regardless of $TPchl_s$ there will be a point in time with minimal Pchl variation, which is defined as t_{minvar} at the following Pchl concentration: $Pchl_{minvar}$.

Very young, small cucumbers already show considerable amounts of greening. It may therefore be assumed that TPchl can be quickly transformed into Pchl. Therefore, when full-grown cucumbers are harvested it is likely that the concentration of Pchl is already decreasing. From here we will assume that harvest takes place at or after t_{minvar} (upper left-hand-side plot of Figure 3). Harvest at t_{minvar} is preferable because cucumbers belonging to that batch will exhibit minimal variability after harvest. Also, harvest at t_{minvar} will result in cucumbers having maximal $Pchl_0$ values, resulting in maximal keeping-quality during storage. t_{minvar} is independent of $TPchl_s$ but will depend on k_f and k_d. Analysing cucumber colour data, the numerical values of all reaction rate constants, including k_d, were very similar for a number of cultivars (Schouten, Tijskens and Van Kooten 2002). It is therefore logical to assume that, next to k_d, k_f does not show much difference between cultivars. In that case the time after anthesis with minimal variation in Pchl concentration will be t_{minvar}, regardless of cultivar when grown at the same temperature.

The variation in $Pchl_0$ values, caused by variation in light conditions during the preharvest period, is minimal at t_{minvar}. After t_{minvar} the variation increases and reaches maximal variation in Pchl at t_{maxvar}, and then decreases again. When the variation in $Pchl_0$ is considered starting from t_{minvar}, then the variation in the Pchl concentration appears to be symmetrical around t_{maxvar} regardless of $TPchl_s$ (left-hand-side plot of Figure 3). This variation pattern may be approximated by describing the Pchl equation (Eq. 3) by a decreasing logistic function between $Pchl_{minvar}$ and 0, expressed in t_m, the maturity for an individual cucumber (Eq. 4):

$$Pchl_{log}(t_m) = \frac{Pchl_{minvar}}{1 + e^{(Pchl_{minvar} \cdot t_m)}} \qquad \text{with } t_{minvar} \le t_m \quad (4)$$

When variation is assumed on t_m, minimal variation is reached at $t_m = -\infty$. After $t_m = -\infty$ the variation increases, reaching the maximal variation at $t_m = 0$, and then decreases



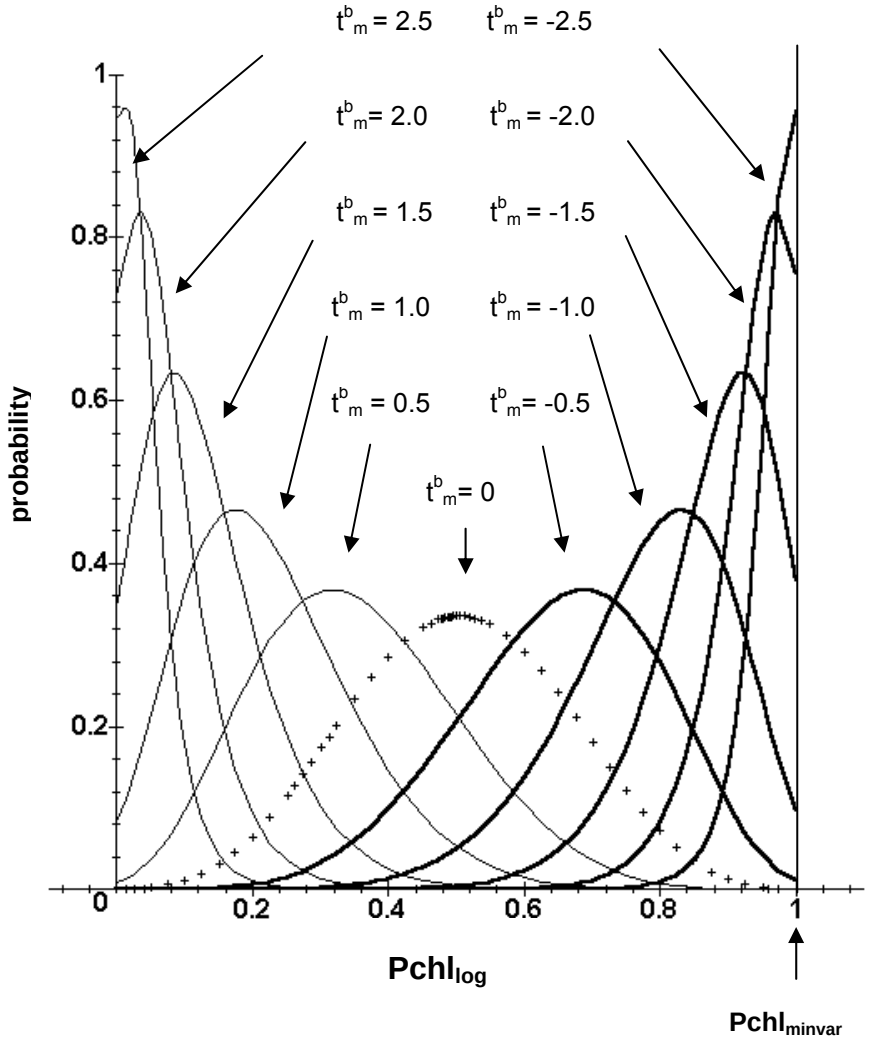


Figure 3. Upper left-hand-side plot: Simulated precursor behaviour (Pchl) assuming that each cucumber is grown at slightly different light conditions, applying Eq. 3. The upper right-hand-side plot shows the behaviour of the logistic function ($Pchl_{log}$) assuming each cucumber has a variation in t_m, applying Eq. 4. Lower plot: Simulation of Pchl distributions for one batch harvested at different maturity stages, indicated by t^b_m. Simulation was carried out using $Pchl_{minvar}$ =1, and σ= 0.5, applying Eq. 7

again (upper right-hand-side plot of Figure 3). The variation pattern is symmetrical around t_m=0. This variation pattern, using the logistic function, is similar to the variation pattern observed for the Pchl equation when t_{minvar} is substituted with t_m=-∞ and t_{maxvar} is substituted with t_m=0. So, although the actual behaviour of the Pchl equation (Eq. 3) and the logistic function (Eq. 4) over time differ considerably, the variation patterns seem to be very similar.

As Pchl also plays a decisive role in the batch keeping-quality, it is of interest to know how the behaviour of batches in time is with respect to the Pchl concentration. To do that the concept of biological variation has to be used. This might be defined as the composite of (biologically based) properties that differentiate individual units of a product (Tijskens and Konapacki 2003). Here it is proposed that biological variation can be applied on the level of t_m. This means that a batch can be characterized by variation in t_m, indicated by t^b_m (batch maturity) with standard deviation σ (batch variation). The connected open symbols of Figure 4 show the distributions of all individual values of $Pchl_0$ gathered per batch. Those distributions are shown as function of a class of $Pchl_0$ and expressed as frequency, the fraction of cucumbers in that class. To describe this mathematically, a description has to be given of the *probability* that a fraction of the batch is within a specific class (Eq. 5):

$$\Pr(\text{Pchl}(t) \in (q_a, q_b]) = \Pr\left(\text{Pchl}(t^{b}_{m}) \le q_b\right) - \Pr\left(\text{Pchl}(t^{b}_{m}) \le q_a\right) \qquad (5)$$

where q_a and q_b are used to describe the class borders, expressed in Pchl values. This probability function may be described in terms of a distribution function ψ which describes the variation in t_m. This involves a number of mathematical steps which are omitted here for clarity (Eq. 6):

$$\Pr(\text{Pchl}(t) \in (q_a, q_b]) = \psi\left(\text{Pchl}^{-1}(q_a) - t^{b}_{m}\right) - \psi\left(\text{Pchl}^{-1}(q_b) - t^{b}_{m}\right) \qquad (6)$$

Out of convenience it was assumed that t_m was normally distributed with average t^b_m and standard variation σ. The probability that a fraction of the batch is within a specific class can now be expressed in terms of Pchl, t^b_m, σ and $Pchl_{minvar}$, when the (inverse) version of $Pchl_{log}$ (Eq. 4) is substituted (Eq. 7):

$$\Pr(\text{Pchl}(t) \in (q_a, q_b]) \approx \Phi\left(\frac{\ln\left(-\frac{q_a - \text{Pchl}_{minvar}}{q_a}\right) - t^{b}_{m} \cdot \text{Pchl}_{minvar}}{\text{Pchl}_{minvar} \cdot \sigma}\right) - \Phi\left(\frac{\ln\left(-\frac{q_b - \text{Pchl}_{minvar}}{q_b}\right) - t^{b}_{m} \cdot \text{Pchl}_{minvar}}{\text{Pchl}_{minvar} \cdot \sigma}\right) \qquad (7)$$

with Φ the normalized cumulative normal distribution function. An indication of the batch behaviour for one batch varying in maturity is shown in the lower plot of Figure 3.

Results and discussion

Shape of distributions

The autumn batches of cultivars ‘Beluga’ and ‘Volcan’ show the skewed $Pchl_0$ distributions for batches with generally a low Pchl concentration. (Figure 4). On the other hand the $Pchl_0$ distribution of the spring batch of cultivar ‘Volcan’ also shows a

skewed distribution (but mirrored) typically for a batch with a general high Pchl concentration. The shape of the distributions varies between two limits in whose vicinity they are skewed. Between those limits the distribution adopts almost the shape of the normal distribution, for instance for the autumn batch of cultivar 'Borja'. The first limit is the one at a value of 0 for $Pchl_0$ and the second limit at a maximum value of $Pchl_0$, $Pchl_{minvar}$.

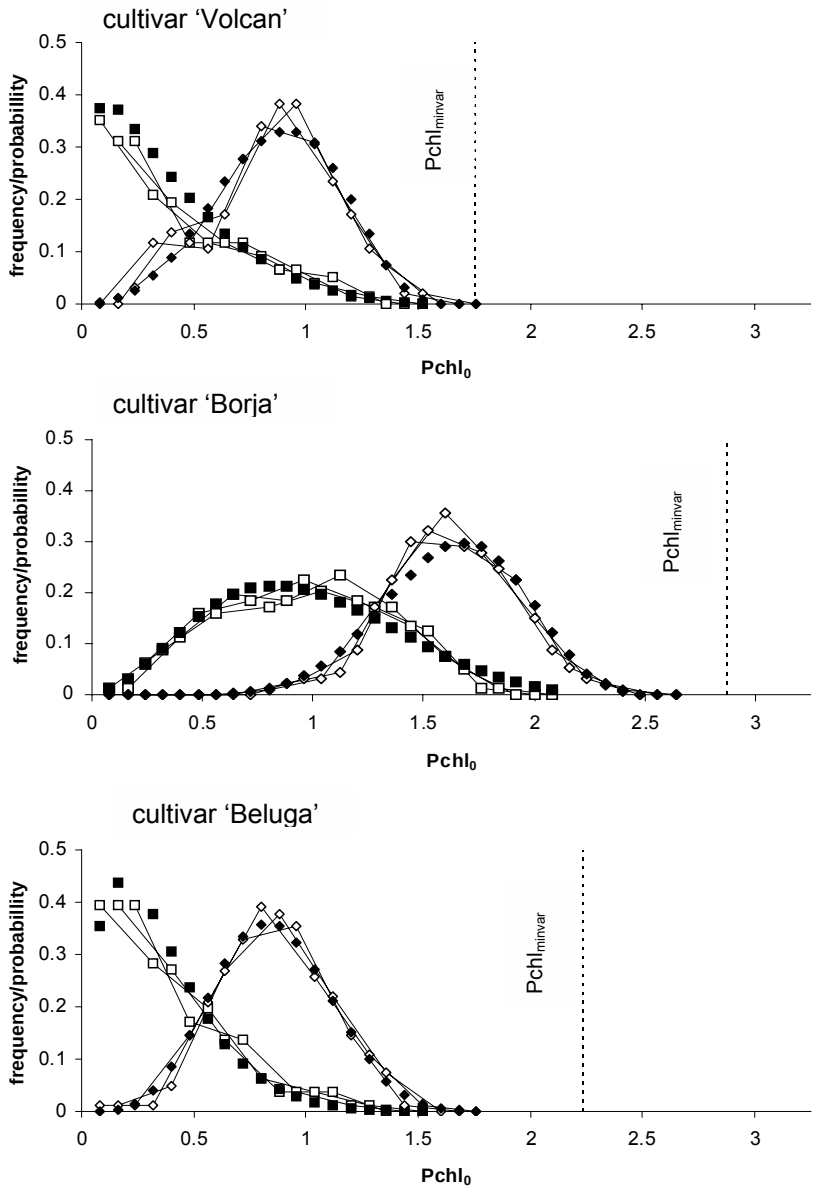


Figure 4. Pchl distributions at harvest ($Pchl_0$) for two batches per cultivar. Per cultivar one batch was harvested in the autumn season (□,■) or the spring season (◊,♦). Open symbols indicate the Pchl distribution obtained from colour data, closed symbols the distribution from batch-model estimations

Estimation of $Pchl_{minvar}$

Figure 4 shows the $Pchl_0$ distribution per batch estimated on colour data (connected open symbols) and analysed using the logistic batch-model formulation of Eq. 7 (closed symbols). Distributions of all batches were analysed in one optimization to obtain $Pchl_{minvar}$, t^b_m and σ per batch. $Pchl_{minvar}$ estimations were similar per cultivar,

so that for the second analysis t^b_m and σ were estimated per batch and $Pchl_{minvar}$ per cultivar (Table 1). For cultivar 'Volcan' σ varied substantially over the growing seasons, whereas this was not the case for the two other cultivars (Table 1). Only during the spring season for cultivars 'Volcan' and 'Borja' negative values for t^b_m were encountered. This means that, compared to the other batches, these batches were of much better maturity. The influence of $Pchl_{minvar}$, which is the largest for cultivar 'Borja' and the smallest for cultivar 'Volcan' (Table 1, Figure 3), is substantial with regard to batch keeping-quality. When a correction for maturity and σ per batch is carried out, the best cultivar with respect to batch keeping-quality is 'Borja', followed by 'Beluga' and finally 'Volcan'. Estimating $Pchl_{minvar}$ for new cultivars could be a tool for cucumber-breeding companies to obtain cultivars with an increased keeping-quality.

Biological variation

Normally, biological variation is treated like an ever-present nuisance that should be minimized as much as possible. The most commonly used technique to deal with biological variation is sorting and grading on external quality attributes (Tijskens and Konapacki 2003). Simple colour measurements on cucumber colour are, however, not enough for keeping-quality predictions, as the ability to stay green cannot be assessed. For cucumbers it turned out that the precursor of colour components was determining batch keeping-quality. Pchl distributions were obtained by collecting the Pchl concentration of all individual cucumbers of all batches. Existing literature on chlorophyll decay and synthesis pointed to the importance of light conditions during growth as source of biological variation. By building in this source of biological variation and testing it with the experimental Pchl distributions, prior knowledge was incorporated into the probablity function (Eq. 7).

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