Relation between Cultivar and Keeping Quality for Batches of Cucumbers

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Abstract

This paper focuses on the characterisation of the cultivar effect in batches with respect to batch keeping quality based on colour measurements only. To do so, a correction for the maturity per batch had to be made for two batches per cultivar. This left the cultivar information with respect to the batch keeping quality. This method to extract cultivar information out of cucumber batches is shown for six batches from three cultivars ('Volcan', 'Beluga' and 'Borja') over two growing seasons. The method consist of describing the batch keeping quality determining property on an individual level and then translating it to a batch level, thereby introducing and describing biological variation. It turned out that the best cultivar, with respect to batch keeping quality, was 'Borja', followed by 'Beluga' and finally 'Volcan'. This paper demonstrates how to use the biological variance present in all biological batches; to take *advantage* of the biological variation instead of seeing and dealing with it as if it were a nuisance.

INTRODUCTION

On first sight, it seems impossible to recognise whether fresh cucumbers are from the same cultivar. Still, cucumber-breeding companies have sometimes large programs to develop new populations with genetically distinguishable properties. Those properties may well be keeping quality or resistance against pests. This paper focuses on the characterisation of the cultivar effect with respect to keeping quality.

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 Jolliffe, 1995). 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. For individual cucumbers this is not possible, because of the unknown stage of maturity (Schouten et al., 1997). However, this is different when batches (one grower, one harvest and of the same cultivar) of cucumbers are considered. On a batch level, next to information on all individuals belonging to that batch, 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 reaches the predefined colour limit (Schouten and Van Kooten, 1998).

This paper focuses on the characterisation 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 on the level of the individual cucumber, the second is the batch level which describes the batch itself, but also a cultivar dependant 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 were harvested 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% RH.

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 per 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 colour were separately averaged over all pixels that belong to the cucumber image. Colour measurements started 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 photo-toxic 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 pre-harvest is restrictive for the concentration of chl and CHL formed during postharvest.

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), this is chl (blue green), and the colourless precursor Pchl. Colour is defined as the sum of CHL and chl. Fig. 1 shows the proposed mechanism for these compounds during synthesis and breakdown of CHL. chl holds a special position 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 Fig. 1 can be expressed in mathematical form by coupled differential equations, one for each process (Schouten et al., 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 Pch and CHL present at harvest time (Schouten et al., 2002).An indication of the colour behaviour in time decay for three hypothetical cucumbers, differing only in $Pchl_0$, is depicted in Fig 2. The colour model does not contain or need cultivar factors.

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 et al., 2002). Batch keeping quality depended on season and cultivar, 'Borja' having the highest and 'Volcan' having the lowest keeping quality (Table 1). As expected, the *average* concentration of $Pchl_0$ per batch was closely related to the batch keeping quality (Table 1).

Behaviour of Pchl during pre- and postharvest for Individual Cucumbers

During postharvest Pchl is broken down into chl according to the mechanism shown in Fig. 1. During preharvest 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 (PT) is transformed into Pchl, and the subsequent decay of Pchl. From this proposed mechanism, the behaviour of Pchl over time can be extracted using the fundamental rules of chemical kinetics. The set of differential equations is given in Eq. 1-2.

$$\frac{\partial PT}{\partial t} = -k_T \cdot PT \tag{1}$$

$$\frac{\partial Pchl}{\partial t} = k_T \cdot PT - k_f \cdot Pchl \tag{2}$$

where k_T is the reaction rate for the formation of Pchl from PT. This set of differential equations can be solved analytically for constant external conditions. Given that no Pchl was present at the start of the cucumber growth, Pchl can be expressed as follows (Eq. 3).
where PT_0 is the initial concentration of PT present at the start of cucumber growth. An

$$Pchl(t) = \frac{k_T \cdot PT_0 (-e^{-k_T \cdot t} + e^{-k_f \cdot t})}{k_T - k_f} \tag{3}$$

indication of the behaviour of Pchl in time may now be simulated for different cucumbers, each differing in PT_0 and assuming a constant temperature during pre- and

$$t_{max} = \frac{\ln\left(\frac{k_T}{k_f}\right)}{k_T - k_f} \tag{4}$$

postharvest. Surprisingly, the maximum in Pchl occurs at t_{max} independently of PT_0 (Eq. 4).
t_{max} depends only on the reaction rate constants k_f and k_T. During the analysis of the colour data of cucumbers calibrating the postharvest colour model it was observed that all reaction rate constants were identical for a number of cultivars (Schouten et al., 2002). It may be assumed that, next to k_f, k_T does not show much difference between cultivars. In that case PT_0 is determinant for the behaviour of Pchl in time and t_{max} will be constant for all cucumbers, irrespective of cultivar, when cucumbers are grown at the same temperature.

As very young cucumbers already show considerable amounts of greening it may be assumed that the ternary complex POR:NADPH:Pchl can be quickly transformed into

Pchl. Therefore, when full-grown cucumbers are harvested it is likely that the concentration of Pchl is already decreasing. Harvest probably takes place somewhere in the range between t_{max} and $t=+\infty$. When the behaviour of Pchl is observed for $t \geq t_{max}$ then the behaviour shows similarities with a decreasing logistic function varying between 0 and P_{max}. This logistic function can be expressed as follows (Eq. 5):

$$Pchl(t) \approx \frac{P_{max}}{1 + e^{P_{max} k \cdot t_{mat}}} \qquad (5)$$

$$\text{with} \qquad t_{max} \leq t_{mat} \leq \infty$$

where P_{max} is defined as the concentration of Pchl at $t= t_{max.}$ P_{max} is assumed to be cultivar specific. By using the logistic function for Pchl(t) instead of Eq. 3 a time transformation is introduced. In Eq. 3, t stands for the time from the start of cucumber growth, but in Eq. 5 it had been replaced by t_{mat}, the maturity of a cucumber. t_{mat} varies between $-\infty$ and ∞. At $t_{mat}= -\infty$ the optimal maturity is reached as it is equivalent to the concentration of Pchl at t_{max} (P_{max}) and at $t_{mat}= \infty$ the minimal maturity is reached as at that point no Pchl is left. When no Pchl is left the cucumber will start to lose colour quickly (Fig. 2).

Behaviour of Logistic Batches in Time

As Pchl plays also 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 Konopacki, 2001). Here, it is proposed that biological variation can be applied on the level of t_{mat}. This means that a batch can be characterised by variation in t_{mat}, indicated by t_{mat}^b with standard deviation σ.

The connected open symbols of Fig. 3 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 faction of the batch, represented by $Pchl^b$, is within a specific class (Eq. 6).

$$\Pr(Pchl^b(t_{mat}) \in (q_0, q_1]) = \Pr\left(Pchl^b(t_{mat}) \leq q_1\right) - \Pr\left(Pchl^b(t_{mat}) \leq q_0\right) \qquad (6)$$

where q_0 and q_1 are used to describe the class borders, expressed in Pchl values. The aim is to translate batch function $Pchl^b$ in terms such as t_{mat}^b, σ and P_{max}. This translation involves a small number of mathematical steps which are omitted here for clarity (Eq. 7).

$$\Pr(Pchl^b(t_{mat}) \in (q_0, q_1] = \psi\left(Pchl^{-1}(q_0) - t_{mat}^b\right) - \psi\left(Pchl^{-1}(q_1) - t_{mat}^b\right) \qquad (7)$$

Ψ stands for the cumulative distribution function which describes the variation in t_{mat}. The next step is to choose which mathematical description should be used for Ψ. In Fig. 3 the $Pchl_0$ values are expressed as faction of all cucumbers that belong to a batch, i.e. values between 0 and 1, so for Ψ a normalised function should be chosen. Out of convenience it was assumed that Ψ was normally distributed. The probability that a faction of the batch, represented by $Pchl^b$, is within a specific class can now be expressed in terms of Pchl, t_{mat}^b, σ and P_{max}, the cultivar specific parameter, when the approximated (inverse) version of Pchl (Eq. 5) is substituted (Eq. 8).

$$\Pr(\mathrm{Pchl}^{b}(t_{mat}) \in (q_0, q_1]) = \Phi\left(\frac{\ln\left(-\frac{q_0 - P_{max}}{q_0}\right) - t_{mat}^{b}}{k \cdot P_{max} \cdot \sigma}\right) - \Phi\left(\frac{\ln\left(-\frac{q_1 - P_{max}}{q_1}\right) - t_{mat}^{b}}{k \cdot P_{max} \cdot \sigma}\right) \quad (8)$$

with Φ the normalised cumulative normal distribution function. An indication of the batch behaviour for one batch varying in maturity is shown in Fig. 4.

RESULTS AND DISCUSION

Shape of Distributions

The autumn batches of cultivars 'Beluga' and 'Volcan' show the skewed distributions for batches which have almost the minimum $Pchl_0$ concentration. On the other hand the distribution of the spring batch of cultivar 'Volcan' shows also a skewed distribution (but mirrored) typically for a batch which has almost the maximum concentration of $Pchl_0$. The shape of the distributions varies between two limits in which 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 $Pchl_0$. This maximum value, P_{max}, may be specific for each cultivar.

Estimation of P_{max}

Fig. 3 shows the $Pchl_0$ distribution per batch estimated on colour data (connected open symbols) and analysed using the logistic batch model formulation of Eq. 8 (closed symbols). Distributions of all batches were analysed in one optimalisation to obtain the logistic rate k over all batches, P_{max} per cultivar, t_{max}^{b} and σ per batch (Table 1). The non-linear regression routine of Genstat (release 3.2, Lawes Agricultural trust, Rothamsted Experimental Station, UK.) was used. Genstat has a convenient standard function build in for Φ.

For cultivar 'Volcan', σ varied substantially over the growing seasons, while this was not the case for both other cultivars (Table 1). Only during the spring season for cultivars 'Volcan' and 'Borja' positive t_{mat}^{b} were encountered. This means that, compared to the other batches, these batches were of much better maturity. When only σ and t_{mat}^{b} are concerned, the spring batch of cultivar 'Volcan' is the best batch. However, the influence of P_{max}, which is the largest for cultivar 'Borja' and the smallest for cultivar 'Volcan' (Table 1, Fig. 3) is substantial. 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'.

Biological Variation

Normally, biological variation is treated like an ever present nuisance which should be minimised as much as possible. Most commonly used technique to deal with biological variation is sorting and grading on external quality attributes (Tijskens and Konopacki, 2001). 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. Building in biological variation on the level of t_{mat} of the precursor function might be possible for a host of other products, provided that process-based models are available or can be generated to describe the behaviour of the quality attribute *and* the precursor. In this paper the first practical application on how to take *advantage* of the biological variation is presented.

One problem exists applying this biological variation incorporation technique. The function describing the behaviour in time of the precursor (Eq. 3) needs to be continuously increasing or decreasing, otherwise the inverse does not exist. This inverse is needed when incorporating process based information into the batch model (Eq. 7). Likely, most process-based descriptions in physiology do not comply with this condition. Therefore a way to circumvent this condition had to be made by transforming the exact Pchl function (Eq. 3) to an approximated version (Eq. 5).

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Tables

Table 1. Overview of parameters belonging to the six batches. k and P_{max} are common for all batches and per cultivar, respectively.

cultivar	season	batch keeping quality (days)	k	average $Pchl_0$	sigma		t^b_{mat}		P_{max}	
					estimate	s.e.	estimate	s.e.	estimate	s.e.
'Volcan'	autumn	3.8	0.024	0.11	23.34	2.12	30.09	1.06	1.752	0.106
'Volcan'	spring	6.8		0.59	4.959	2.31	-6.87	3.86		
'Borja'	autumn	8.4		0.69	13.77	1.12	7.99	1.17	2.869	0.194
'Borja'	spring	13.1		1.54	15.59	1.20	-6.76	3.08		
'Beluga'	autumn	4.3		0.12	7.466	1.55	28.502	0.633	2.230	0.204
'Beluga'	spring	7.2		0.65	6.757	1.46	5.06	2.74		
R^2_{adj} (%)	93.2									
n	282									

Figures

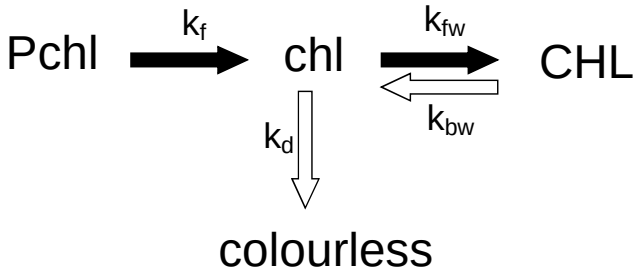

Fig. 1. Model representation of the last part of the chlorophyll pathway for cucumbers stored in the dark. Closed arrows indicate chlorophyll synthesis and open arrows catabolism. Indicated are the reaction rate constants.

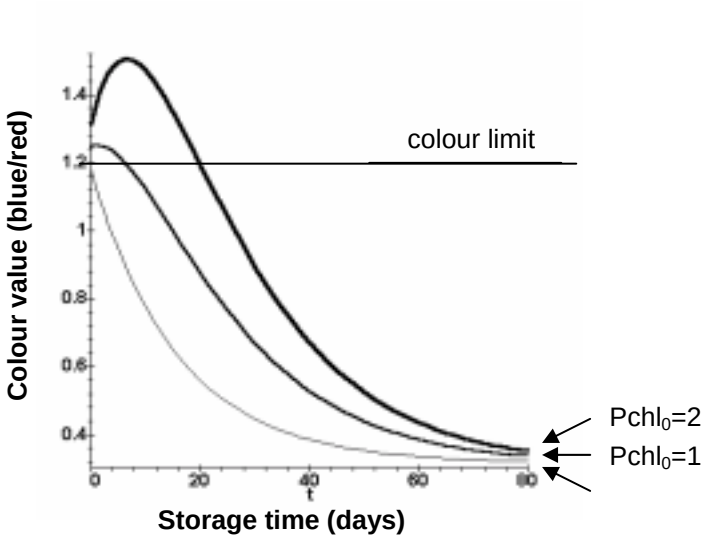

Fig 2. Colour behaviour for of three cucumbers differing in $Pchl_0$. Indicated is the colour limit. The cucumber with the smallest concentration of $Pchl_0$ has an intercept with the colour limit at 0, the medium concentration at 8and the highest concentration at 20 days. This intercept is defined as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

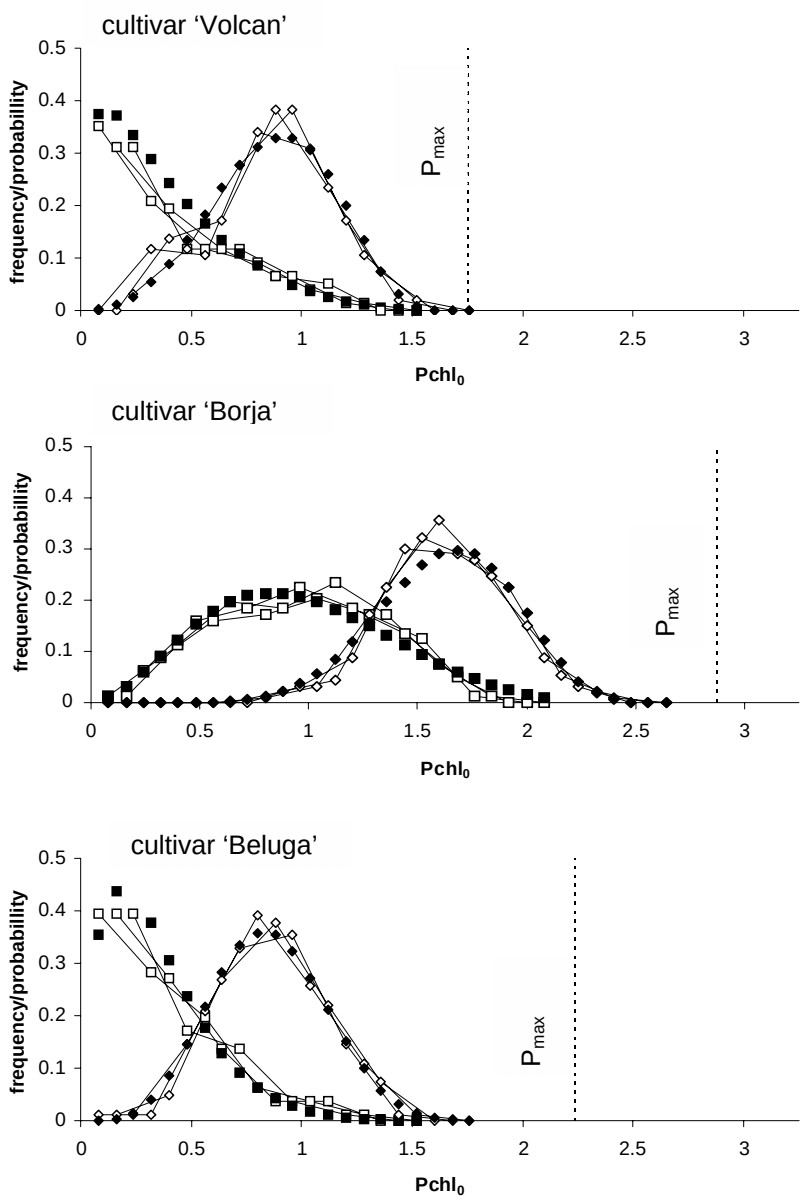

Fig 3. Distribution of Pchl concentrations at harvest ($Pchl_0$) for two batches per cultivar. Per cultivar one batch was harvested in the autumn season (□,■) or the spring season (◊,♦). Open symbols indicate the Pchl distribution obtained from colour data, closed symbols the distribution from batch model estimations.

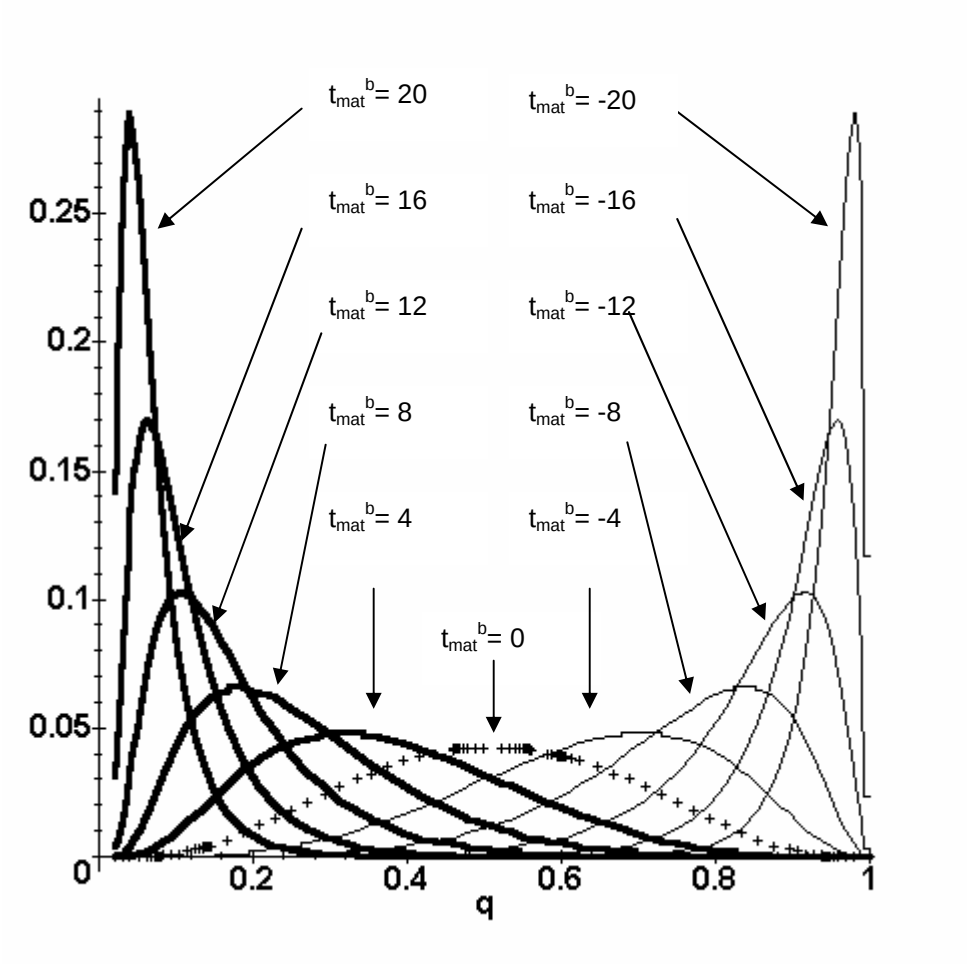

Fig. 4. Simulation of Pchl distributions for one batch harvested at different maturity stages, indicated by t_{mat}^{b}. Simulation was carried out using P_{max} =1, k=0.15 and σ=5, applying Eq. 8.