Physiology of biological variation

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Abstract

In agricultural products, variation exists in quality attributes between batches. Examples of this biological variation are well known and the general response is trying to suppress it as much as possible; to create uniformity using pre- and postharvest methods. This thesis shows a methodology that takes advantage of the biological variation, instead of treating it as a nuisance. This biological variation methodology was applied to understand the expected keeping quality of batches.

The methodology currently consists of three steps. Firstly, repeated non-destructive measurements of quality properties of individuals need to be applied to find out how the quality attribute changes over time without having to worry about biological variation. Secondly, kinetic models need to be constructed that show the quality attribute changing over time as a combination of simultaneously occurring processes that, ideally, have a strong physiological background. The last step consists of translating the kinetic model that describes the behaviour of the quality attribute of individuals to batches using stochastics. This methodology is applied for cucumbers and strawberries.

Cucumber. The keeping quality for a cucumber, defined as the time the colour remains acceptable to the consumer, depends on the state of the chlorophyll metabolism. A generic model was build that describes the postharvest colour behaviour in time and temperature for individual cucumbers, irrespective of growing conditions and cultivar. The model enables prediction on the batch keeping quality, on the basis of initial colour measurements only.

Strawberry Postharvest life of strawberries is largely limited by Botrytis cinerea infection. A colour model was built that describes the simultaneous development of the red colour and the anti-fungal function of individual strawberries over time. Batch keeping quality predictions could be derived on the basis of initial colour measurements or from the time between harvest dates.

Batch model. The batch model describes the influence of one source of biological variation, here assumed to be variation in light conditions during the preharvest period, on the distribution of precursor concentrations by combining (product specific) kinetic models and a generic stochastic part. The batch model described batch behaviour in terms of current maturity, biological variation and maximal maturity towards keeping quality of cucumbers and strawberries. Applications of biological methodology may be numerous: proposing protocols for keeping quality predictions, characterisation of cultivar specific influences on keeping quality or, in general, starting of a new field that is concerned with the 'hidden' information that is present in all biological batches.

Contents

STELLINGEN

1. Nauwkeuriger meten levert betere, en uiteindelijk fundamentele, modellen op (Dit proefschrift).

2. Variabiliteit is maar mooi een lastig probleem (Pelayo et al. PBT, 2003).

3. Vooraf kritiek eisen en incasseren is een voorwaarde voor succesvol publiceren.

4. Genomics heeft een excellente toekomst als er een solide koppeling komt met fundamenteel fysiologisch modeleren.

5. Mannen weten hun zaakjes goed te verbergen. (Willard, Nature, 2003)

6. Full cost accounting heeft geleid tot zowel de verzelfstandiging van de NS als tot onbetaalbaar WUR onderzoek.

7. Wetenschap is saai zonder Wim T. Schippers, de meester van de ontregeling.

Stellingen behorende bij het proefschrift:

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Rob Schouten
Wageningen, 9 januari 2004

1

General introduction

In agricultural products, variation exists in quality attributes between individuals. For instance, apple fruit from one harvest will consist of apples that were shaded within the tree and of apples that were directly exposed to sunlight. As a consequence, a batch of apples shows variation in skin colour. This is an example of biological variation partly due to the position of the apple in the tree. In general, biological variation may be described as the composite of biological properties that differentiate individual units of a batch (adapted from Tijskens and Konapacki, 2003a). Examples of biological variation are well known and the general response to biological variation is trying to suppress it as much as possible; to create uniformity using pre- and postharvest methods (Tijskens et al., 2003). To stay with the apple example, after harvest the apples may be transported directly to an auction where they are sorted and graded on weight and colour to create sub-batches of about the same colour and size. Later, a mixed 'batch' may be generated when sub-batches of the same colour and size class are put together from different orchards. At the present state of technology, sorting can be accomplished, both rapidly and accurately, on external properties like colour, weight, shape and external defects. There are two important issues associated with sorting. When e.g. apples are sorted on colour, they are sorted on the current colour, not on expected colour retention. So, apples from different orchards, regions, and different harvest dates might be mixed having about the same colour. However, the apples from each orchard, region and harvest date may have a different colour development over time, leading to a significant colour variation when reaching the consumer. Also, an ongoing trend is that consumers put more emphasis on internal properties like taste, flavour and the presence of 'health promoting' substances. Sorting and grading on external properties does not supply any information on these internal properties. So, when apples from different orchards, regions and harvest dates are mixed having about the same actual colour, the variation in internal properties may be undesirably large.

A way to prevent these problems associated with sorting and grading may be to sort only on defects, and grade on the batch level. A batch is considered as all individuals with a common growth history. In practice, a batch consists often of all individuals from the same cultivar, grown at the same location (orchard, greenhouse) and from the same harvest date. The variation in properties observed on the batch level is of biological origin, being a derivative of past growth conditions. The biological variation on the batch level is unique and extracting this 'fingerprint' might assess current behaviour and predict future behaviour of the batch. In the postharvest trajectory, biological variation may help to establish a 'best before date' or suitability for export. In the preharvest trajectory, biological variation may help to decide on optimal orchard specific harvest dates and suitability for long time storage of apples and pears. Key point is the assessment and use of biological variation in this respect. Fundamental research on biological variation

methodology is almost non-existent. Almost, because this thesis tries to show some key issues that enable the development of biological variation methodology. The importance to use and understand biological variation can be shown when clear economic gains may be expected. Therefore, biological variation was measured and applied to understand the expected keeping quality of batches.

Keeping quality is connected to quality. Quality is a difficult property, as everyone uses a slightly different set of criteria to interpret the quality of a product. In order to have some practical grip on quality related issues, the concept of acceptability was introduced (Tijskens, 2000). When somebody decides on the acceptability of a product, the quality is compared to a criterion, the quality limit. If the quality exceeds that limit, the product is accepted, and otherwise rejected (Wilkinson and Tijskens, 2002). So, acceptability of a product depends on product quality and on the level of the acceptance limit. The acceptance limit is primarily defined by economical and psychological factors; the quality of a product is largely defined by its intrinsic properties. Acceptability is directly related to the keeping quality of a product. For fruits and vegetables, product properties such as colour, firmness and taste change over time. Keeping quality is the time until the product attribute drops below the acceptance limit at any dynamic or static condition. So, keeping quality combines two aspects of product acceptance, the acceptance limit and product quality, into a generally applicable index of quality (Tijskens and Polderdijk, 1996). Keeping quality and shelf-life are terms often used interchangeably and are indeed closely connected. Shelf-life is the keeping quality under standardised storage conditions (Tijskens, 2000). The concept op keeping quality enables quality research to be formally separated into two fields. Consumer attitudes towards regional food preferences (Verlegh and Steenkamp, 1999), sensory evaluation (Munoz, 2002) and customer value processes (Payne and Holt, 2001) are recent examples of research efforts aimed at the acceptance limit. Here the focus will be on the other field, the factors that influence product behaviour. Product behaviour shows generally a decay in quality attributes after harvest. Within the time the product remains acceptable, it goes through the horticultural production chain. Knowledge of the expected keeping quality at the start of the horticultural chain will be of great benefit to participants. For instance, consumers may be able to get guaranteed high quality products or producers may be able to export, instead of supplying products to the domestic market.

Aim of the thesis

The aim of this thesis is the development and application of biological variation methodology. The development is shown with regard to the batch keeping quality of strawberry and cucumber only. However, as the key issues are generic in nature, the grand aim is to provide generic tools for the analysis of batches of fruits and vegetables.

Methodology

Methodologies to pursue the aim of this thesis are primarily modelling techniques (kinetic and stochastic) in combination with non-destructive measuring techniques. Key issues to develop the biological variation methodology are:

(i) repeated non-destructive measurements of quality properties of all individuals in a batch (longitudinal data)
(ii) building physiological kinetic models
(iii) building of a stochastic model capable of combining kinetic models and information on biological variation to predict keeping quality on a batch level

ad (i) Non-destructive measurements

Repeated non-destructive measurements on individuals are essential to generate accurate knowledge concerning the development of a quality related property. This may be illustrated by stating an example from the workshop on biological variance (Tijskens et al., 2001). This example shows the simulated firmness decay of a tomato; the aim is to find the process behind the decay. The firmness left for each tomato, after a varying storage period, is measured destructively (left hand-side of Fig 1.1), starting with (seemingly) identical tomatoes. According to these experiments, the behaviour of the replicates resembles an exponential decay, but there is quite a lot of deviation from the proposed exponential curve. What could be the reason? Experimental errors in determining the firmness could be the case, however, it is also possible that the experimental set-up is to blame. Let's assume we restart the experiment, but change the experimental set-up. The difference is that the firmness is measured repeatedly over time of the same tomatoes (right hand-side of Fig. 1.1). The effect is that initial variation in firmness between tomatoes is still present in this experiment, but that the magnitude remains unchanged every time the firmness is measured. This means that using non-destructive repeated

measurements, the (exponential) behaviour over time of the attribute under investigation might be extracted, as now variation is present between individuals, not within individuals.

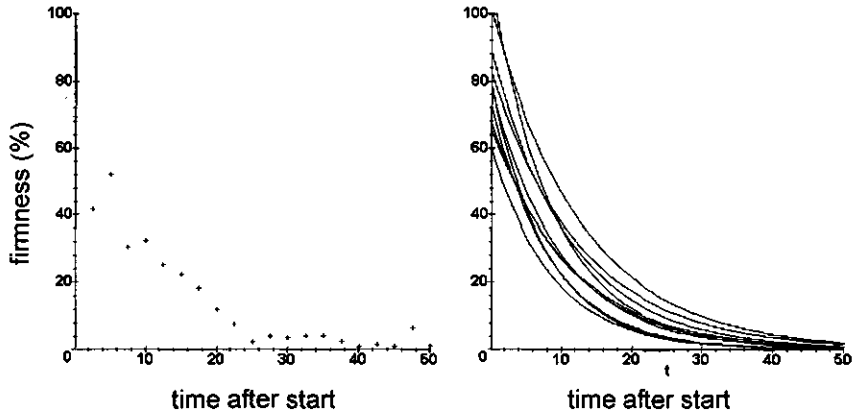

Fig. 1.1. Simulated firmness decay for tomatoes examined using separate tomatoes observed after a variable storage time (left hand-side) or observed continuously (right hand-side).

ad (ii) Kinetic modelling

Kinetic modelling is a useful technique in relation to quality changes as they represent biochemical and physical reactions that often proceed at a certain rate and with certain kinetics. Kinetic modelling enables the description of these changes quantitatively. Also, kinetic modelling is a powerful tool to unravel basic reaction mechanisms (van Boekel and Tijskens, 2001). Understanding these basic mechanisms is an essential issue for the description and understanding of biological variation. To have a better understanding of kinetic modelling, traditionally used to describe general chemical reactions, let's examine the example of the consecutive reaction. The simplest consecutive reaction describes the formation of a compound B from compound A which then reacts further to the final end compound C: (Eq. 1.1)

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad (1.1)$$

Let's assume the development of the concentration of compounds A, B and C can be measured repeatedly and non-destructively. The upper left plot of Fig. 1.2 simulates the development in time for three different initial concentrations of compound A. Hypothetical values for the reaction rate constants k_1 and k_2 are used and it assumed that no initial concentrations of B and C are present. Over time, compound A is consumed and turned

into compound B, which shows an initial rise in concentration but later a decay in concentration as compound B is turned into compound C (first column of Fig 1.2).

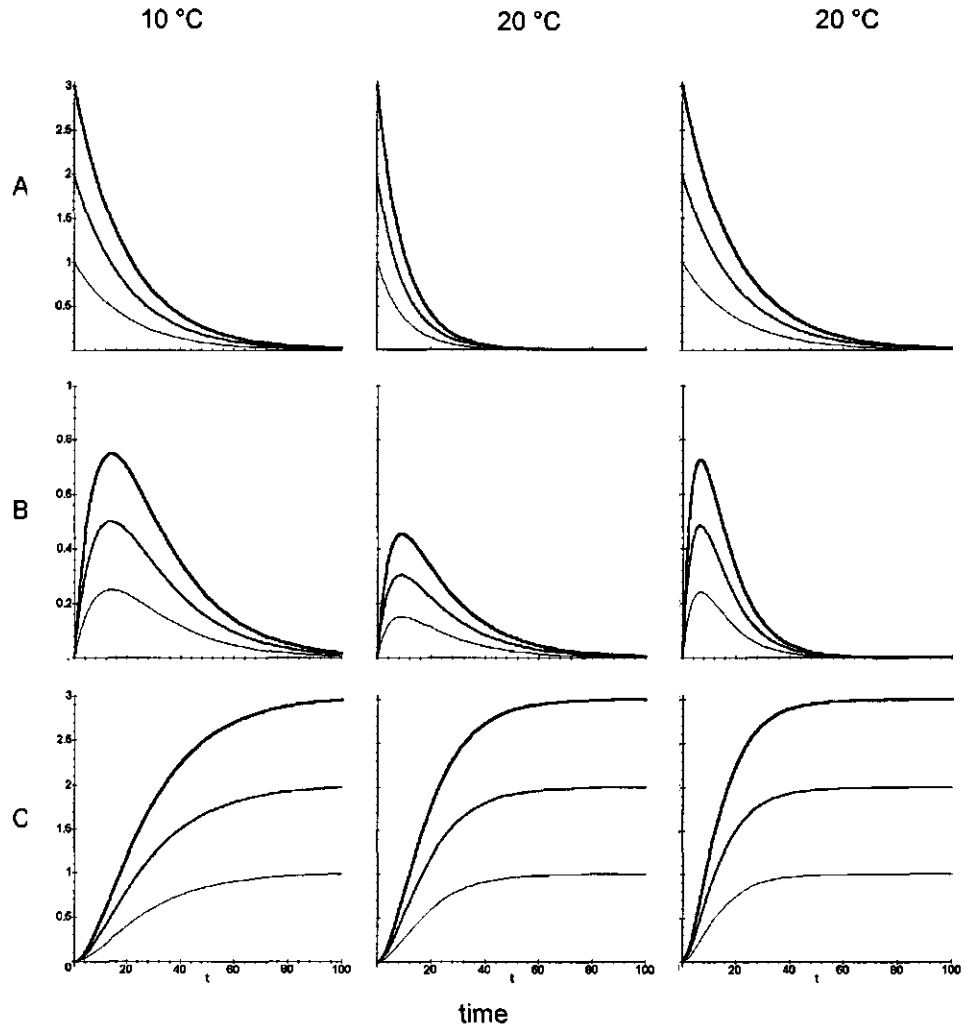

Fig. 1.2. Concentration profiles based on the consecutive reaction A -> B -> C simulated at 10°C (left hand-side row) for three samples differing in initial concentration of A. The middle and right-hand columns show behaviour of the same samples, but now at 20 °C. The difference in concentration profiles between column two and column three is caused by different temperature dependencies only.

Temperature effects can be incorporated using e.g. Arrhenius' Law. In case of the consecutive reaction example there are two reaction rate constants, each with a temperature dependency according to Arrhenius' Law. Both the second and the third column in Fig. 1.2 show the development in time of the concentration of compounds A, B and C using the same initial concentrations. However, now the consecutive reaction is not carried out at 10 °C, as was shown in the first column, but at 20 °C. In the second column the reaction rate constant k_1 at 20 °C is set to twice the value of the reaction rate constant at 10 °C combined with reaction constant k_2 that is almost not dependent on temperature. For the third column the temperature dependencies for the reaction rate constants are reversed compared to those of the second column, leading to clear differences in concentration profiles for especially compounds A and B. The usefulness of kinetic modelling becomes clear when asked to analyse plots as shown in Fig. 1.2. Concentration profiles of column 1 and column 2 can be analysed simultaneously, applying so called multi-variate, multi-response non-linear regression analysis, to obtain a small number of parameters that fully describe the complex, non-linear behaviour seen in the plots. These are:

- three initial concentrations of compound A
- two kinetic parameters for the reaction rate constants at 10 °C (k_1, k_2)
- two kinetic parameters for the temperature dependency of k_1 and k_2

How applicable is kinetic modelling when trying to describe physiological reactions connected to keeping quality? Let's examine the colour development of green apples. The experimentally observed s-curve like behaviour (Tijskens and Konapacki, 2003b) may be used to calibrate a generic autocatalytic model (Eq. 1.2). Colour decay is slow initially when the enzyme (Enz) concentration is limiting, picks up later on, and finally slows down again when the colour concentration is limiting (left hand-side of Fig. 1.3).

$$\text{colour} + \text{Enz} \rightarrow 2\,\text{Enz} \qquad (1.2)$$

This colour model 'fits' very well on apple (Tijskens and Konapacki, 2003b) and tomato data (Tijskens and Evelo, 1994). However, it is known from literature that tomato and apple are very different products physiologically. For instance, it is known that chlorophyll, responsible for the green colour in the skin of green apples, is very sensitive to ethylene. Furthermore, ethylene itself will be generated autocatalyticaly. Now, a new hypothetical model may be proposed to specifically describe colour behaviour of apples (Eq. 1.3). This model also 'fits' the data very well (right hand side of Fig. 1.3), but is now based on actual physiological knowledge.

chlorophyll + ethylene → ethylene (1.3)
ethylene → 2 ethylene

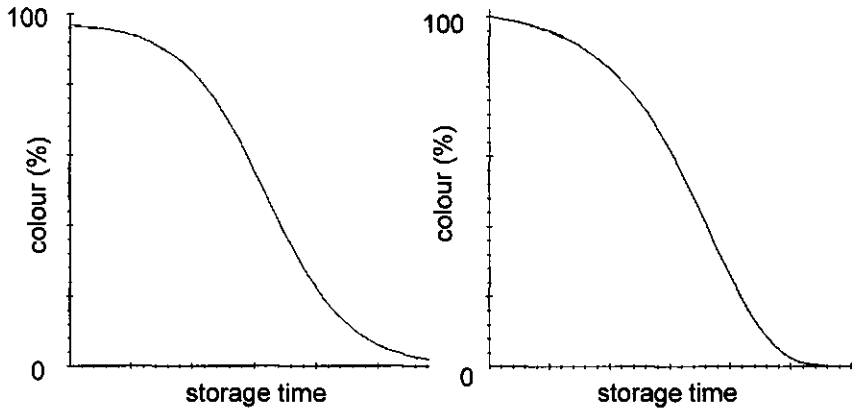

Fig. 1.3. Comparison of a autocatalytic model (left hand-side (Eq. 1.2)) with the hypothetical apple colour model (right hand-side (Eq. 1.3)) for describing colour development over time.

What reason is there to use a complex physiological model (Eq 1.3) instead of simple generic model (Eq. 1.2) when experimental data 'fit' equally well on all models? The processes described by a physiological model are actually representative of processes occurring in the products. Consequently, environmental changes affecting these processes may be described correctly using this type of model. Furthermore, physiological models may guide further research. For instance, batches of apples may show very different colour behaviour during long time storage, while all batches were harvested having about the same initial colour. Instead of investigating the small colour differences at harvest, it might be much more useful to measure the ethylene distribution at harvest for each apple batch. The first approach might be a consequence of using the generic model, the second approach of using the physiological model.

ad (iii) Stochastic modelling

Physiological kinetic models are useful to describe physiological processes for individual products. However, biological variation is considered a batch property. How to describe biological variation with physiological kinetic models? Let's assume the batch of green

apples again, this time with the aim to predict the colour of all apples belonging to a batch after a period of long time storage provided the physiological kinetic model (Eq. 1.3) can be applied. This can be accomplished by doing two experiments using repeated non-destructive measurements. The first experiment is aimed to extract the kinetic parameters of the colour model and uses 50% of all apples of the batch (first sub-batch):

- measure the ethylene concentration and the colour non-destructively for all apples of the first sub-batch at harvest.
- store each equal parts of the sub-batch at different temperatures and measure the ethylene concentration at regular intervals during the storage period.
- calibrate the apple colour model using the gathered experimental data in combination with Arrhenius' Law to obtain kinetic parameters.

During the second experiments the colour and ethylene levels at harvest are measured for all apples of the second sub-batch and combined with the kinetic parameters to predict the colour of all apples after long time storage. This procedure is labour intensive and sensitive to measurement errors at harvest. It would be much more convenient to use the biological variation contained in the batch because of the shared orchard, cultivar and harvest date. Let's assume that significant biological variation can be attributed to the ethylene production of the apples. The colour of the harvested apples will be influenced by the variation in ethylene. Some apples produce only very small amounts of ethylene, having almost no adverse effect on the colour, resulting in colour retention. Other apples will produce more ethylene resulting in rapid colour loss. Therefore, the variation in ethylene will have an effect on the colour distribution. It is likely that also considerable colour variation will be present because of the earlier mentioned position of the apples in the tree. Now there are two sources of biological variation, resulting in a complex colour distribution, as now light green apples might be light green because of the position in the tree, because of exposure to large amounts of ethylene, or a combination of both. This batch might be characterised by measuring the ethylene and colour distribution at harvest and analysing these distributions with mathematical tools to separate the effects of ethylene and tree position. The colour of this batch after long time storage may then be estimated based on the batch characterisation at harvest. Unfortunately, these mathematical tools are not available yet.

Batch colour data can be expressed in histograms and these can be represented mathematically by standard statistical probability distributions like the Poisson or the binomial distribution. However, what is the probability distribution for a batch when the individuals of that batch have quality attributes that change over time according to a physiological model? Stochastics, the mathematical subfield consisting of probability and

statistics, might be used to describe batch behaviour. Stochastics theory might combine a kinetic model, for the description of the behaviour of the individuals in the batch, with a probability distribution function for the biological variation that differentiates the individuals in the batch. The development of these mathematical tools might enable the use of the hidden information, the biological variation, for characterisation of e.g. the keeping quality at the batch level.

Overview of the thesis

The research described in this thesis started with the goal to predict the keeping quality of cucumbers based on the colour at harvest. A rather new method, an image analysis system, was used for colour measurements. The reason to use colour measurements for predictions on keeping quality is that the limiting quality attribute for cucumbers is colour. Unfortunately, it seemed that colour at harvest was not a very good indicator of the keeping quality for individual cucumbers (Chapter 3). It was discovered, however, that the shape of colour distributions at harvest, obtained by measuring the colour of all cucumbers in a batch, could be used as indication of the preharvest growth conditions (Chapter 4). Apparently, because of the common growth history of batches, there is information hidden in batches. How to retrieve this 'hidden' information? Is this kind of information available in all products? To answer the first question, an attempt was made to extract, describe and understand the information hidden in colour distributions of cucumber batches (Chapters 5). The second question is a generic question and is not easily answered. It was tackled by searching for a product completely different than cucumber. Strawberry seemed not connected to cucumber as rot incidence, not colour is the limiting quality attribute. There is a connection, however. When strawberries are divided in two groups and one group is subjected to a TL-light treatment the incidence of rot was delayed in the light treated group. Not only the rot incidence was delayed, the colour of the light treated group was also more red (Saks et al., 1996). This means that colour is not only connected to the keeping quality of cucumber batches (Chapter 5), but also to the keeping quality of strawberry batches (Chapter 6). The same approach to describe and understand hidden batch information was then applied to both cucumber and strawberry (Chapter 7).

Colour is perhaps the most ideal property to measure at the moment: it can be measured very accurately and the measurement is non-destructive, enabling repeated measurements. Chapter 2 provides an overview of colour measurements, colour processes, recent colour research and the bottlenecks when trying to convert physiological knowledge into colour applications. Removing these bottlenecks, especially

the absence of physiological colour models and the lack of a methodology to deal with batch variation, are major constituents of this thesis.

Structure of the thesis

Chapter 1. General introduction and aims of this thesis.

Chapter 2. Bottlenecks in colour research. This chapter describes general colour processes and provides an overview of recent research into the manipulation of those processes. Colour measurements, development of colour models that incorporate physiological knowledge and the batch concept are introduced and expanded in later chapters.

Chapter 3. Keeping quality of cucumbers fruits predicted by the biological age. This chapter shows the development of a non-destructive method to measure the colour of cucumbers. It also shows what happens when colour measurements are coupled to a kinetic model without taking physiology into account.

Chapter 4. Keeping quality of cucumber batches: Is it predictable? This chapter investigates the connection between colour distributions of cucumbers and growth conditions and describes batches in terms of keeping quality and maturity.

Chapter 5. Predicting keeping quality of batches of cucumber fruit based on a physiological mechanism. This chapter presents a physiological kinetic colour model that shows that batch keeping quality depends on one specific precursor. Bringing physiology into the kinetic modelling immediately proves its value by enabling prediction of the batch keeping quality solely on the basis of non-destructive measurements.

Chapter 6. Predictability of keeping quality of strawberry batches. This chapter presents another physiological kinetic model, now aimed at the physiological processes responsible for the keeping quality of strawberries. Batch keeping quality is linked to one specific precursor and non-destructive colour measurements can be used to predict the batch keeping quality of strawberries.

Chapter 7. Batch variability and cultivar keeping quality of cucumber and strawberry. This chapter proposes a generic stochastic model for the interpretation of batch behaviour of

both strawberries and cucumbers. The model is validated using experimental data from chapter 5 and chapter 6.

Chapter 8. Discussion. This chapter focuses on limitations and applications of biological variation methodology. Aims for further development are stated.

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2

Bottlenecks in colour research

Introduction

Colour is not directly a quality attribute, but it is strongly related to maturity. Colour may limit the (keeping) quality for products as diverse as cucumbers, bananas and broccoli (green to yellow transition) and tomatoes, cherries, strawberry (green to red transition). Colour development is different for each product. However, for the majority of fruits and vegetables only three types of colour processes are sufficient to describe most colour changes. This chapter describes those processes and provides an overview of recent research into the manipulation of those processes to improve the colour retention. Bottlenecks for applications of the results of colour research are indicated. Solutions for the bottlenecks, such as improved colour measurements, development of colour models that incorporate physiological knowledge and the batch concept are introduced here and expanded in later chapters of this thesis.

Green to yellow physiology

Fruits (and sometimes vegetables) are generally classified either by increased respiration and ethylene biosynthesis (climacteric fruits) or without ethylene biosynthesis (non-climacteric fruits). Although non-climacteric fruits frequently respond to ethylene, ethylene is not required for ripening. In contrast, for climacteric fruits ethylene is necessary for the co-ordination and completion of ripening. This classification into climacteric and non-climacteric types of fruits and vegetables is important when discussing colour physiology, as the physiology is generally less complex and better understood for non-climacteric products.

Chlorophyll is generally considered to be the most important compound responsible for the green colour of fruits and vegetables. Chlorophyll exists in two forms, chlorophyll a and chlorophyll b, which are chemically identical, save for a formyl instead of a methyl group at the 7^1 position. Both forms of chlorophyll are photoreceptors and situated in chloroplasts; chlorophyll a is both reaction centre and antenna chlorophyll, chlorophyll b is found only in antennae (Porra, 1997). For non-climacteric fruits and vegetables chlorophyll degradation is indicative of the transition of chloroplasts to gerontoplasts, a senescence specific form of plastids. The yellowing is due to unmasking rather than biosynthesis of new yellow pigments (Matile et al., 1999). The most likely pathway for chlorophyll a degradation in fruits and vegetables is the cleavage by chlorophyllase resulting in the formation of chlorophyllide (Heaton and Marangoni, 1996) Chlorophyllide will be converted into yellow-brown compounds and finally into colourless compounds according to the pheophorbide a pathway. The first step of chlorophyll b

degradation is the conversion of chlorophyll b to chlorophyll a. This step is thought to be part of a chlorophyll cycle by which the two forms of chlorophyll are balanced in the photosynthetic apparatus (Matile et al., 1999). Chlorophyll degradation is considered a hazardous process that must be strictly co-ordinated as to render chlorophyll photo dynamically harmless. Moreover, chlorophyll is not degraded to reuse its constituents, but to gain access to more valuable materials such as protein nitrogen and lipid carbon. So, in a sense chlorophyll is not so much degraded as detoxified (Matile et al., 1999).

For climacteric-like vegetables like broccoli the green to yellow colour development is different. Broccoli is harvested at an immature stage and this causes considerable stress due to disruption in energy, nutrient and hormone supplies resulting in a very short shelf-life. In asparagus and broccoli, harvesting in a stage of rapid growth causes the tips to lose large amounts of sucrose and undergo major changes in gene expression. This leads to a markedly altered metabolism including protein and lipid loss and amino acid accumulation which may be described as a starvation response (Page et al., 2001). Ethylene has an important role in regulating the yellowing of broccoli since chlorophyll loss is associated with an increase in floret ethylene synthesis. Interestingly, treatment with the plant hormone cytokinin resulted in longer postharvest life as less chlorophyll loss and a delay in asparagine accumulation was observed, although sucrose loss was unaffected (Downs et al., 1997).

For banana, de-greening is clearly different from non-climacteric fruits as, for instance, neither chlorophyllide nor pheophorbide accumulated during ripening. Interestingly, chlorophyll retention in the banana peel was larger at 35 °C than at 20 °C. This may be explained by a temperature sensitive dissociation of chlorophyll from the thylakoid membrane (Drury et al., 1999) or perhaps by the existence of another chlorophyll degradation pathway. IJanave (1997) showed that oxidative enzymes might degrade chlorophyll in vitro. In contrast, a study concerning colour development of bananas during ripening indicated that colour decay increased with increasing temperature by Chen and Ramaswamy (2002).

Green to red physiology

The green to red colour development in climacteric fruits like tomato and red pepper is largely due to the transition of chloroplasts to chromoplasts. Transition of chloroplasts into chromoplasts or gerontoplasts is comparable to as far as chlorophyll breakdown and the disappearance of thylakoids are concerned (Matile et al., 1999). Large amounts of carotenoids, mainly β-carotene and lycopene are synthesized in the chromoplasts. Geranyl-geranyldiphosphate (GGPP) is the precursor of the carotenoids and the

conversion of GGPP to phytoene is the first step in the carotenoid biosynthesis. The next steps de-saturate phytoene and ξ-carotene and produce lycopene, responsible for the red colour of ripe tomato fruit. Lycopene may undergo cyclisation to either β- or α-carotenes. Lycopene accumulation for tomato arises during ripening as a consequence of reduced lycopene cyclisation and the presence of ripening enhanced phytoene synthase (Fraser et al., 2002).

Carotenoids are often part of pigment protein complexes which are associated with chlorophyll to protect the photosynthetic apparatus from oxidative reactions especially under high light-stress (Sandmann, 2002). Carotenoids, especially β-carotene and lycopene, have anti-oxidative properties such as quenching of singlet molecular oxygen and scavenging of free radicals to prevent DNA damage and lipid peroxidation (Bohm et al., 1997).

Postharvest colour development of e.g. non-climacteric strawberries and cherries ranges from light red till deep red and is indicative of anthocyanin production. Anthocyanin production is triggered by three classes of light receptors (UV-A, UV-B and blue light) and is under hormonal, especially of giberrelic acid, control (Mol et al., 1996). For apple, it was found that five anthocyanin enzymes are co-ordinately expressed during red coloration in the skin (Honda et al., 2002). Anthocyanins are one of the end products of the flavonoid pathway which is well characterised. The main pathway transforms phenylalanine into anthocyanins but also flavones, isoflavonoids, phlobaphenes and tannins are produced from the many intermediates (Boss et al., 1996). Flavonols, the direct anthocyanin precursors, are protecting plants from harmful UV irradiation (Boss et al., 1996) and play a role in fruit-pathogen interactions (Jersch et al., 1989).

Recent colour research

Recently, an interesting possibility to affect the ethylene triggering process became available. MCP (1-Methylcyclopropene) inhibits ripening for a host of climacteric fruits and climacteric like vegetables, specifically firmness, but also colour retention. For instance, application of MCP increased the colour retention of broccoli over 20% (Able et al., 2002). Lower ethylene production and respiration, slower loss of firmness, acidity, and less peel colour change was observed for a rapid ripening summer apple during storage (Pre-Aymard et al., 2003). Other colour development retardants with a similar function as MCP exist. Gibberellic acid, a plant hormone, can retain green colour in citrus fruit, whether applied as preharvest spray or postharvest dip treatment (Porat et al., 2001). Ethylene oxide (EO) and sulfur dioxide (SO_2) may be used to prevent ripening in bananas.

Treatment with EO and SO_2 was efficient in extending the shelf life of bananas, showing a fresh appearance, good colour and minimal mould development (Williams et al., 2003).

Cytokinins are believed to delay senescence by maintaining cellular integrity. Cytokinins prevent proteases from the vacuole to leak into the cytoplasm to hydrolyse proteins of the chloroplast. Consequently, cytokinins are connected to chlorophyll retention. Gan and Amasino (1995) devised a strategy of auto regulated cytokinin production using the highly senescence specific SAG12 promoter fused to the ipt gene in transgenic tobacco. The ipt gene encodes isopentenyl phosphotransferase, the enzyme that catalyses the rate limiting step for cytokinin synthesis. The chimaeric P_{SAG12}-IPT gene was only activated at the onset of senescence thereby preventing over expression that led to delayed growth and fertility. This strategy has also been carried out for lettuce cv Evola (McCabe et al., 2001). In the transgenic lines no senescent leaves were present neither at the seedling stage nor during later development. Next to the SAG12 promoter, also other promoters have been used in conjunction with the ipt gene. Chen et al. (2001) applied these chimaeric genes to transform broccoli, resulting in transgenic broccoli with 50% chlorophyll retention after four days storage at 25°C.

For products that show a non-climacteric red colour development caused by anthocyanin production like strawberry, postharvest light or heat treatment might be used to increase the keeping quality. Light treatment was able to overcome poor red colour and 'white shoulders' in two strawberry cultivars while diminishing fruit rot at the same time (Saks et al., 1996). Interestingly, Vicente et al. (2003) applied heat treatments (45 °C for 3 h) to strawberry (cv Selva) in combination with MAP (Modified Atmosphere Packaging). Reduced fungal decay, softening and red colour development was found after a market simulation period, especially when the CO_2 produced during heating was allowed to retain in the package. So, red colour development and reduced fungal decay seems to be equally enhanced by light treatment while heat treatment specifically increases the resistance against fungal decay but not the red colour development.

For tomato, numerous colour mutants exist (reviewed by Gray and Picton, 1994). For instance, Cnr mutant tomato fruits have low levels of carotenoids, phytoene and lycopene. Extracts from ripe fruit showed a reduced ability to synthesise the carotenoid precursor GGPP, but also a lack of phytoene synthase (Fraser et al., 2001). Ronen et al. (2000) investigated two pigmentation mutants in tomato (β and og). Cloning of both genes revealed that β encodes lycopene-ε-cyclase, a key enzyme that converts lycopene to β-carotene. During fruit development the mRNA levels of lycopene producing enzymes phytoene and phytoene desaturase increase, while the mRNA levels of the genes for the lycopene cyclases decline or completely disappear (Ronen et al, 1999). Other interesting mutations towards carotenoid accumulation are the high pigment mutations, hp-1 and hp-2. These mutants show exaggerated photo responses during de-etiolation and higher

lycopene and β-carotene levels in combination with higher chlorophyll levels in immature fruit (Kerckhoffs et al., 1997). Tomato seedling de-etiolation is a phytochrome (red light) response, which can be enhanced by blue light, suggesting that hp-1 may influence phytochrome (Giovannoni, 2001).

Combinations of mutants could be of interest with respect to tomato colour keeping quality. Both hp and og are colour intensifier mutants. The double mutant hp/hp og^c/og^c tomato fruits showed improved red colour and increased shelf-life but also several undesirable effects. The effect on yield, firmness and colour development by combining the mutants, hp, og^c and alc to create all possible homozygous and heterozygous combinations for tomato fruits was investigated. The alc inclusion should improve firmness as alc fruit does not fully ripen off the vine unless picked beyond breaker stage and does not show a climacteric pattern resulting in increased storability up to 300% for homozygous alc/alc fruit. Analysis of intra-allelic additive and dominant interactions within these three loci and their interallelic interactions resulted in a number of genotypic combinations that represented a good compromise between yield, firmness and colour development (de Araújo et al., 2002).

Transgenic research on ripening concerns often both firmness and colour development, especially when related to ethylene production. For example, the major pigments remained undegraded during ripening compared to wild-type melons in ACO antisense Cantaloupe melons (Flores et al., 2001). Henzi et al. (2000) evaluated twelve transgenic broccoli lines containing a tomato antisense ACC oxidase gene. For three of those lines an improvement in head colour was noticed after 96 hours of storage. Transgenic research aimed at slowing colour development has also been carried out. Bacterial phytoene desaturase expressed in tomato did not elevate total carotenoid levels, but did increase the fraction of β-carotene threefold (Romer et al., 2000). Another approach used bacterial phytoene synthase to be overexpressed in tomato applying the PG promoter. It resulted in about a two-fold increase of phytoene, lycopene and β-carotene (Fraser et al., 2002).

Colour applications

Knowledge of colour change of fruits and vegetables has increased substantially over the years which might be illustrated by a total of 1077 hits (articles) over the last three years when using a combination of fruits, vegetables and colour (and color) as keywords for the winSPIRS literature database. Especially transgenic progress is swift due to molecular approaches such as positional cloning, QTL (Quantitative Trait Loci) mapping and genetic engineering (White, 2002). As the colour processes themselves are fairly well described it

might be expected that colour applications based on the obtained physiological knowledge are reported in literature and used in practise. Unfortunately, this is not the case and only very few colour applications exist. For instance, the colour sorting of apples, peaches and mangoes at auctions (http://www.aweta.nl) is based on ad-hoc rules to use colour as a maturity index. Another area of application might be plant breeding. For instance, the 'Borja' cucumber cultivar is described having an extended (colour) shelf-life (www.enzazaden.nl), but this trait is 'generated' similarly to other desirable traits such as disease resistance and high productivity. So, although the physiological colour processes are fairly well known and increasingly factors are discovered modifying these processes, applications are rare in practise. What is the reason for the gap between colour research and colour application?

Bottleneck: colour measurements

Colour measurements are usually performed using devices that are based on the CIE chromaticity colour space (e.g. Minolta Chromameter) or the RGB colour space (e.g. video camera). The vast majority of colour measurements are carried out using the chromameter. The chromameter analyses reflected light from a xenon source and quantifies the light in terms of three colour indices that define the colour space: the a-scale, from green to red, the b-scale, from blue to yellow and the L-scale, from dark to light. Advantages using the chromameter are the relatively low acquisition cost and the calibration procedure that enables comparison of colour measurements with literature sources. A disadvantage of the chromameter is that only small surfaces, often less than a cm^2, can be measured with each measurement. This is not very important when attempting to measure the colour of a solid, planar surface that is both completely uniform in colour and topography. However, the position of the chromameter on the fruit or vegetable has a significant effect on the colour measurement, as they are certainly not uniformly coloured. To deal with this, the chromameter is often used on multiple positions on the surface of the fruit or vegetable.

A rather new development for colour measurements of fruits and vegetables is the application of a RGB system, a RGB video camera in a light controlled container. Colour is expressed as a combination of R (red), G (green) and B (blue) values that vary between 0 and 255. Not every possible colour can be expressed this way, a disadvantage not present when using the CIE chromaticity colour space (Williamson and Cummins, 1983). The advantage, however, is that a video camera easily covers large surfaces enabling colour measurements of whole products. Fig. 2.1 shows examples of cucumber and strawberry colour images (expressed here in grey-scale) that consist of several hundred

thousands pixels. Each pixel corresponds to a separate RGB measurement. Additionally, RGB images can be edited routinely using colour recognition software to measure the colour of specific parts of fruits and vegetables (Fig. 2.1, lower right-hand side).

Fig. 2.2 shows a comparison of colour measurements carried out using a chromameter and a RGB colour measurement system for following the individual colour development of tomatoes (Tijskens and Evelo, 1994) and cucumbers, respectively. The cucumber plot shows a more fluid colour development patterns that are likely more based in physiology than those observed in the tomato plot.

Fig. 2.1. Left-hand side plot shows a flat 3D picture, showing the whole skin of a cucumber. Automated image editing software generates the flat 3D picture from a cucumber that is placed on two small hooks with one mirror behind and two side mirrors beside the cucumber. Lower right-hand side plot shows the effect of colour recognition software that classifies the different strawberry colour parts: calyx, flesh (dark red and light red), seeds and background from the original strawberry picture (upper right-hand side plot).

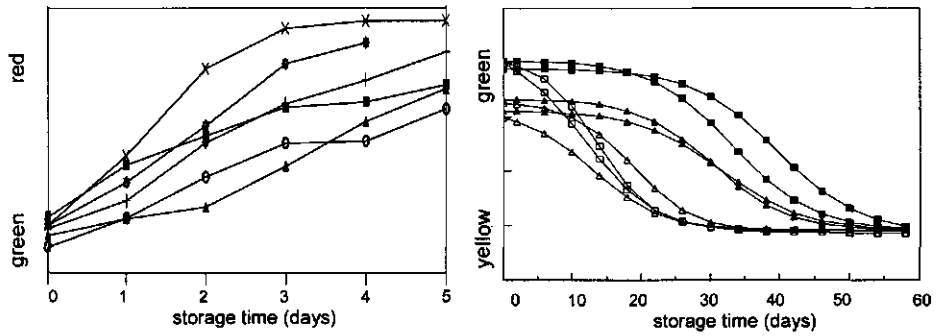

Fig. 2.2. Repeated colour measurements of individual tomatoes (left-hand side) and cucumbers (right-hand side) over time. Colour of tomatoes is expressed as the a value of the Lab scale Lab (CIE chromaticity colour space), the average of three separate measurements on the equator of individual tomatoes (Tijskens et al., 1994). The right-hand plot shows repeated RGB measurements over time for individual cucumbers expressed as the ratio of the Blue (B) to Red (R) intensities from one single RGB colour measurement.

Thus, one of the bottlenecks for transferring physiological knowledge into colour applications is the current practise of measuring colour with the chromameter. Fortunately, a solution is available: a RGB video camera in a light controlled environment. Another solution might be the use of reflectance spectra (spectrophotometer combined with an integrating sphere) in the near future. Reflectance spectra are increasingly used to assess the plant's physiological status (Penuelas and Filella, 1998) with the appearance of fast and cheap spectrophotometers. Polder et al. (2002) showed that spectral images offer more discriminating power than standard RGB images for measuring ripeness stages of tomatoes and Merzlyak et al. (2003) linked spectral reflectance spectra of several apple cultivars to chlorophylls, carotenoids and anthocyanins. The benefit is the recording of all colour components with the spectrophotometer simultaneously. However, it is quite difficult to interpret the multitude of peak patterns, as indicated by the ad-hoc approach applied by Merzlyak et al. (2003).

Bottleneck: modelling

Models may be used to translate knowledge into colour applications. The most basic ones are classification models that use simple rules for e.g. colour grading at auctions or neural networks to grade fruit colour images into quality classes (Nakano, 1997). A somewhat more advanced type of model focuses on the colour of a product after a shelf-life period on the basis of the current colour. For instance, Jollife and Lin (1997) noticed that rapid elongation, high photochemical quenching of chlorophyll fluorescence and a high initial colour of cucumbers at harvest correlated with high (colour) shelf-life and developed a multiple linear regression model to predict shelf life. These approaches focus on classification of the current colour, not the expected colour when the consumer is reached; they lack any physiological knowledge and show often a poor performance.

A second type of colour models describes the colour development over time. Tijskens and Polderdijk (1996) noticed that, in literature, the decrease over time of a single quality attribute (e.g. colour) is frequently described by one of four following basic types of mechanisms: linear, Michaelis-Menten, exponential or logistic. Indeed, several articles apply one of these four types to describe the colour development over time. For instance, the green to yellow development of bananas has been described as a combination of a logistic and a linear function (Chen and Ramaswamy, 2002). Colour change of cooked potatoes was described using exponential kinetics (Nourian et al., 2003) and the green to red development of tomato fruits (Tijskens and Evelo, 1994) has been modelled using a logistic function. Often these (semi-) empirical models describe the colour development very well, but the model parameters have the strong tendency to

change per batch, per cultivar and per season. In other words, these models lack predictive power. The reason is that parameters belonging to these colour models are ad-hoc parameters unlinked to the underlying physiological processes.

A third type of models describes the actual processes underlying the phenomenon (e.g. colour changes in a product), rather than the phenomenon itself using a system of problem decomposition (Sloof, 2001). The rules for building these kinetic models are rooted in chemical dynamics and thermodynamics theory (Atkins, 1986). For instance, Van Boekel (2000) presents a model on chlorophyll degradation of fermented olives. Existing knowledge of chlorophyll degradation pathways was used to build a model that describes chlorophyll degradation as several simultaneously occurring processes. The parameters of the model are specific for the (bio-)chemical processes and therefore have the same value for each repetition. They should, therefore, be independent of batch or season when measured at constant temperature. This type of model has been used to describe physiological phenomena as diverse as respiration in fruits and vegetables (Hertog et al., 1998), temperature and pH effects of lipase and peroxidase activity in hazelnuts (Seyhan et al., 2002), activity of pectin methyl esterase during blanching of peaches (Tijskens et al., 1999) and the effect of pH on the colour degradation of blanched broccoli (Tijskens et al., 2001). So, kinetic modelling can be used to translate the vast knowledge available in literature into generic mathematical descriptions. Kinetic modelling might therefore be suitable to bridge the gap between research and application. Unfortunately, no kinetic models have been incorporated into (commercial) applications yet, with one notable exception: a recommendation for long term storage of potato batches (http://www.tolsma.com/advies.html). This recommendation is based on a kinetic model that describes storage behaviour of potato tubers in terms of accumulation of reducing sugars. The model was calibrated and validated applying long term storage experiments over a wide range of storage temperatures for several seasons and cultivars (Hertog et al., 1997).

So, one of the bottlenecks for transferring physiological knowledge into colour applications is the lack of kinetic colour models. Why are there no kinetic colour models for fresh produce? One reason might be the often insufficient knowledge about the colour processes during growth (Tijskens, 2003) in combination with a lack of understanding how harvest affects these processes. For instance, the reason why colour decay of harvested cucumbers sometimes starts quickly and sometimes starts much later, independent of the initial colour (right hand-side plot of Fig. 2.2) may be 'solved' for a postharvest researcher by pointing at likely preharvest factors. Differences in initial conditions are 'generated' during the preharvest phase by mainly differences in growth conditions. How growth conditions affect the postharvest colour behaviour quantitatively is, unfortunately, unknown. Preharvest research has focussed extensively on cultivation

techniques and dry matter production without focussing on how quality attributes, like colour, are generated during growth. Solving this bottleneck for application of colour research would require the generation of kinetic colour models that describe the impact of growth conditions, the effect of harvesting, and the effects of storage on colour generation and decay.

Bottleneck: batch variation

One of the areas for colour applications may be the assessment of large amounts of produce in terms of predicted internal or external quality attributes. Growers do not continually present products to the horticultural production chain, but do so batch-wise to optimise the labour distribution within their companies. In principle, a batch may contain all maturity shades possible without those that are considered immature (will be harvested later) and those considered over mature (already harvested). Interestingly, there are indications that this is not the case. For instance, the right hand-side plot of Fig. 2.2 shows different types of colour development for cucumbers, either with a higher or lower initial colour or either with shorter or longer colour retention. Each type shows only two cucumbers but is representative of actual cucumber batches. Another example of batch variation concerns cucumber batches grown at the same time in one commercial greenhouse with standard growing and storage conditions. In the left hand-side plot of Fig 2.3 comparisons of batch keeping quality percentages are shown for always two batches (160 pairs) with the same genetic background (homozygous lines) but grown at two different locations within the greenhouse. The batch keeping quality percentage is a measure of postharvest colour retention at the batch level (Chapter 4). It might be expected that batches from the same genetic background have similar postharvest behaviour. On the other hand, distinct differences in batch keeping quality percentages are also visible (left hand-side plot of Fig 2.3). This indicates that even within a greenhouse, batch variation is at work due to perhaps differences in temperature and sunshine profiles. However, not only the genetic background and the growth location within the greenhouse are of importance. For instance, the right hand-side plot of Fig. 2.3 shows a comparison of the batch keeping quality percentages for always two batches (20 pairs) with the same genetic background harvested from stem and vine parts of cucumber plants. In this case batch variation is also clearly visible: vine batches have a smaller keeping quality range than stem batches. These examples indicate that batches may be regarded as unique fingerprints of past (preharvest) experiences.

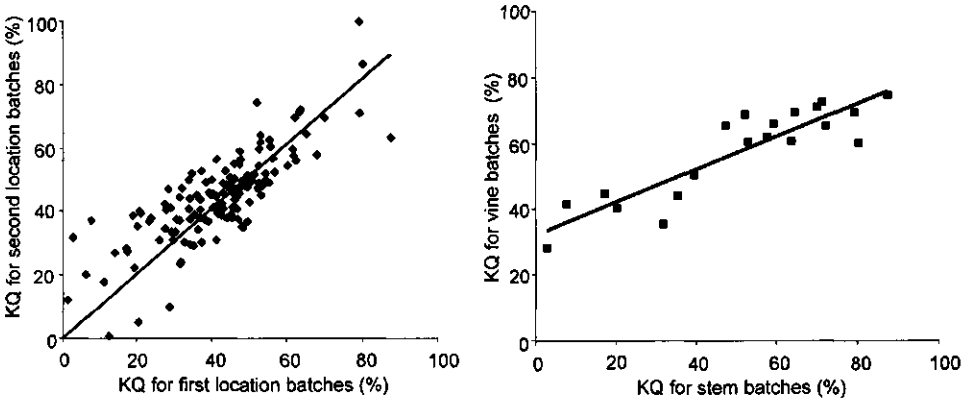

Fig. 2.3. The left hand-side plot shows the comparison of the keeping quality (KQ) percentages for batches grown at two different locations in one greenhouse that have an identical genetic background (160 homozygous lines). The right hand-side plot shows the comparison of keeping quality percentages harvested from the stem or vine part of cucumber plants for twenty batches that have an identical genetic background (20 homozygous lines).

A way to deal with the large amounts of data, for instance colour measurements from all individuals of a batch would be to deal with them as one entity. To do so, the colour measurements could be recorded and expressed as a colour histogram, for instance like the left hand-side plot of Fig. 2.4. Although the *shape* of the histogram can be 'guestimated' as fairly normal , measuring a larger portion of the batch would result in a better estimation (Fig. 2.4, middle plot). If enough colour measurements are carried out, it might be concluded that colour for this batch is normally distributed. This means all colour measurements may be expressed mathematically applying the normal (Gaussian) distribution (http://mathworld.wolfram.com/NormalDistribution.html) transforming the discrete histogram representation into a continuous batch probability distribution. The Gaussian distribution is widely used to describe histograms, even when the shape of the histogram does not appear to be normal by declaring that the sample size is apparently too small. In that case standard robust statistical techniques such as ANOVA can be applied. When the shape of a histogram is clearly not normal, it is common practise to use the binomial or the Poisson probability distributions (http://mathworld.wolfram.com/BinomialDistribution.html, http://mathworld.wolfram.com/PoissonDistribution.html). At first instance, it appears that the Gaussian, binomial and Poisson probability distributions may be suited to describe actual colour of fruits and vegetables batch. Let's assume that that colour development over time of all individuals in a batch can be approximated by an exponential or logistic mechanism (Tijskens and Polderdijk, 1996). Because the cumulative binomial distribution resembles a logistic function, logistic development of all individuals in a batch is often coupled to the binomial distribution. Similarly, exponential

behaviour of individuals of a batch may be linked to the Poisson distribution (Tijskens, 2003). As discussed earlier (see Bottleneck: modelling), colour can often be described by exponential or logistic mechanisms. Subsequently, the binomial and Poisson probability distributions will often 'fit' well to batch behaviour of fruits and vegetables. However, the binomial and Poisson distributions are discrete distributions that are not suited to describe continuous measurements. Also, the mechanisms have no physiological meaning (see Bottleneck: modelling) and therefore neither do these probability distributions. Each specific combination of colour processes working in all individuals of a batch will determine not only the kinetics but also the type, shape and dynamic behaviour of the colour batch distribution. In other words: each kinetic model will have a unique distribution.

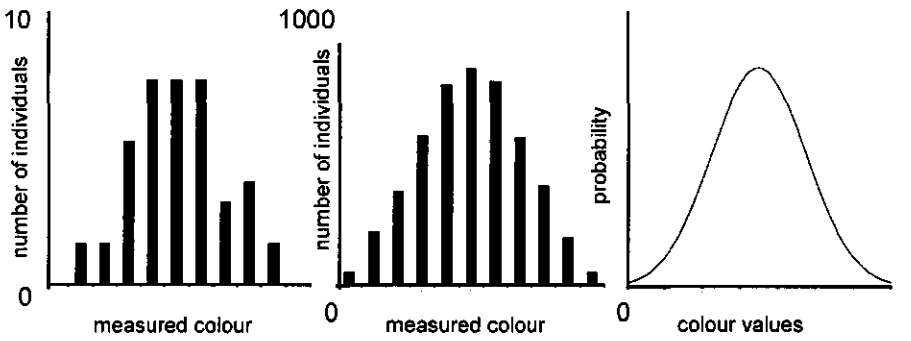

Fig. 2.4. Example of the expression of colour measurements as histograms from a medium sized (left hand-side plot) and a large (middle plot) sample of a batch. The right-hand side plot shows the probability density function for the entire batch if it is assumed that the colour measurements are normally distributed.

A kinetic model describing behaviour of individuals during preharvest and postharvest is necessary for building physiological batch models. However, this is not the only prerequisite. A second condition is that differences between individuals must be attributed to circumstances that cause variation in growth within each batch. For instance, the biological cause for colour variation in apples might be, amongst others, the variability in sun exposure due to position of apples at the tree (see Chapter 1). In principle, the combination of the distribution of the apples in the trees combined with a kinetic colour model should result in an apple batch model. This batch model might describe colour distributions of apple batches differing in e.g. preharvest light conditions, storage times and storage temperatures. In general, variation in light conditions during preharvest may seem a good candidate as a source of biological variation. However, (micro-)climate,

position in the truss (bananas, truss tomatoes) and nutrient levels might also be considered sources of biological variation. Combining the source(s) of biological variation and kinetic colour models to a batch model requires a methodology that is currently unavailable. This bottleneck may well be solved by stochastics, the mathematical subfield consisting of probability and statistics, and result in the generation of generic statistical procedures for the creation of batch models.

So, interpretation of batch variation in terms of predicted internal or external quality attributes is severely restricted right now by both the lack of kinetic colour models *and* the absence of the methodology to translate these kinetic models into colour batch models. Solving both bottlenecks will require a significant effort, but may also generate a very significant benefit in terms of translating physiological knowledge into colour applications.

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3

Keeping quality of cucumber fruits predicted by the biological age

Rob E. Schouten, Els C. Otma, Olaf van Kooten and L.M.M. Tijskens
Postharv. Biol. Technol. 12, 175-181, 1997.

Abstract

In the absence of defects, colour is one of the few practical criteria for assessing cucumber (*Cucumis sativus* L.) quality. However, cucumbers with the same colour at harvest can exhibit large differences in quality upon reaching the consumer. Photochemical yield (Φ_{PSII}), photochemical quenching (q_P) and a photosystem I evaluation (k_e), as internal quality measurements, were used in combination with an external quality measurement, represented by the colour, to test if the keeping quality could be predicted on basis of these measurements. Keeping quality is defined as the time for a cucumber to reach a certain predefined colour limit. To test the hypothesis, colour changes for 2000 cucumbers were measured during storage at 20 °C and 100% RH. The colour data were fitted to a model where a correction for biological age (C_{ba}) was applied to each individual cucumber. The correction for the biological age could be linked to the measured Φ_{PSII}, q_P and k_e values. Statistical analyses resulted in values for the maximum (green) and minimum colour (yellow) for each cucumber. The minimum colour could be correlated with different cultivars and the maximum colour with plant growing conditions. After a suitable transformation by a neural network, C_{ba} could be estimated based on the values of Φ_{PSII}, q_P and k_e and the initial colour. Predictions on the keeping quality by the model resulted in an explained variance of 74% (R^2_{adj}=0.74).

Introduction

In the absence of any visual defects, colour is one of the few practical criteria for assessing cucumber quality after harvest at present. A dark green cucumber is expected to have a longer shelf-life than a light green cucumber (Lin and Ehret, 1991). Longer shelf-life has been associated with high initial chlorophyll content in the peel (Lin and Jolliffe, 1995). However, cucumbers of the same colour at harvest can exhibit large differences in colour upon reaching the consumer. A number of pre- and post-harvest factors have been implicated (Anon., 1988).

In the Netherlands, cucumbers are classified into quality classes based on shape, uniformity, defects and colour. For export, a dark green cucumber with good colour retention is required. Keeping quality is currently determined by daily inspection of cucumber samples stored at 18 °C and 70% RH, but this is laborious and expensive. A more useful criterion for classifying cucumbers would be assessment of expected keeping quality, defined as the time taken from the initial colour to a predefined colour limit (Tijskens and Polderdijk, 1996).

A model was developed in which colour change could be treated mathematically as a decreasing enzyme. A central role in this model is the concept of the correction for 'biological age', i.e. a term describing the maturity of the fruit. This enables the description of the colour development of the cucumber in every initial maturity stage by a single equation. It was our intention to test, by analysis of photosynthetic performance of individual fruits, whether the maturity stage of individual fruits could be evaluated. This would enable the discrimination with regard to keeping quality between cucumbers having an identical colour at harvest. Photosynthetic performance is measured by non-destructive optical techniques.

Material and Methods

Cucumbers

Cucumber (*Cucumis sativus* L.) plants of three cultivars ('Enigma', 'Flamingo', and 'Jessica') were planted at the end of July and at the end of August 1995 at the experimental research station in Naaldwijk (PBG). The plants were grown hydroponically at a density of 1 (= low plant density) or 3 plants m^2 (= high plant density). Two nutrient solutions of 1.5 (= low EC) and 7 dS m^{-1} (= high EC) were applied (Janse, 1995). Cucumbers of

marketable size and colour were harvested once a week, transported to ATO within 2 h and stored in the dark at 20 °C and 100% RH. Date of harvest (= day 0) was recorded for each individual cucumber. More than 2000 cucumbers, harvested in 10 weeks, were monitored during storage.

Optical measurements

Three photosynthetic measurements were performed on day 1 after harvest, near the point, at the middle and near the neck position of individual cucumbers. Photochemical yield (Φ_{PSII}) and photochemical quenching (q_P) were measured with modulated chlorophyll fluorescence on a commercially available PAM-2000 (Walz, Effeltrich, Germany).

Φ_{PSII}, the photochemical quantum yield, is a measure for the efficiency of light usage for photosynthetic electron transport by photosystem II (Genty et al., 1989). Under non-photorespiratory conditions, a linear relationship exists between Φ_{PSII} and the quantum yield of CO_2 fixation (Genty and Harbinson, 1996). Photochemical quenching, q_P, is a direct measure of the fraction of open photosystem II reaction centres (Van Kooten and Snel, 1990). Cucumbers were adapted for 30 min. to a light intensity of 11 µmol m^{-2} s^{-1} before they were measured at an actinic light intensity of 40 µmol m^{-2} s^{-1}. During 4 min., Φ_{PSII} was measured every 30 s, utilising a saturating light pulse of 13000 µmol m^{-2} s^{-1} for a duration of 0.8 s. After the last pulse, a LED array emitting 710 nm light was switched on for 15 s for the measurement of F_0' (Van Kooten and Snel, 1990) and subsequent calculation of q_p.

The rate constant for photosynthetic electron transport, k_e, is measured by a light induced absorbance change around 820 nm. (ΔA_{820}). The ΔA_{820} measures the oxidation state of P700, the specialised chlorophyll reaction centre of photosystem I (Harbinson and Woodward, 1987; Schreiber et al., 1988). The rate limiting step for photosynthetic electron transport, which determines k_e, precedes P700. So, by measuring how quickly oxidised P700 is reduced by the photosynthetic electron chain in the light, it is possible to calculate the rate constant for photosynthetic electron transport k_e. This is done by transiently oxidising a portion of the P700 pool using a short (1 ms) flash and measuring its relaxation (Genty and Harbinson, 1996).

Colour measurements

Image analysis was used for the colour measurements. The system, developed at ATO, consists of a colour video camera (JVC KY-F30 3CCD) in a container with a controlled light environment, connected to a personal computer. After a measurement, the cucumber image is separated from the background, and the light intensities for the red, green and

blue colour are separately averaged over all the pixels that belong to the cucumber image. Colour was measured twice a week, starting on day 1, until yellowing was complete or until decay of the cucumber was imminent.

A simple visual scale on a card, showing predefined colour stages of the cucumber, (Central Bureau of Fruit and Vegetable Auctions in the Netherlands (CBT)) was used to calibrate the image analysis system. The colours on the card range from 1 (= light yellow) to 9 (= dark green). Four product experts jointly classified 94 cucumbers in 16 classes with values between 1 and 8.5 according to the colour card. The colour of those cucumbers was also measured by the image analysis system.

Data analysis

Statistical analysis of the colour data was performed by multiple nonlinear regression (Genstat 5, Rothamsted Experimental Station, Oxford, UK). The equations and mathematical description of the model were developed using MAPLE V (Waterloo Maple Software, Waterloo, Canada). A back propagation Neural Network (NeuralWare, Inc., Pittsburgh, USA) was used to improve the keeping quality predictions. To train the neural network for all the examples generated, ten-fold cross-validation was used. The optimal neural network for analysis of the photosynthetic measurements combined with the initial cucumber colour data contains an input layer with four units and one 'hidden' layer with two units. The optimal neural network for analysis of only the initial colour data contains one input layer with four units.

Results and discussion

Image analysis

To compare colour card ratings with image analysis system measurements a broad range of colours from fresh and stored cucumbers were obtained. A ratio of the blue to the red intensity accounted for 98.3% of variability (Fig. 3.1). The blue/red ratio is comparable with the ratio of Hunter values (a/b) (Thorne and Segurajauregui Alvarez, 1982).

A colour card value of 5 was chosen as a minimum for adequate keeping quality after a shelf-life period expressed in days unless microbial spoilage occurred.

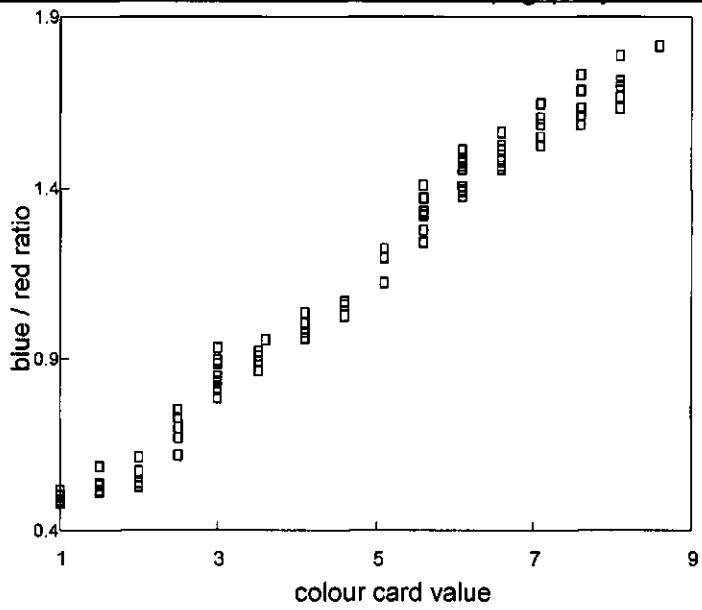

Fig. 3.1. The (linear) relationship between colour determination by image analysis or colour card. The colour values vary between very dark green (8.5) and light yellow (1).

Modelling

Based upon the observed sigmoid behaviour of the colour change over time, a simple model is proposed which results in the description of the colour data in terms of a logistic function. The model is based on the disappearance of the green colour (Gr) by an enzyme (Enz) which, in time, becomes more available (Eq. 3.1). The increase in this enzyme activity is considered to be a crude description of the process of senescence. The following kinetics can then be derived (Eq. 3.2).

$$Gr + Enz \rightarrow 2\ Enz \tag{3.1}$$

$$\frac{d}{dt} Gr = -k \cdot Gr \cdot Enz \tag{3.2}$$

The differential equation (3.2) can be solved by applying the mass conservation law to resolve the enzyme term and equation (3.3) is obtained after the addition of an offset term, Gr_{plus}.

$$Gr(t) = Gr_{plus} + \frac{Gr_{min} - Gr_{plus}}{C_{ba}\ e^{t \cdot k \cdot (Gr_{min} - Gr_{plus}) + 1}} \qquad (3.3)$$

with

$$C_{ba} = \frac{Gr_{min} - Gr_0}{Gr_0 - Gr_{plus}}$$

Gr_{min} represents the maximum (dark) green colour possible at minus infinite time, Gr_{plus} the maximum (light) yellow colour at infinite time. Gr_0 is the initial colour of the cucumber at t=0; i.e. the time when the photosynthetic parameters are measured. Equation 3.3 closely resembles the equation used by Tijskens and Evelo (1994) to fit colour data from tomatoes. Here the same principle of correction for biological age (C_{ba}) is used to describe the colour development in every initial maturity stage by the same equation. C_{ba} is the dimensionless ratio between the covered and the not covered trajectory of the curve which gives an indication about the maturity stage by means of the difference between Gr_{min} and Gr_0. The general pattern of the logistic function and the bearing of the function parameters are shown in Fig. 3.2.

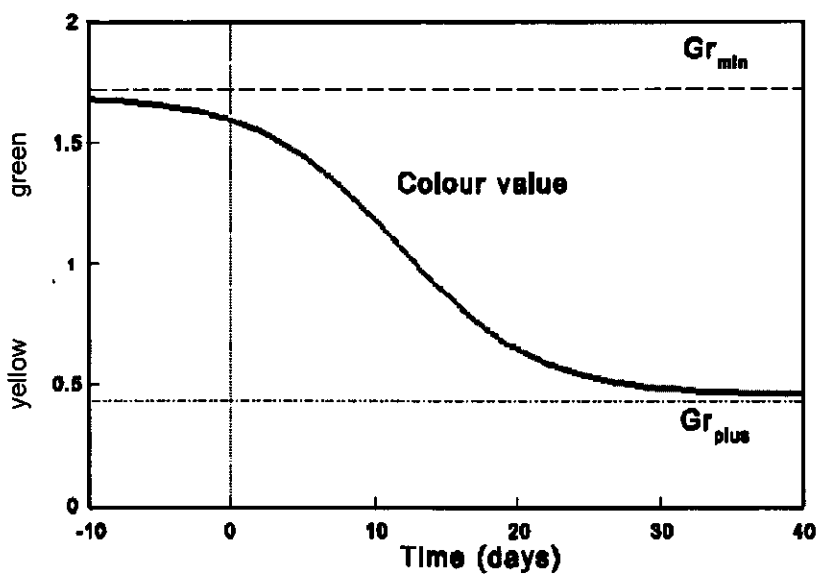

Fig. 3.2. Typical logistic curve for description of colour development, showing infinite colour limits: Gr_{min} (maximum green colour), Gr_{plus} (maximum yellow colour).

Application of the model

Although the vast majority of the cucumbers passed the colour limit without spoilage, data from only about 500 cucumbers were suitable to define the sigmoid curve, especially Gr_{plus}, with sufficient accuracy. To get a rough idea of the model behaviour and the parameters in the iterative nonlinear regression analysis, the colour data were first analysed for each cucumber separately. The blue/red ratio was used, without transformation, for Gr(t) in equation 3.3. Initial estimates for the parameters Gr_{min}, Gr_{plus} and k were found by applying the standard Genstat sigmoid curve. For Gr_0, the first colour measurement (day 1) was used. The explained variance (R^2_{adj}) was very high, between 95-100%, for each cucumber separately. However, the model presupposes an identical reaction rate (k) for the process of colour change over all maturity stages. When the same reaction rate was estimated in common for all cucumbers, similar values for R^2_{adj} were obtained. In Table 3.1, the cumulative number of cucumbers ranked according to R^2_{adj}, are shown for the nonlinear regression analysis with a common reaction rate. It is clear that the colour development for cucumbers can be described with a common reaction rate in a logistic function (Table 3.1).

R^2_{adj}	number of cucumbers with common reaction rate	common reaction rate and Gr_{plus}
>99%	421	388
>98%	490	479
>97%	501	491
>96%	501	498
<96%	3	6

Table 3.1. Cumulative number of cucumbers fitted with a common k, (first column) or with a common k and a common Gr_{plus} (second column) ranked according to R^2_{adj} in the non-linear regression analysis.

Correlations were found between the different parameters from the nonlinear regression and cultivar, plant density and plant nutrients conditions by analysis of variance. Gr_{min} could be correlated to plant nutrients and plant density (Table 3.2). This indicates that the most favourable growing conditions for the cucumber plants (low plant density, high EC)

were concomitant with the largest value of Gr_{min}. The less favourable growing conditions (high plant density, low EC) were concomitant with the smallest value of Gr_{min}. Gr_{plus} could be correlated with the different cultivars (Table 3.3). This indicates that after the disappearance of the green colour cultivar specific combinations of yellow colour components were present.

Gr_{min}	Low plant density	High plant density
Low EC	1.641	1.596
high EC	1.770	1.703

Table 3.2. Values for Gr_{min}, expressed as colour card value, from the nonlinear regression analysis with a common reaction rate for different growing conditions.

Cultivar	Gr_{plus}
'Enigma'	0.465
'Flamingo'	0.475
'Jessica'	0.499

Table 3.3. Values for Gr_{plus}, expressed as colour card value, from the nonlinear regression analysis with a common reaction rate for different cultivars.

However, the actual differences in Gr_{plus} between the cultivars were very small. Therefore a common Gr_{plus} for all cucumbers was applied. Multiple nonlinear regression, now with a common value for both k and Gr_{plus} was performed. The calculated values for R^2_{adj}, given in the second column of Table 3.1, were again high. This indicates that all biological variation in the cucumbers can be attributed to Gr_{min}. This also means that the process of colour change in cucumbers can be predicted, provided a reliable method of assessing Gr_{min} is available and provided Gr_0 is measured with sufficient accuracy.

Incorporation of optical measurements

If the three photosynthetic parameter measurements (Φ_{PSII}, q_P and k_e) could be used to describe the process of colour change, then the prediction of the keeping quality depends only on the colour limit. Generally, Φ_{PSII}, q_p and k_e provide an indication of the state of the energy metabolism of the cucumber. It can be assumed that this state of energy metabolism corresponds to the biological age of the detached fruit. Multiple linear regression analysis of the logarithm of C_{ba} (dependent variable) against Φ_{PSII}, q_p and k_e for all three measurements on the cucumber, combined with Gr_0, (10 independent variables) resulted in a correlation with an explained variance of 71%. Omitting one of the photosynthetic parameters meant a considerable loss of correlation. Applying a neural network to predict the logarithm of C_{ba} on the basis of photosynthetic parameters and Gr_0 increased R^2_{adj} to 77%. By predicting C_{ba} with this neural network and assuming a constant k and Gr_{plus} common to all maturity stages and cultivars, the keeping quality could be predicted by the model. This resulted in a value for R^2_{adj} of 74% between the predicted and the graphically determined keeping quality (Fig. 3.3). If the applied neural network was solely based on the initial colour data, the calculated R^2_{adj} between the predicted and the graphically determined keeping quality was only 53%. This is somewhat comparable to the correlation (R^2= 23%) between the initial colour at harvest and the shelf- life reported by Lin and Ehret (1991).

For the prediction of C_{ba} the photosynthetic parameters alone were not sufficient and Gr_0 had to be included. A correlation between Gr_0 and the logarithm of C_{ba} was found with a value for R^2_{adj} of 55%. This means that, in general, a dark green cucumber will be younger (lesser biological age) than a light green cucumber. This is consistent with the view of Lin and Ehret (1991) who proposed that factors inducing rapid fruit growth (low plant density, high EC), would result in younger fruit at harvest. On the other hand, this low value for R^2_{adj} indicates a large biological variation. This biological variation could be captured, apparently rather well, by including the photosynthetic parameter measurements to the prediction of C_{ba}.

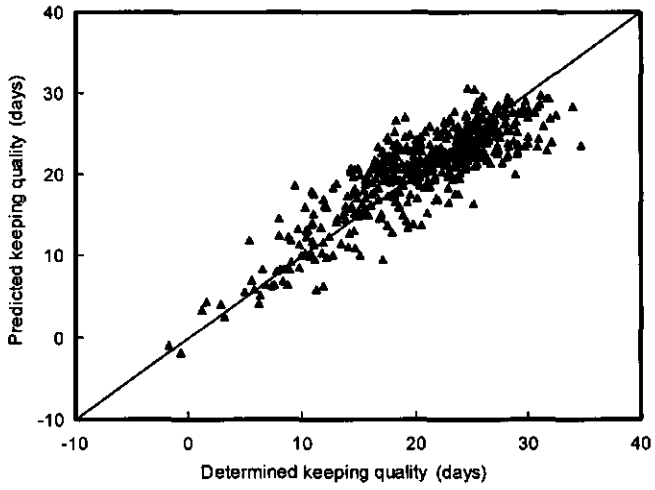

Fig. 3.3. Scatterplot of the graphically determined keeping quality versus predicted keeping quality. Accounted variability is 74.2% for a total of 504 cucumbers. The standard error of the Y estimate is 3.17 and the X coefficient is 0.979 ± 0.007.

Prediction of quality classes

To illustrate the difference between keeping quality predictions based on initial colour and photosynthetic measurements and those based on initial colour measurements alone, all cucumbers were classified into 4 classes of keeping quality. This classification is based on the graphically determined keeping quality for each cucumber. Neural network calculations were applied to predict the number of cucumbers that belong in each quality class on the basis of initial colour alone (colour-NN) or with photosynthetic measurements included (photosynthetic-NN). From Fig. 3.4 it is clear that the number of correctly predicted cucumbers in each keeping quality class for the photosynthetic-NN is larger than the total number of wrongly predicted cucumbers in that class. For the colour-NN, this is observed only for one keeping quality class (>21 and <28 days). Also, for the colour-NN a small amount of cucumbers deviated more than one keeping quality from the correct keeping class. This is not the case for the photosynthetic-NN. So, in contrast to the present practice, prediction of keeping quality on the basis of initial colour alone is not reliable, but in combination with photosynthetic measurements such predictions are feasible.

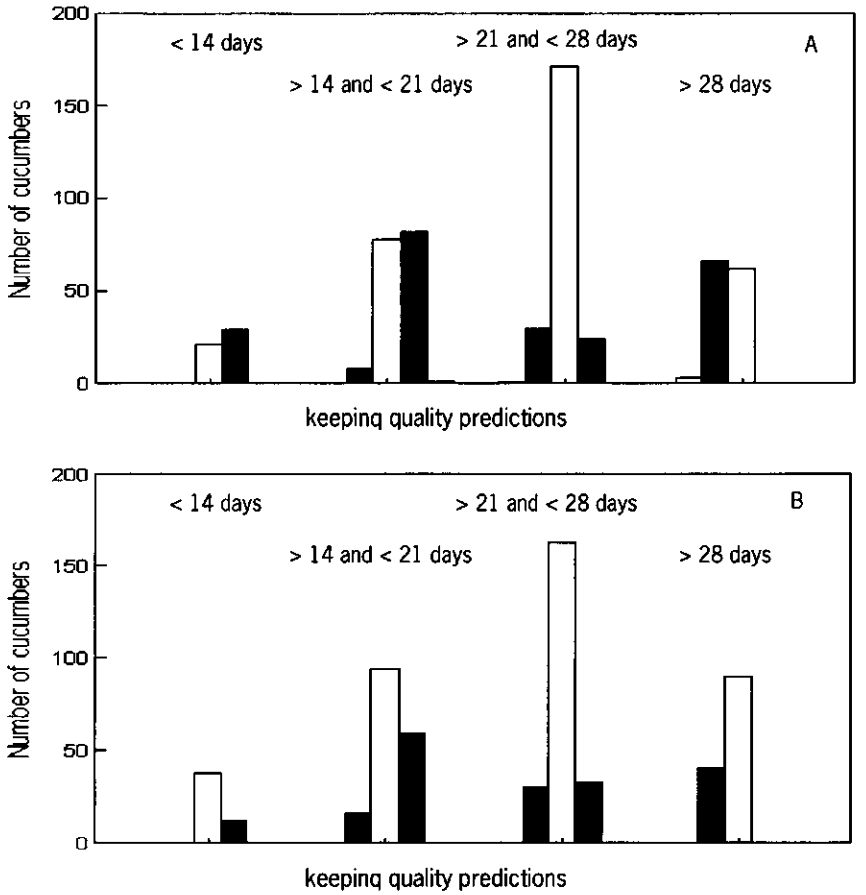

Fig. 3.4. Predictions on the number of cucumbers in each cucumber keeping quality class by the colour neural network (Fig. 4A) or by the photosynthetic neural network (Fig. 4B). Correctly predicted cucumbers in each keeping quality class are denoted by open bars. Wrongly predicted cucumbers for each class (closed bars) could belong to class(es) with either longer or shorter keeping quality.

Conclusions

Colour development can be described by a logistic function and a common reaction rate and a common value for the final cucumber colour after storage for all maturity stages and cultivars tested. The initial cucumber colour is related to the growing conditions (plant density and plant nutrients) of the cucumber plant. By applying the concept of correction for biological age, colour development can be described independent of maturity of the

cucumber. The correction for biological age could be assessed by combining an accurate initial colour measurement and three photosynthetic parameter measurements (Φ_{PSII}, q_P and k_e), making predictions on the colour development possible. By defining the keeping quality of a cucumber as the time to reach a colour limit, predictions on keeping quality could be made. The keeping quality could be predicted with a calculated R^2_{adj} of 74% for cucumbers differing in cultivar and growing conditions.

Acknowledgements

This study is supported by the Central Bureau of Fruit and Vegetable Auctions in the Netherlands (CBT). We would like to thank Jan Janse of the experimental research station in Naaldwijk (PBG) for the production of the cucumbers at rigidly defined conditions. Furthermore, the contribution of Roger Willems, responsible for the development of a versatile colour image analysis system, is acknowledged.

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4

Keeping quality of cucumber batches: is it predictable?

Rob E. Schouten and Olaf van Kooten
Acta Horticulturae 476, 349-355 (1998)

Abstract

The prediction of the keeping quality for a cucumber, the time the colour remains acceptable, is until now not possible because of the unknown stage of maturity of the fruit. This is a fundamental problem common to all products that have a decrease of the limiting quality attribute according to the autocatalytic mechanism. For individual cucumbers this problem remains unsolved, but on a batch level an approach to obtain the stage of maturity of a batch is described. The stage of maturity of a batch of cucumbers can be obtained by observing the skewness of the colour distribution. This is demonstrated for four batches differing in growing conditions. The colour distributions obtained from high nutrient density treatments indicate an early stage of maturity because of the typical skewness. The approach to predict the stage of maturity from colour distributions was extended to predict the batch keeping quality, the number of days for which 95% of a batch has an acceptable colour. For four growing conditions the batch keeping quality for cucumbers was calculated by fitting colour data to a model that allows for biological variation between cucumbers. The hypothesis is that characterisation of the colour distribution provides sufficient information to specify the batch keeping quality. This is under further investigation.

Introduction

A generally accepted definition of keeping quality is the time a commodity remains acceptable. In a constant environment, with a given value of initial quality and of a quality limit, the first attribute to become unacceptable will always be the same. The four most common mechanisms of decrease of the quality attribute encountered are (Tijskens and Polderdijk, 1996):

a) zero order reactions having linear kinetics
b) Michaelis-Menten kinetics
c) first order reactions having exponential kinetics
d) autocatalytic reactions having logistic kinetics

The keeping quality in a constant environment can be represented as $f(Q)/k$ with k as the reaction rate of the process of quality decrease. f(Q) contains the initial quality (Q_0) and the quality limit (Q_l). It depends on the mechanism of the process of quality decrease of the specific attribute (Table 1 from Tijskens and Polderdijk, 1996). For a number of products, measurement of the initial quality (Q_0) is sufficient to predict the keeping quality when values for k are available. These are products with the limiting quality attribute decreasing according to the linear/Michaelis-Menten and exponential mechanism (Table 4.1). Unfortunately, this is not the case for products decreasing according to the autocatalytical mechanism because no information can be obtained about Q_{min}, the maximally possible quality at minus infinite time (Table 4.1). Therefore, an attempt to predict the keeping quality of a product with a limiting quality attribute decreasing according to this mechanism should start with a method of evaluating Q_{min}.

For cucumbers the limiting quality attribute is colour. Stored at 20 °C this is the case for 85% of all cucumbers (unpublished data). An approach is described to define, describe and predict the keeping quality of batches of cucumbers.

The definition of the batch keeping quality is derived from the practical classification routines used at auctions in the Netherlands. From a large batch, often comprising over thousand cucumbers, four boxes of twelve cucumbers are randomly selected and judged on colour. When more than two cucumbers (~ 4.2%) have a colour considered too yellow, the whole batch is considered unacceptable. Because of this the batch keeping quality can be defined as the number of days for which 95% of a batch has an acceptable colour.

kinetic mechanism	f(Q)
linear/Michaelis-Menten[a]	Q_0-Q_L
exponential	$\ln(Q_0-Q_L)$
autocatalytic[b]	$Ln(Q_{min}-Q_L)/Q_L \cdot Cba$
	$Cba = (Q_{min}-Q_0)/Q_0$

[a] If the amount of substrate is much larger than the specificity facor K_m, which is probably the case intial region of decay then Michaelis-Menten reduces to a linear mechanism.

[b] Q_{min} represents the maximum quality at minus infinite time.

Table 4.1. Overview of respective quality functions.

Description of the batch keeping quality is accomplished by fitting colour data to a model that assumes a colour change from green to yellow according to a logistic function. Logistic behaviour is the consequence of the autocatalytic mechanism of quality decrease. During the fitting procedure, some of the model parameters are allowed to vary for individual cucumbers while others are assumed to be specific for a given growing condition and cultivar. This system allows the individual cucumber, the growing condition and the cultivar to have their specific effects on the batch keeping quality.

For the prediction of the batch quality, a measure of Q_{min} of the batch is necessary. While information about Q_{min} for individual cucumbers could not be obtained, it is assumed that information regarding the distribution of Q_{min} can be obtained from the skewness of the colour distribution for batches of cucumbers. The difference between the colour distribution and the distribution of Q_{min} is regarded as a measure of the stage of maturity of the batch. Hypothesis is that the colour distribution contains sufficient information, like the skewness, to predict the batch keeping quality. This would allow discrimination with regard to batch keeping quality on the basis of very simple measurements, namely the colour of each cucumber in the batch.

Material & Methods

Cucumbers

Cucumber (*Cucumis sativus* L.) plants of three cultivars ('Enigma', 'Flamingo', and 'Jessica') were planted at the end of July and at the end of August 1995 at the experimental research station in Naaldwijk (PBG). The plants were grown hydroponically at a density of 1 (= low plant density) or 3 plants m^{-2} (= high plant density). Two nutrient solutions of 1.5 (= low EC) and 7 dS m^{-1} (= high EC) were applied (Janse, 1995). Cucumbers of marketable size and colour were harvested once a week, transported to ATO within 2 h and stored in the dark at 20 °C and 100% RH. Date of harvest (=day -1) was recorded for each individual cucumber. More than 800 cucumbers, harvested in 9 weeks, were monitored during storage.

Colour measurements

Image analysis was used for the colour measurements. The system is developed at ATO and consists of a colour video camera (JVC KY-F30 3CCD) in a container with a controlled light environment, connected to a personal computer. During a measurement, the cucumber image is separated from the background and the light intensities for the red and blue colour are separately averaged over all the pixels that belong to the cucumber image. The ratio of the blue to red intensity has a very high correlation with a colour card, showing predefined colour stages of the cucumber (Central Bureau of Fruit and Vegetables in the Netherlands) and was used for cucumber colour assessment (Chapter 3). Colour was measured twice a week, starting on day 0. This was repeated until the process of yellowing finished, until bacterial spoilage was eminent or rubber necks (Janse, 1995) appeared.

Data analysis

Equations and mathematical description of the model were developed using MAPLE V (Waterloo Maple Software, Waterloo, Canada). Calculations of the batch keeping quality have been performed with MATLAB 4 (The Mathworks, Inc., Natick, USA). Distributions were fitted using Tablecurve 2.01 (Jandel Scientific, San Rafael, CA).

Results & Discussion

Colour distribution

Prediction of keeping quality for individual cucumbers on the basis of colour only is not possible because of the unknown Q_{min}. This also applies for cucumber batches, but now extra information is available. By not only measuring the colour but also noting the colour distribution additional information is available to characterise the batch keeping quality. It is assumed that the maturity of a batch can, indirectly, be measured by examining the colour distribution. The maximal green colour distribution (f_{max}) is assumed to be normally distributed. This can be regarded as the variation in chlorophyll content mainly due to growing conditions (Chapter 3). At harvest a batch of cucumbers with distribution f_0 will be available. This batch does not consist of cucumbers with a colour greener than is allowed by f_{max}, but can have cucumbers with a colour more yellow than is available in f_{max}. This can result in skewness of f_0 but only when f_0 and f_{max} are partly overlapping, thereby changing f_0 from normal to skewed (Fig. 4.1a). This happens when a batch is in an early stage of maturity. However, when a batch is in a later maturity stage then f_0 will not be limited by f_{max} and no skewness in f_0 will be encountered (Fig 4.1b). This behaviour of f_0 can be described by the binomial distribution and is a consequence of the autocatalytic mechanism. The binomial distribution, however, cannot be used for practical applications because f_0 is a mathematically continuous and not a discrete function. For practical applications a continuous function is needed, like the Gamma distribution. In Fig. 4.2 the f_0 colour distributions are shown for different treatments. The considerable skewness of f_0 for the high EC treatments indicates an early stage and the absence of the skewness for the low EC + low plant density indicates a later stage of maturity.

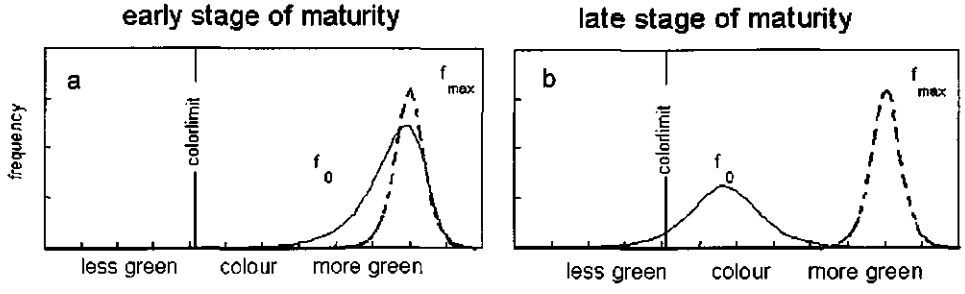

Fig. 4.1. Schematic effect on the shape of the colour distribution f_0, with a given constant maximally green distribution f_{max}, in case of an early (Fig. 4.1a) or a late stage of maturity (Fig. 4.1b) of a batch.

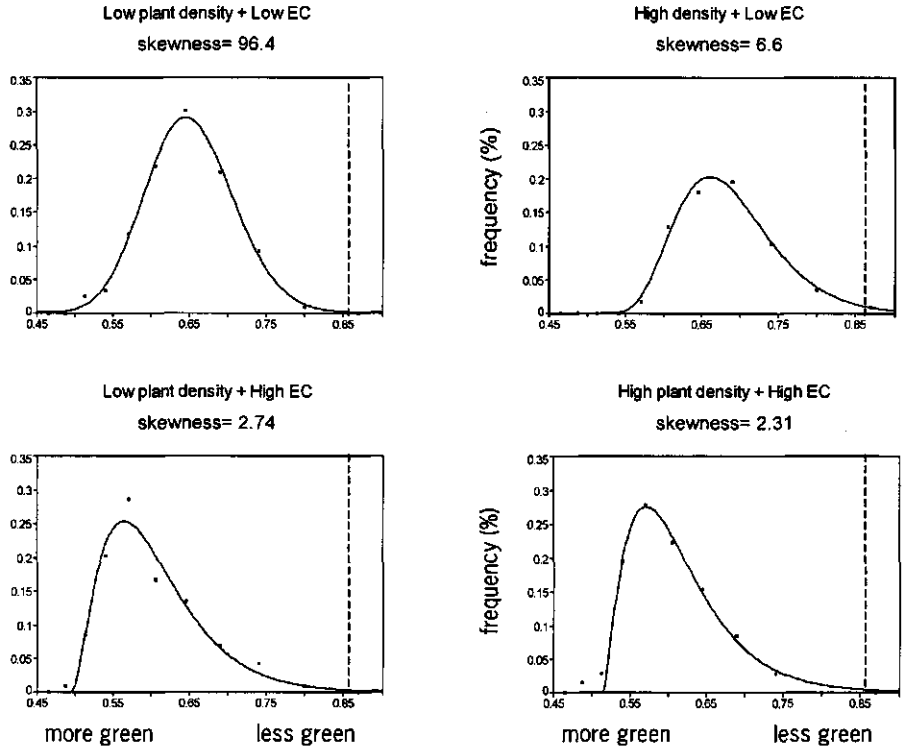

Fig. 4.2. Colour distributions for four different treatments. The colour limit (dashed line) is indicated. The distribution is fitted to the Gamma density function. Skewness, one of the characteristics of the Gamma density function, is indicated for each of the four treatments.

Model description

For the development of the model an approach is used in which both the random effects of biological variation between individual units and the fixed effects of treatment and cultivar are incorporated in one non-linear model (Wilkinson et al.,1997). The change of colour in time of cucumbers can be modelled adequately using a logistic function. The model is based on a simple degreening process where the green colour (Gr) is broken down by an enzyme system (Enz) which increases in time. The increase in enzyme activity is considered to be a crude description of the process of senescence. This results in the following kinetics, with reaction rate k, which can be solved for individual cucumbers (Eqs. 4.1 and 4.2).

$$Gr + Enz \rightarrow 2\,Enz \qquad (4.1)$$

$$Gr = Gr_{plus,c} + \frac{Gr_{min,c,gr,i} - Gr_{plus,c}}{1 + \frac{Gr_{min,c,gr,i} - Gr_{0,c,gr,i}}{Gr_{0,c,gr,i} - Gr_{plus,c}} e^{t\,k_{c,gr}(Gr_{min,c,gr,i} - Gr_{plus,c})}} \qquad (4.2)$$

Gr_{min} mathematically represents the maximum (dark) green colour possible at minus infinite time and practically represents the maximally possible chlorophyll concentration at the specific growing condition. Gr_{plus} mathematically represents the maximum (light) yellow colour at (plus) infinite time and practically represents the colour when no chlorophyll is left. Gr_0 is the colour of the cucumber at $t=0$.

From Fig. 4.3a, where the actual degreening process for several cucumbers from a batch of the cultivar 'Enigma' is shown, it is clear that the colour decreases towards a common asymptotic value. The variation in colour is described in the model (Fig. 4.3b, Eq. 4.2) by building in three sources of variation. First source of variation is the cultivar depending variation (index c) (Chapter 3). Second source of variation is caused by the growing condition (index gr). The rate constant k depends both on treatment and cultivar but does not vary with individual cucumbers. Third source of variation is the random individual variation (index i) for each cucumber due to the genetic variation and variation in preharvest conditions for cucumbers of the same treatment and cultivar.

The keeping quality of a batch can be defined as the number of days for which 95% of a batch still has an acceptable colour (Fig. 4.3). To estimate the keeping quality of a batch, all colour data are fitted to the model using non-linear regression. Data for all cucumbers of the same cultivar are fitted simultaneously as these cucumbers have at least one parameter in common, namely Gr_{plus}. Only 40 cucumbers per treatment were used in the fit procedure because of computational limitations. Cucumbers were randomly selected when they reached the colour limit without bacterial spoilage to have sufficient data for the last part of the degreening process. Estimations of model parameters are presented for cucumbers differing only in treatment, not in cultivar. The cultivar effect turned out to be small from earlier model calculations (Chapter 3). The blue/red ratio was used, without transformation, for Gr in equation 4.2. For Gr_0 the first colour measurement (day 0) was used.

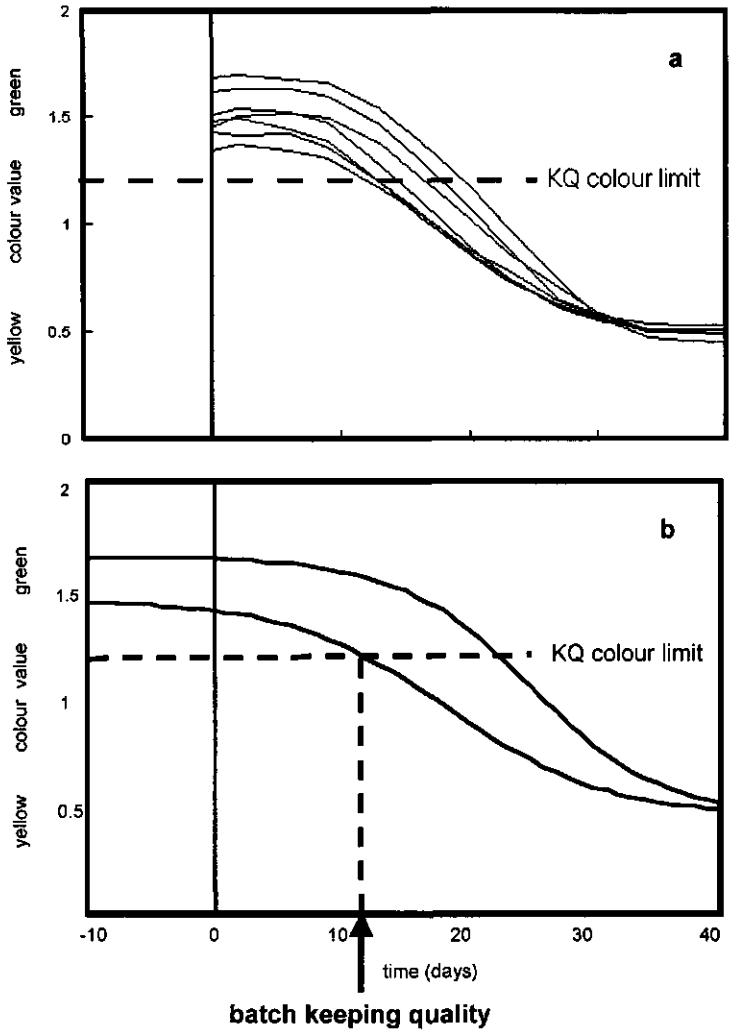

Fig. 4.3. The degreening process for a part of a real batch with indicated colour limit. In Fig. 4.3b the 90% confidence interval is showed. The batch keeping quality, the number of days for which 95% of the cucumbers of the batch still has an acceptable colour.

The statistics of parameter estimation is presented in Table 4.2. The reaction rate, k, is correlated with EC, the concentration of nutrient solution for the cucumber plant, indicating that a preharvest condition has a clear effect on the degreening process. Interestingly, a batch grown with a high value of EC results in a slow reaction rate of the degreening process (Table 4.2). A high value of EC also corresponds to a batch in an early stage of maturity having a colour distribution with considerable skewness (Fig. 4.2).

Probably, the reaction rate is an indication of the rate at which the colour distribution f_0 evolves from f_{max}. Therefore, younger fruit has probably a lower rate of yellowing. According to table 4.2 the most favourable growing condition (high EC, low plant density) has the highest- and the least favourable growing condition (low EC high plant density) has the lowest batch keeping quality. It is, however, not clear why the batch in the latter stage of maturity (Fig. 4.2) and the less favourable growing conditions (low EC, low plant density) has a batch keeping quality comparable to the batch in the early stage of maturity (Fig. 4.2) with the more favourable growing condition (high EC, low plant density), respectively 14.3 and 15.9 days (Table 4.2). Therefore, skewness of the colour distribution is not sufficient to describe the batch keeping quality. Hypothesis is that a colour distribution contains sufficient information to specify the batch keeping quality. It is assumed that the colour distribution can be described according to the Gamma distribution (Fig. 4.2). Parameterisation of the Gamma distribution enables the quantification of the colour distribution. It is likely that a suitable combination of parameters, such as a measure of skewness, median and a measure of standard deviation (Fig. 4.2) has a high correlation with the batch keeping quality.

treatment	low plant density/low EC	high plant density/low EC	low plant density/high EC	high plant density/ high EC
k	0.158	0.161	0.119	0.122
Gr_{plus}	0.458	0.468	0.434	0.449
batch KQ	14.3	8.4	18.3	15.9

Table 4.2. Overview of the parameter estimates. Results are shown for cucumber batches at different growing conditions. k depicts the reaction of the degreening process, Gr_{plus} is the maximum yellow colour, expressed as colour card value and batch KQ is the batch keeping quality expressed in days. All estimates differ significantly from each other.

This approach of predicting the batch keeping quality on the basis of colour measurements is not possible without the accuracy in colour measurement obtained with the image analysis system. Products with a limiting quality attribute according to the autocatalytic mechanism will benefit from this approach. Particularly, products with colour as quality limiting attribute, like tomatoes, (Tijskens and Evelo, 1994) should be suitable.

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5

Predicting keeping quality of batches of cucumber fruits based on a physiological mechanism

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Abstract

The keeping quality for a cucumber, defined as the time the colour remains acceptable to the consumer, depends on the state of the chlorophyll metabolism. By building a physiological model of the chlorophyll metabolism for cucumbers and using colour data from cucumbers stored at 12 °C, 20 °C and 28 °C, the parameters of the model were estimated with time and temperature simultaneously as explaining variables. Colour data were expressed as ratios of the separate intensities of the red, green and blue (RGB) values from a 3CCD digital camera, namely blue/red (B/R) and blue/green (B/G). The explained variance obtained was more than 93%. The model structure is generic in nature and describes the postharvest colour behaviour in time and temperature for cucumbers, irrespective of growing conditions and cultivar.

For six batches, from three cultivars over two growing seasons, the batch keeping quality, i.e. the time that 95% of all cucumbers in a batch had an acceptable colour, was obtained at 20 °C. A batch was defined as all cucumbers from one grower, one cultivar and one harvest. It is shown that, for batches grown in the spring season, high (colour) keeping quality related to high resistance against bacterial spoilage. Model parameters were estimated using either B/R or B/G colour data. Comparing the model parameters, it was deduced that the average initial colour measurement, expressed as the ratio of the red and green intensities (R/G), would be indicative for the batch keeping quality. It was possible to predict batch keeping quality, on the basis of initial colour measurements only, with percentage variance accounted for (R^2_{adj}) of 94%. As the basis of the batch keeping

quality predictions is a generic model, it might be possible to predict batch keeping quality for many other products which have green colour as the limiting quality attribute.

Introduction

Long shelf life of cucumbers has been associated with high chlorophyll content in the peel (Lin and Jolliffe, 1995). Predicting cucumber yellowing by using image analysis at harvest time and using a statistical multiple regression approach turned out to be unsatisfactory (Jolliffe and Lin, 1997). The basic problem is that cucumbers of the same colour at harvest can exhibit large differences in colour when reaching the consumer. Predicting the keeping quality for individual cucumbers, i.e. the time the colour remains acceptable to the consumer, is seriously hampered by the unknown biological age, i.e. maturity at harvest, of the fruit. An attempt to establish the biological age by combining colour measurements with non-destructive photochemical measurements was only moderately successful (Chapter 3).

The approach described in this paper focuses on the use of colour measurements only to predict keeping quality on the batch level. 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 (Chapter 4). The aim of this paper was to develop a colour model, based on physiological processes in the peel of cucumbers, that allows the prediction of keeping quality at batch level. The physiological processes used in the model are part of the chlorophyll pathway and specifically describe the build up and breakdown of colour components. Behaviour of colour components can be followed in time by repeatedly measuring the same cucumber with image analysis. As colour measurements are fast and non-destructive, predictions of batch keeping quality can easily be translated to practical solutions for the horticultural production chain.

Material and methods

Cucumbers

Two sets of cucumbers (*Cucumis Sativus* L.) were used. The first set consisted of eight batches of 45 cucumbers each from auction Bemmel (The Netherlands) harvested between the 6th of June and the 21st of August 1996. The cucumbers of cultivars 'Mustang', 'Tyria', 'Ventura' and 'Korinda' were of marketable size and colour. Cucumbers

were transported within 2h to the measuring facility and individually tagged at the lightest green side. Every batch was divided into three sub-batches of 15 cucumbers each, and stored in the dark at 100% RH at 12 °C, 20 C° and 28 °C, respectively.

The second set of 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 the 29th of September 1998 (autumn season) and three batches were harvested the 10th of 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. About 1/3 of the cucumbers from the autumn season suffered mechanical damage at the proximal position during transport and those were dipped (2 s) in a 0.05% chlorine solution on the day of arrival at the measuring facility to stop excessive bacterial spoilage.

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 Chapter 3. Colour measurements took place once (12 ºC), twice (20 ºC) and, thrice (28 ºC) per week. Colour measurements took place per cucumber and were expressed as the ratios of the blue/red (B/R) values, the blue/green (B/G) values and the red/green (R/G) values from the separate intensities of the red, green and blue (RGB) values. After a measurement the light intensities for the red, green and blue colour are separately averaged over all pixels that belong to the cucumber image.

Tijskens et al. (2001) found that expressing colour as the ratio –a*/b* in the CIE-Lab system reduced the variance in the data considerably. The a* and the b* value express colour of the 'green-red' and the 'blue-yellow' colour perception, respectively. The a* value probably relates to the R and G values in the RGB system while the b* value probably relates to the B value in the RGB system. Using the ratio -a*/b* in the CIE-Lab system as a kind of internal standardisation probably also applies to the ratios B/R and B/G in the RGB system.

Colour measurements started one day after arrival at the measuring facility and ended when yellowing was complete or decay of the cucumber was imminent. Only the darkest side of the cucumber was measured. Cucumbers were measured twice on the first measuring day (day 0) and once on all other measuring days. On day 0 the cucumber

was re-measured if the difference between the two colour measurements exceeded 0.5% of the mean value. This was done to avoid experimental outliers that could hamper the keeping quality predictions based on the colour measurements only.

Rot assessment

In strawberries, spoilage by fungal growth may occur as soon as the tissue gives the opportunity to do so (Hertog et al., 1999). When growing conditions are identical for batches of cucumbers, the occurrence of bacterial spoilage in a batch may be caused by low resistance against spoilage and not so much by the variation in the spoilage pressure between the batches.

When bacterial spoilage was observed during storage of cucumbers belonging to the Spanish data set, the day of occurrence, and whether rot at the proximal or distal position of the cucumber was encountered was recorded. Main criterion was whether the cucumbers were affected or not, rather than the degree. Affected cucumbers were removed.

Batch keeping quality

As colour is the first quality attribute to become unacceptable for the majority of cucumbers, the keeping quality can be defined as the time it takes for an individual cucumber to reach a predefined colour limit. On a colour card, used to grade cucumber at Dutch auctions, colours are shown ranging from 1 (light yellow) to 9 (very dark green). For a cucumber to be acceptable at an auction the colour should be above colour card value 5. A linear relationship was found between colour card and B/R measurements. The B/R colour limit is 1.202 (Chapter 3). Batch keeping quality can be defined as the time it takes before 5% of the cucumbers in a batch reaches the colour limit (Chapter 4).

Model development

Conceptual model

The colour model is based on knowledge obtained from literature regarding the processes of synthesis and degradation of chlorophyll in terms of colour compounds. Synthesis of

chlorophyll for cucumbers during storage is taken into account because an increase in the green colour was often observed during the first days of storage.

POR (protochlorophyllide oxidoreductase) is a photo-enzyme which catalyses the reduction from protochlorophyllide to chlorophyllide, the direct precursor of chlorophyll (Lebedev and Timko, 1998). The specific POR gene, por B, is expressed both in seedlings and in adult plants (Porra, 1997). For lightgrown cucumber seedlings it was found that the levels of POR mRNA decreased when placed in the dark (Kuroda et al., 1996). A ternary complex of POR B:NADPH:Pchl has been observed. For POR A, which is a form of POR that is only responsible for the initial greening of tissues, such complexes have been implicated to be a safe form of Pchl storage as to prevent photo-toxic events when illuminated (Porra, 1997). This concept might also exist for POR B as the sequence identity between POR A and POR B is quite high, 75% (Porra, 1997). Here the assumption is made that the amount of the complex POR B:NADPH:PChl formed during pre-harvest is restrictive for the amount of chlorophyllide formed during postharvest.

During senescence in fruits and vegetables chlorophyll a can be cleaved by chlorophyllase resulting in the formation of chlorophyllide, or chlorophyll a may loose its Mg^{2+} ion to form pheophytin. Both chlorophyllide and pheophytin will be converted into pheophorbide which will be turned into colourless compounds. The initial catabolite varies from commodity to commodity, but most likely the first pathway is predominant in fresh produce and the second path predominant in processed produce (Heaton and Marangoni, 1996).

From a modelling point of view, only compounds with colour and their precursors are of interest. Next to chlorophyll itself (blue green), this is chlorophyllide (blue green), pheophytin (olive brown) and pheophorbide (olive brown) and the colourless precursor protochlorophyllide. The colour of a cucumber will consist partly of the compounds pheophytin and pheophorbide. In this paper the assumption is made that the concentration of these compounds is fairly constant over time due to a steady state between generation from chlorophyll and decay to colourless compounds, resulting in a constant concentration level of these compounds over time. This may well be the case since an offset term had to be included in a previous model on the colour change of individual cucumbers (Chapter 3). This leaves only chlorophyllide, chlorophyll and the precursor protochlorophyllide to be considered in the model. Fig. 5.1 shows the proposed mechanism for these compounds during synthesis and breakdown of chlorophyll (CHL) under the assumption that all chlorophyll is broken down to chlorophyllide. Chlorophyllide (chl) holds a special position as it is an intermediate in both synthesis and breakdown. The initial amount of Pchl, as part of the ternary complex POR B:NADPH:PChl, (Pchl) is depicted as crucial and governing the colour behaviour.

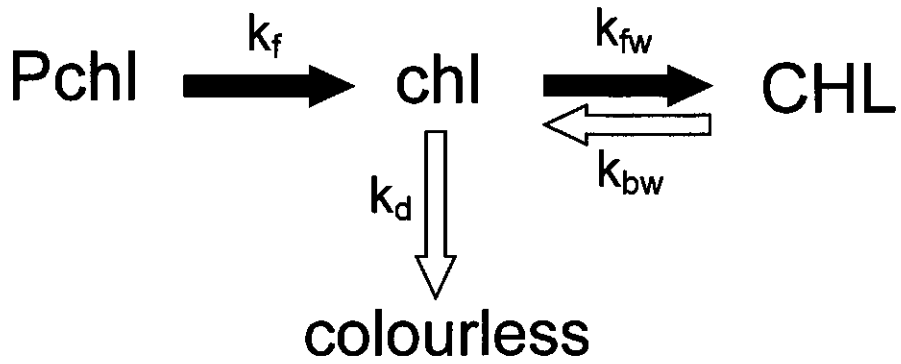

Fig. 5.1. Model representation of the last part of the chlorophyll pathway for cucumbers stored in the dark based on combined literature sources. Closed arrows are used to indicate chlorophyll synthesis and open arrows are used for the chlorophyll catabolism. Pchl, chl and CHL stand for protochlorophyllide, chlorophyllide and chlorophyll, respectively. Indicated are the different reaction rate constants.

Mathematical model

From the mechanism, shown in Fig. 5.1, the colour changes over time can be extracted following the fundamental rules of chemical kinetics. The set of differential equations is given in Eq. 5.1-5.3.

$$\frac{dPchl}{dt} = -k_f \cdot Pchl \tag{5.1}$$

$$\frac{dCHL}{dt} = k_{fw} \cdot chl - k_{bw} \cdot CHL \tag{5.2}$$

$$\frac{dchl}{dt} = k_f \cdot Pchl - k_{fw} \cdot chl + k_{bw} \cdot CHL - k_d \cdot chl \tag{5.3}$$

where k_f, k_{fw}, k_{bw} and k_d are the reaction rate constants for the formation of chl, the formation of CHL, the decay of CHL and the decay of chl, respectively. This set of differential equations can be solved analytically for constant external conditions, but this will yield enormously wieldy expressions, which are not really suitable for statistical analysis. Assuming, however, that chl is in constant steady state with Pchl and CHL (Eq. 5.4) the set of differential equations can be solved into a more condensed form (Eq. 5.5-5.6):

$$chl(t) = \frac{k_f \cdot Pchl(t) + k_{bw} \cdot CHL(t)}{k_{fw} + k_d} \tag{5.4}$$

$$Pchl(t) = Pchl_0 \cdot e^{-k_f \cdot t} \tag{5.5}$$

$$CHL(t) = Pchl_0 \cdot \frac{k_f \cdot k_{fw} \cdot \left(-e^{-k_f \cdot t} + e^{-\left(\frac{k_{bw} \cdot k_d \cdot t}{k_{fw} + k_d} \right)} \right)}{k_f \cdot (k_{fw} + k_d) - k_{bw} \cdot k_d} + CHL_0 \cdot e^{-\left(\frac{k_{bw} \cdot k_d \cdot t}{k_{fw} + k_d} \right)} \tag{5.6}$$

where $Pchl_0$ and CHL_0 are the initial (t=0) concentrations of Pchl and CHL. The development of the green colour in cucumbers over time, COLOUR(t), can be attributed to the sum of the concentrations of CHL(t) and chl(t) as both have a blue green colour together with an offset term, $COLOUR_\infty$ (Eq. 5.7). $COLOUR_\infty$ describes the colour of the cucumber when all green colour components have vanished at infinite time and is probably composed of the yellow components pheophytin and pheophorbide. Finally, Eq. 5.4 to Eq. 5.6 can be substituted into Eq. 5.7 to obtain the green colour development of cucumbers in terms of $COLOUR_\infty$, $Pchl_0$ and CHL_0 concentrations (Eq. 5.8).

$$COLOUR(t) = COLOUR_\infty + CHL(t) + chl(t) \tag{5.7}$$

$$COLOUR(t) = COLOUR_\infty + CHL_0 \cdot \left(\frac{\left(k_f \cdot k_{fw}^2 + \left(-k_{bw} \cdot k_d + 2 \cdot k_f \cdot k_d \right) \cdot k_{fw} - k_{bw} \cdot k_d^2 + k_f \cdot k_d^2 \right) \cdot factor1}{factor2} \right) + Pchl_0 \cdot \left(\frac{k_f \cdot \left(-k_{fw}^2 + (-k_d + k_f) k_{fw} - k_{bw} \cdot k_d + k_f \cdot k_d \right) \cdot e^{-k_f \cdot t} + \left(k_{fw}^2 + k_{fw} \cdot k_d \right) \cdot k_f \cdot factor1}{factor2} \right)$$

with

$$factor1 = e^{\left(\frac{-k_{bw} \cdot k_d}{k_{fw} + k_d} \right) \cdot t}$$

$$factor2 = k_f \cdot k_{fw}^2 + \left(\left(-k_d - k_f \right) \cdot k_{bw} + 2 \cdot k_f \cdot k_d \right) \cdot k_{fw} + k_{bw}^2 \cdot k_d + \left(-k_f \cdot k_d - k_d^2 \right) \cdot k_{bw} + k_f \cdot k_d^2$$

(5.8)

Temperature dependence

Each of the mentioned reaction rate constants (k_f, k_{fw}, k_{bw} and k_d) depend on temperature, presumably according to Arrhenius' law (Eq 5.9):

$$k_i = k_{i,ref} \cdot e^{\frac{E_i}{R_{gas}} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T} \right)} \qquad (5.9)$$

with R_{gas} as the gas constant (8.314 J $mol^{-1}K^{-1}$). The parameter $k_{i,ref}$ stands for the reaction rate constant at the arbitrarily chosen reference temperature T_{ref} (K). The energy of activation E_i expresses the dependence of the reaction rate constant k_i on temperature, with i=f,fw,bw or d.

Statistical analysis

Experimental data on colour development were analysed statistically using the non-linear regression routine of Genstat 5 (release 3.2, Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The equations and mathematical description of the colour model were developed using Maple V (release 4, Waterloo Maple Software, Waterloo, Canada). The set of colour data of the Dutch cucumbers was analysed using the model formulation of Eq. 5.8 together with the temperature dependence according to the Arrhenius equation (Eq. 5.9). These colour data were analysed using temperature and time simultaneously as explaining variables estimating the initial conditions ($Pchl_0$ and CHL_0) per cucumber and the kinetic parameters (k_f, k_{bw}, k_{fw}, k_d, E_f, E_{bw}, E_{fw}, and E_d) per batch. The set of colour data of the Spanish cucumbers was analysed using the model formulation of Eq. 5.8 and the kinetic parameters obtained from the analysis of the Dutch data set to obtain estimates of $Pchl_0$ and CHL_0 per cucumber. The reference temperature for the Arrhenius equation (T_{ref}) was in all analyses 285 K (12 °C).

Results & discussion

Kinetic parameters for the colour model

The set of Dutch cucumbers, consisting of eight batches, was used to fit the colour data to obtain the kinetic parameters of the colour model. Due to memory limitations of Genstat only 21 fruits, i.e. seven fruits per storage temperature, from each batch could

be analysed simultaneously. Consequently, fruits longest in storage were chosen for inclusion in the analysis. B/R and B/G measurements from the separate intensities of the RGB system obtained from the 3CCD digital camera were used, without transformation, for COLOUR(t) in Eq. 5.8.

During preliminary analysis an almost identical value for $COLOUR_\infty$, using either B/R or B/G colour data, was estimated for all batches. An earlier modelling approach showed that the final cucumber colour was slightly dependent on cultivar, but could very well be taken as a constant (Chapter 3). Subsequent analysis was performed with a fixed value for $COLOUR_\infty$. Separate analysis of each of the eight batches showed a constant ratio between k_{fw} and k_{bw}, but actual values differed over batches. Therefore, k_{fw} was fixed at 1 with no energy of activation (E_{fw}=0) and k_{bw} and E_{bw} were estimated.

Separate analysis of each of the eight batches showed a high percentage variance accounted for (R^2_{adj}), on average more than 96%, using either B/R or B/G colour data. The analysis for the four batches of 'Mustang' revealed about the same variation in the parameters as was encountered in the analysis of the colour data for the two batches of 'Ventura' and the batches of 'Tyria' and 'Korinda' (data not shown). So, as far as the parameter values were concerned, no distinction based on cultivar could be made or had to be made to analyse the data.

The average values for the parameters from the separate analyses of eight batches are shown in Table 5.1. Using the average parameter values, the percentage variance accounted for (R^2_{adj}) was more than 93% per batch using either B/R or B/G colour data. Comparing the parameters estimated from the B/R and B/G colour data, no clear difference was visible for k_{bw} and E_{ss}. On the other hand, k_f has a larger value and k_d has a somewhat larger value using B/R compared to using B/G colour data.

The experimental result that some reaction rate constants depended on the type of absorbed light, as indicated by the parameters estimated using B/R and B/G colour data (Table 5.1), is probably indicative of the different types of chlorophyll, chlorophyll a and chlorophyll b, in the cucumber. B/R and B/G measurements might, next to chlorophyllide, primarily assess mixtures of chlorophyll a and chlorophyll b in different ratios. Both types of chlorophyll absorb light in the blue-green and the red part of the spectrum. However, the maximum absorption for chlorophyll b in the blue-green part is shifted towards the green part of the spectrum compared to chlorophyll a. The maximum absorption of chlorophyll b in the red part is also shifted towards the green part of the spectrum compared to chlorophyll a (Hader and Tevini, 1987). This might indicate that for a B/R measurement the mixture of chorophyll a and chlorophyll b contains more chlorophyll a and less chlorophyll b than for a B/G measurement.

Ratio	B/R		B/G	
parameter	value	s.e.	value	s.e.
$k_{bw,ref}$	0.1537	0.0175	0.1575	0.0138
$k_{d,ref}$	0.3744	0.0451	0.3269	0.03162
$k_{f,ref}$	0.04848	0.00577	0.03591	0.00306
E_{bw}	20535	4489	20635	3342
E_d	20868	4240	19887	3251
E_f	49834	4972	58829	3708
$COLOUR_\infty$	0.316		0.312	
T_{ref}	285 K	(12°C)		
R^2_{adj}	93.5%		95.0%	
N	1261		1261	

Table 5.1. Parameter estimates and their standard error (s.e.) for the analysis of the Dutch colour set. The average values of eight separate analyses, including four different cultivars, are shown.

Experimental and simulated data, i.e. applying the estimated parameters from Table 5.1 and the estimated initial concentrations of Pchl and CHL, for five arbitrary cucumbers per temperature from 'Mustang' are shown in Fig. 5.2. For a number of cucumbers, the synthesis was larger than the loss of colour compounds, as indicated by an increase during the early stages of storage. Other cucumbers are showing predominantly loss of colour components. Fig. 5.2 shows a good agreement between experimental data and model calculations. However, sometimes there is a lack of fit with regard to the initial rise in colour and the colour at the end of the storage period. This may be caused by the assumption that all chlorophyll is broken down by chlorophyllase instead of chlorophyll being also broken down to pheophytin (Heaton and Mahorini, 1996). Another reason for lack of fit at the end of storage period may be the emergence of dark areas on some of the cucumbers, the first signs of rot.

The biological age of a cucumber is reflected by the difference of the maximum green colour and the colour at harvest in a simple logistic model for colour loss (Chapter 3). In the current model, the biological age is reflected by the initial amount of Pchl. As the current colour model is based on a physiological colour mechanism and the colour model was analysed satisfactorily on colour data from different cucumber cultivars it may be

expected that the model is generic. This means that the colour model can be used for describing colour behaviour for other marketable cucumbers irrespective of their growing conditions and cultivar.

Determination of the keeping quality of batches

Batch keeping quality was determined for the batches of the Spanish set of cucumbers (Table 5.2). First the colour data were analysed with the colour model to estimate the values of $Pchl_0$ and CHL_0 per cucumber using the kinetic parameters from the Dutch set of cucumbers (Table 5.1). 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. Batches, grown in autumn, had a 3 to 5 days shorter batch keeping quality than batches of the same cultivar grown in spring. Generally longer batch keeping quality is observed in the spring season and this seemed to reflect the somewhat higher initial average B/R. However, when the standard deviation of initial B/R is taken into account this relation is obscured (Table 5.2) and keeping quality predictions based on B/R seem impossible. Batch keeping quality depended on season and cultivar, 'Borja' having the highest and 'Volcan' having the lowest keeping quality.

Cultivar	season	number of cucumbers	initial average colour	st. dev. initial colour	batch keeping quality (days)
Volcan	autumn	80	1.47	0.02	3.8
Borja	autumn	86	1.53	0.02	8.4
Beluga	autumn	86	1.44	0.01	4.3
Volcan	spring	97	1.48	0.02	6.8
Borja	spring	99	1.55	0.02	13.1
Beluga	spring	85	1.46	0.01	7.2

Table 5.2. Overview of the Spanish set of cucumbers. Colour is expressed as B/R with indicated standard deviation (st. dev.).

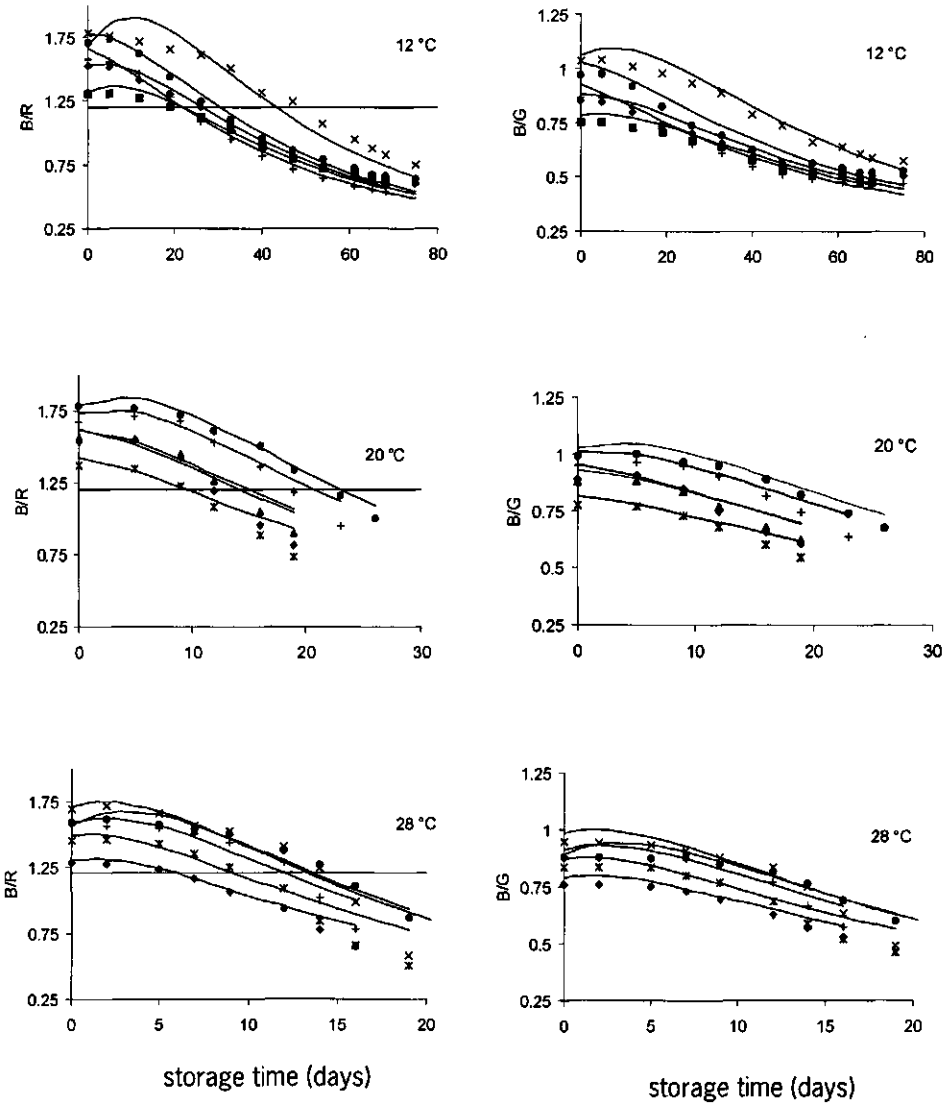

Fig. 5.2. Plots of fitted (solid lines) and experimental (symbols) colour changes during storage of five arbitrarily selected cucumbers of cultivar 'Mustang' at 12°C (top row), 20°C (middle row) and 28°C (bottom row). For plots on the left and right colour is expressed as B/R and B/G, respectively. The B/R colour limit is indicated.

Rot incidence

In Fig. 5.3 the spoilage behaviour of the three batches from the spring season of the Spanish data set are shown. Data collected for the autumn batches were neglected, since those cucumbers were treated with a chlorine solution. Spoilage occurs much more frequently at the proximal position than at the distal position of the cucumber, probably because of infections due to easy access of spoilage organisms when cut at harvest time. Batches of 'Borja' showed less spoilage both at the proximal and distal position than batches from other cultivars. Since batches of 'Borja' also showed a much higher batch keeping quality than the other two batches this suggests that batch keeping quality is also an indication of overall batch resistance to spoilage.

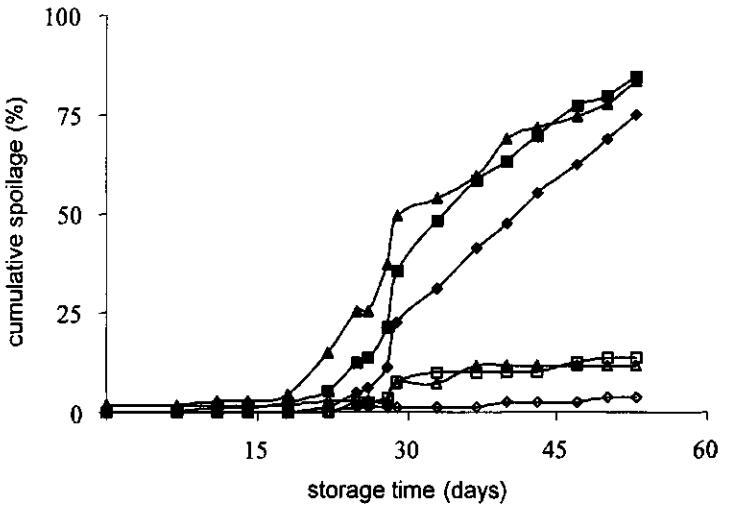

Fig. 5.3. Plot of the observed cumulative spoilage in time at 20°C for three cucumber batches of the spring season from the Spanish data set. Indicated is rot occurring at the proximal (closed symbols) and the distal (open symbols) position of the cucumber for the cultivars 'Volcan' (square), 'Beluga' (triangle) and 'Borja' (diamond).

Predicting batch keeping quality

Fig. 5.4 shows the estimates for CHL_0, $Pchl_0$ and chl_0 per season and per batch using either B/R ($CHL_{0\ BR}$, $Pchl_{0\ BR}$ and $chl_{0\ BR}$) or B/G colour data ($CHL_{0\ BG}$, $Pchl_{0\ BG}$ and $chl_{0\ BG}$) analysing the Spanish data set. The chl_0 values were obtained by subtracting CHL_0 and $COLOUR_\infty$ from $COLOUR_0$ (Eq. 5.7).

The linear relationship between the $Pchl_0$ values using either B/R or B/G colour data is striking (R^2_{adj}= 96% for all batches, Fig. 5.4, middle row). One might assume that both estimates of $Pchl_0$ are an assessment, differing in a calibration factor, of the concentration of POR B:NADPH:PChl, the protochlorophyllide ternary complex (see earlier). This would mean that the precursor of both chlorophyll a (predominately measured by a B/R) and chlorophyll b (predominately measured by B/G) have the same precursor. This is more or less confirmed in literature. Breakdown products of chlorophyll a were only detected during senescence of flowering plants, although both chlorophyll a and chlorophyll b disappeared (Matile et al., 1996). It is known that chlorophyll b can be converted into chlorophyll a, for instance in etiolated cucumber cotyledons (Ito and Tanaka, 1996). If chlorophyll b is formed out of chlorophyll a and if the first step of chlorophyll b breakdown is the re-formation of chlorophyll a then only one common protochlorophyllide is necessary (Fig. 5.5). Although this discussion is undecided, only protochlorophyllide a has been found in barley (Scheumann et al., 1999). If only one form of colourless protochlorophyllide exists then it should not make a difference whether it is estimated from B/R or B/G colour data.

The percentage variance accounted for (R^2_{adj}) between the CHL_0 estimates using B/R and B/G colour data is only 63% for all batches (Fig. 5.4, upper row). This might be expected as chlorophyll a and chlorophyll b absorbs slightly different light in the green and red part of the spectrum as was discussed in the Results and discussion section.

Because of the high percentage variance accounted for (R^2_{adj}= 86% for all batches, Fig. 5.4, lower row) between chl_0 estimates using B/R and B/G colour data, it might be assumed that both estimates of chl_0 are an assessment, differing in the same calibration factor as discussed for $Pchl_0$, of the concentration of chlorophyllide. This might be the case as, next to one form of protochlorophyllide, also only one form of chlorophyllide is necessary when chlorophyll a is the precursor of chlorophyll b. On the other hand, chl a and chl b, have been found in light grown leaves of several plant species (Rise and Goldsmidt, 1990). Alternative breakdown of chlorophyll b to chlorophyllide b instead of first decaying to chlorophyllide a might have been the cause (Fig. 5.5).

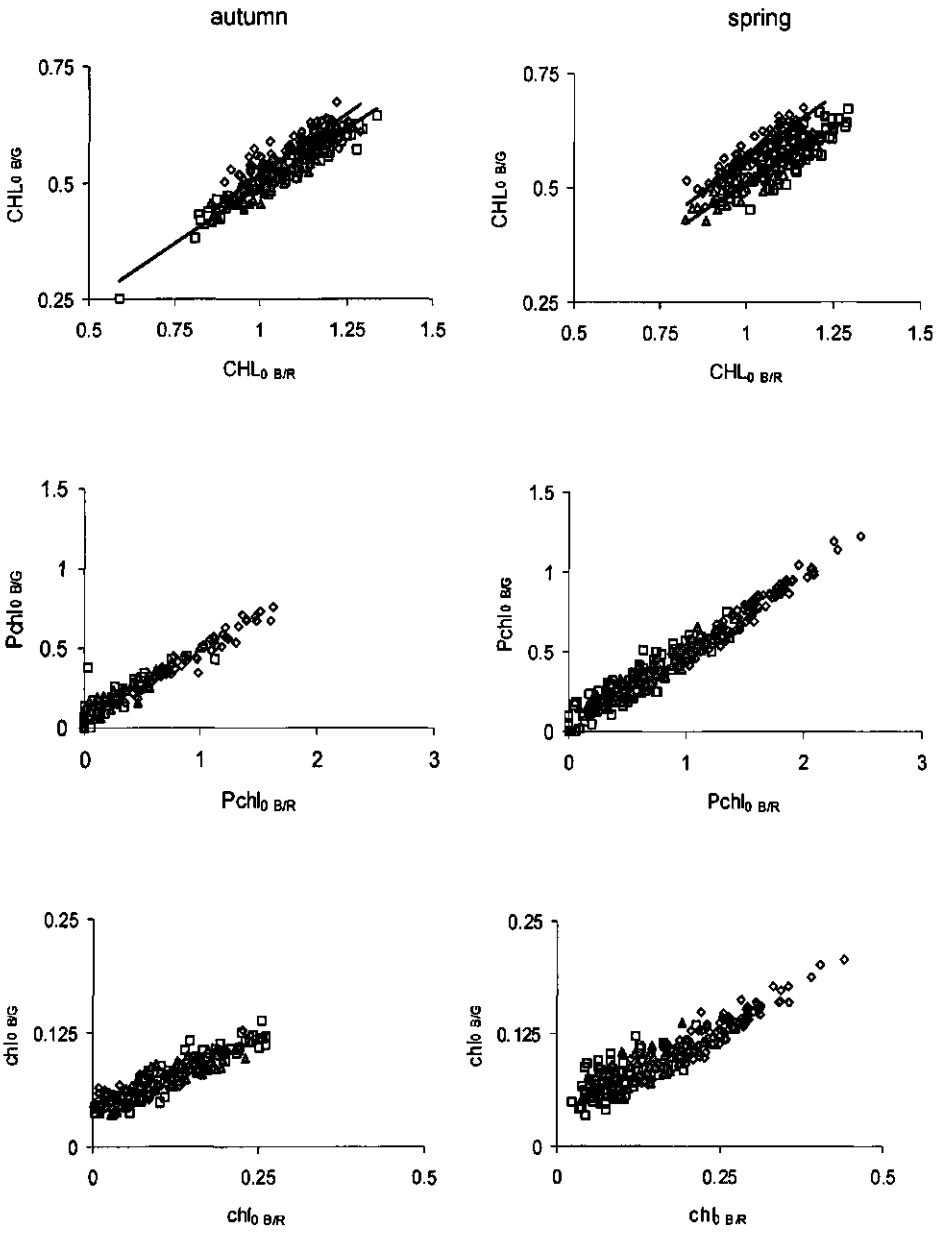

Fig. 5.4. Plots of CHL_0 (upper plots), $Pchl_0$ (middle plots) and chl_0 (lower plots) estimated using B/R and B/G colour data. The plots on the left and right are from autumn and spring season, respectively, for batches of 'Volcan' (square), 'Beluga' (triangle) and 'Borja' (diamond) of the Spanish data set. The slope between the CHL_0 estimates using B/R and B/G colour data, with an intercept through the origin, is indicated per batch. Storage temperature for all batches was 20°C.

The average value of $Pchl_0$ was larger for the batches from the spring compared to the autumn season (Fig. 5.6, upper plot). Also, the average value of $Pchl_0$ for the batches of 'Borja' was, per season, considerably larger than for the other batches. The slope between the CHL_0 values, estimated from B/G and B/R colour data per batch, seemed to be steeper for high keeping quality batches; the indicated slopes for the batches of 'Volcan' and 'Beluga' are largely coincided each season (Fig. 5.4, upper plots). In fact, the slope between the CHL_0 values, estimated using B/G and B/R colour data, per batch had a high correlation with the batch keeping quality (R^2_{adj}= 98%, Fig 5.6, middle plot).

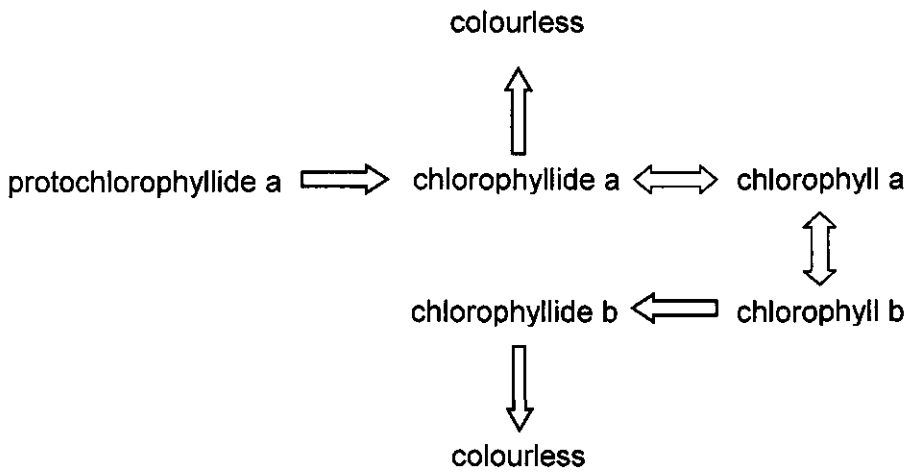

Fig. 5.5. Proposed scheme of the last part of the chlorophyll pathway with differentiation between the different types of colour components, a or b, and their relations.

This may be explained by interpreting the effect of the different values for the reaction rate constants k_d and k_f, estimated using either B/R or B/G colour data, on the colour model (Fig. 5.1). The reaction rate constants k_f and k_d were smaller using B/G than using B/R colour data (Table 5.1). This means that, when Pchl is converted into colour components, more time will be spend in the steady state between chl and CHL as both creation (k_f) and breakdown (k_d) of steady state components is smaller using B/R than using B/R colour data. This effect is stronger the more initial Pchl is present.

When the more complex chlorophyll mechanism shown in Fig. 5.5 is used the role of the different chlorophyll's may become clearer with respect to the difference in k_f and k_d. The value of k_f may represent the ratio of chlorophyll a and chlorophyll b creation from Pchl. As chlorophyll b is created from chlorophyll a (Fig. 5.5) it may be expected that k_f is smaller, when estimated from B/G than from B/R colour data as a B/G measurement

indicates more chlorophyll b and less chlorophyll a than for a B/R measurement (see the Results and discussion section). The value of k_d may represent the ratio of chlorophyll a and chlorophyll b decay to colourless compounds. As chlorophyll b first has to be broken down to chlorophyll a (Fig. 5.5) it may be expected that k_d is smaller when estimated from B/G than from B/R colour data.

The slope between CHL_0 values, estimated from B/R and B/G colour data, was indicative of the amount of $Pchl_0$ and therefore for the keeping quality per batch. The slope between chl_0 values, estimated from B/R and B/G colour data, had apparently no relation with batch keeping quality (Fig. 5.4, lower row). As the green colour consists of CHL and chl, the slope between initial B/G and initial B/R, should also be steeper for high keeping quality batches. The slope between initial B/G and initial B/R, or stated in a simpler form, initial R/G, was correlated with the batch keeping quality with a percentage variance accounted for of 94% (Fig. 5.6, lower plot).

Practical application

The prediction of the batch keeping quality is rooted in a physiological mechanism. The processes covered in the model are generic in nature. Therefore it might be possible to provide batch keeping quality predictions based on initial colour measurements for other products which have the green colour as limiting quality attribute, e.g. broccoli, green beans and Brussels sprouts. The procedure to set-up a system for batch keeping quality predictions would consist of the following steps:

1. Establish the appropriate colour limit per product. Although the colour limit was defined as the acceptable colour for the consumer it may be suitable to use higher values of this colour limit in earlier parts of the horticultural production chain.
2. Purchase an image analysis system including a 3CCD camera.
3. Storage of about ten batches per product, varying in growing conditions and cultivar under laboratory circumstances and measuring the colour repeatedly with image analysis until yellowing is complete or decay is imminent.
4. Analysis of the colour data with the colour model to establish the kinetic parameters per product.
5. Determine the batch keeping quality for the stored batches (see the Results and discussion section).
6. Calibrate the image analysis system. Calibrating digital cameras from different manufacturers may prove important as the 3CCD chips all have their specific temperature and colour frequency characteristics.

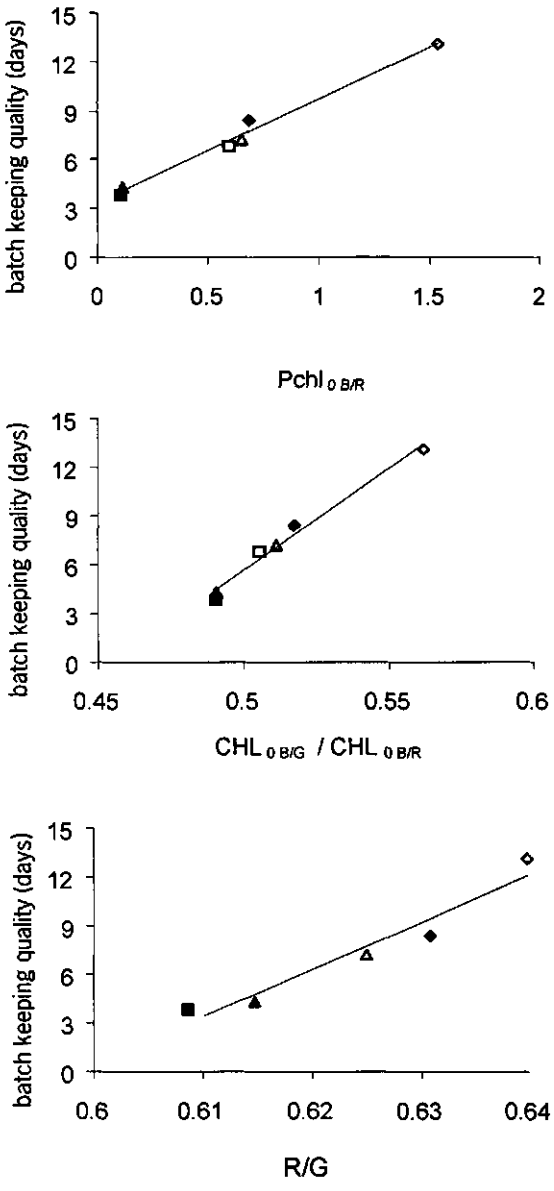

Fig. 5.6. The upper plot shows the average initial Pchl estimated from B/R colour data per batch, the middle plot shows the slope between CHL_0 estimated from B/G and B/R colour data per batch and the lower plot shows the initial R/G per batch against the batch keeping quality. Plots shows symbols belonging to batches from 'Volcan' (square), 'Beluga' (triangle) and 'Borja' (diamond) in the autumn (closed) and the spring season (open). Storage temperature for all batches was 20°C.

These steps have to be carried out only once per product. Hereafter predictions per batch on the keeping quality differing in cultivar and growing conditions may be expected. Per batch a representative number of individuals should be measured, ideally more than 100.

The only substantial limitation for integrating batch keeping quality predictions in the horticultural production chain is the way it is organised nowadays. The current practice at auctions is mixing batches that enter the auction within a certain time period and transferring them to the next part of the chain as one joint 'batch'. As this method functions only on a batch level the organisation of the logistic chain should be changed considerably.

Conclusions

A colour model was developed based on a physiological mechanism obtained from literature under the assumption that no precursor (Pchl) would be synthesised after harvest. The colour model was calibrated on colour data from different cultivars at different storage temperatures. The model was used to describe the colour behaviour of six batches of different cultivars, different growing seasons and growing regions. High amounts of precursor at harvest ($Pchl_0$) were estimated for batches with high keeping quality. Differences in $Pchl_0$ were linked to differences in initial chlorophyll levels and initial colour measurements, expressed as RGB values from a 3CCD digital camera. Because differences in $Pchl_0$ could be linked to initial colour measurements, predictions on batch keeping quality were possible. As the processes described in the model are generic in nature, batch keeping quality predictions based on initial colour measurements could also be feasible for other products with the green colour as a limiting quality attribute. Practical application would require a considerable change in the organisation of the horticultural logistic chain as it should be based on single batches as opposed to mixed batches.

Acknowledgements

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6

Predictability of keeping quality for strawberry batches

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Postharv. Biol. Technol. 26, 35-47 (2002).

Abstract

Postharvest life of strawberries is largely limited by *Botrytis cinerea* infection. It is assumed that there are two factors influencing the batch keeping quality: the Botrytis pressure and the resistance of the strawberry against infection. The latter factor will be discussed in this article. A colour model is presented that describes the development of red colour and anti-fungal function of individual strawberries over time. The model was fitted to colour data from strawberries grown at two different nutrient densities (EC) and stored per batch at 5 °C, 10 °C or 16 °C and constant vapour pressure deficit after harvest. A batch was defined as all strawberries from one EC and one harvest. Spoilage per batch was recorded daily during storage. The fitted initial spoilage per batch was found to relate to the fitted average amount of precursor of both the colour and the anti-fungal compounds. Batch keeping quality could be derived from the initial batch colour distributions for the low nutrient batches. Also, batch keeping quality correlated highly with the time between harvest dates for the low EC batches. An explanation for the ability to link colour distributions to keeping quality without having to use a term describing the pH is presented from pigment HPLC measurements of sub-batches. Indications that the colour distributions of high EC batches may be subject to substantial co-pigmentation are presented. For a practical implementation of predicting keeping quality of batches additional research is needed, especially regarding different Botrytis pressures between greenhouses.

Introduction

Strawberries are very perishable, mainly due to spoilage by the fungus *Botrytis cinerea* (Browne et al., 1984; Saks et al., 1996). The main criterion for spoilage is whether the strawberries are affected or not, rather than the degree of decay. Quality losses can be expressed in terms of batch keeping quality (Tijskens and Polderdijk, 1996; Chapter 4) and can be defined as the time it takes for the first strawberry in a consumer package of 20 strawberries to become rotten (Hertog et al., 1999). The aim of this article is to develop a method capable of predicting the keeping quality of batches of strawberries on the basis of physiological processes and non-destructive measurements.

The physiological processes are part of the flavonoid pathway that describes both the colour and anti-fungal compounds generated by the strawberry. Another process that might interfere with Botrytis growth is the softening due to maturation of the strawberry which may be beneficial for the rapid spread of Botrytis in the strawberry tissue.

Non-destructive measurements can be very beneficial for model building as they enable repetitive measurements on the same strawberry. Additionally, the batch can still enter the horticultural production chain after establishing the keeping quality. Destructive measurements, however, can have a supportive role in model development when sub-batches, gathered at different storage times from the batch, are analysed. Their role can be to confirm whether quality limiting processes covered in the model actually occur and to identify the possible cause for unexpected behaviour.

There are two reasons to aim for a batch approach. Firstly, individual products are, from an economical view, not useful for participants in the agro-chain because of the sheer volume of product moved. Secondly, sometimes it is not possible to get the necessary data regarding quality decay by measuring an individual product. The extra information within a batch, because of their shared harvest date, cultivar and grower may be sufficient (Chapter 4).

Material and methods

Strawberries

Strawberry plants (*Fragaria* x *ananassa* Duchesne cv. Elsanta) were purchased from a local supplier and planted in a greenhouse compartment in July 2000. Three plants were grown per 7.5 litre pot. Plants were watered every 2 h during the day with a volume of 0.7 litre using drip irrigation. Natural antagonists were applied weekly against thrips, mites and other pests. Plant debris was removed once a week. Plants were arranged in

two rows of 18 pots with either a high or low nutrient density solution according to specifications of a local growth media supplier. The actual nutrient density of the drainage, expressed as EC (electrical conductivity) level is shown in Fig. 6.1. A batch was defined as all strawberries harvested on the same day from the same nutrient density. Fruit were harvested when the predetermined number of about 130 strawberries with no white patches had been reached per nutrient density. After harvest, strawberries were placed in a cardboard box covered with air filled polystyrene and carried by hand to the measuring facility within 30 min. A total of 12 batches were harvested during August and September 2000. Only regularly shaped fruit without signs of Botrytis were placed on plastic discs (Ø 28 cm) with 24 holes (3.5 x 2.2 x 0.4 cm). Dimensions of the disc were designed to hold 24 strawberries, arranged in a circular pattern, without touching each other and without changing position during the experiments. To ensure this, a small clay roll, fixed to the disc with glue, was used to support each strawberry.

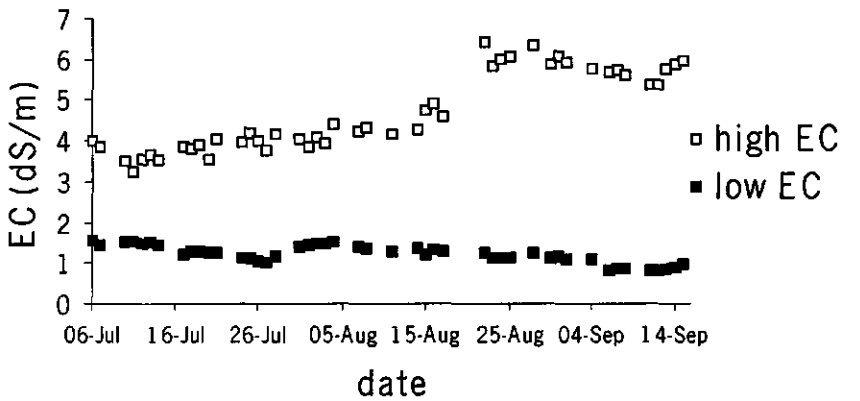

Fig. 6.1. The variation in EC during cultivation.

Storage and rot assessment

Strawberries were stored per batch on discs at 5 °C, 10 °C or 16 °C in 4 m^3 climate chambers (Weiss Umwelttechnik, Germany) at a constant vapour pressure deficit of 100 Pa in the dark. The quality of strawberries was visually assessed per batch by counting the number of strawberries affected by Botrytis on a daily basis. Affected strawberries were removed. No discrimination was made on the basis of the level of decay.

Assessment was done within 10 min per batch to minimise the time for a batch to be outside the climate chamber.

Non-destructive measurements

Measurements took place daily, every two or three days for batches at a storage temperature of 16 °C 10 °C or 5 °C, respectively. Fruit were subjected to temperature adjustment to compare firmness measurements. Strawberries stored at 5° C were placed for 1.5 h in the 10°C and 1.5 h in the 16 °C chamber. Strawberries stored at 10 °C were placed for 1.5 h at 16 °C. Finally, strawberries placed or stored at the 16 °C were held for 30 min at room temperature.

Firmness was measured with a Zwick Z2.5/TS1S materials testing machine (Ulm, Germany) using a cylinder (Ø 16 mm) to compress the strawberry to 0.30 N with a constant speed of 20 mm/min. Preliminary trials were carried out to determine non-destructiveness of the applied force. Discs were placed on a turntable and each individual fruit was measured.

Colour measurements were carried out in a controlled light environment with a 3CCD video camera (Hitachi HV-C20) connected to a PC. A constant light environment in the colour box was created with the aid of 12 TL bulbs and a diffuser plate. Discs with 24 fruit were placed on a turning device in the colour box and each fruit was measured individually. By the use of specialised colour learning software (ATO, Wageningen) strawberry images could be separated into flesh, seeds, calyx, clay and background colour. Light intensities for the red, green and blue (RGB) values were separately averaged over all pixels that belong to the different parts of the strawberry.

Destructive measurements

Batches were divided into four sub-batches of equal size and equal content based on initial firmness and colour measurements as to assess pigment and pH changes at different moments in time during storage. The first sub-batch was taken out at the day of harvest. The second sub-batch was taken out at day 11, 8 and 6 for batches stored at 5 °C, 10 °C and 16 °C respectively, indicating batches with 10-20% rotten strawberries. The third sub-batch was taken out at the day 18, 11 and 8 for batches stored at 5 °C, 10 °C and 16 °C respectively, indicating batches with 50-60% rotten strawberries. The fourth sub-batch was kept in the climate chambers until all strawberries were rotten. Sub-batch 1-3 were stored at –85 °C.

For pigment extraction purposes strawberries were placed overnight at 5 °C to defrost and in the morning each fruit was blended and a sample of 1 g per fruit was

mixed with 2 ml acetone for 10 min before being filtered through G. Schut & Zonen V255 paper using a 2 ml syringe. The filtrate was evaporated using a speedvac (1.5 h) and redissolved in 1 ml 3% formic acid and passed through a C18 Sep-Pack cartridge previously activated by 10 ml methanol followed by water and 3% formic acid. Pigments were recovered with 2 ml methanol 3% formic acid. A sample of 20 µL was analysed by HPLC (modified from Gil et al., 1997).

Pigments were separated using a HPLC system (Dionex P580 pump) consisting of a photodiode array detector (Dionex UVD 3405) with an autosampler (Marathon, Spark) operated by Chromeleon software. A reverse phase C18 Hewlett Packard, LiChrospher 100 column (250 x 4 mm; particle size 5 µm) was maintained at 35 °C. Running conditions were according to Gil et al. (1997). The flow rate was 1 ml/min and detection was performed at 520 nm. Reproducibility of the HPLC analysis was ± 5%.

Pigment compounds were identified by chromatographic comparison with pelargonidin-3-glucoside (Pg-3-gl) and cyanidin-3-glucoside standards purchased from Carl Roth GmbH&Co. (76185 Karlsruhe, Germany). The acylated form of Pg-3-gl was quantified using Pg-3-gl as standard.

Alkaline hydrolysis of the pigments was used to identify the acylated form of Pg-3-gl for 5 individual strawberries. After standard extraction of the pigments the sample was evaporated in a speedvac (1h) and redissolved in 5 ml 10% aqueous NaOH. The mixture was flushed with N_2 and hydrolysed for 8 min in the dark. Pigments were recovered as their red oxonium salt by adding 10% aqueous HCl until pH 4 was reached (modified from Hong and Wrolstad, 1990). After passing through a C18 Sep-Pack cartridge, the standard HPLC method was used.

pH measurements per strawberry were conducted on defrosted and blended strawberries using a micro pH meter (CG820, Schott Geräte, Germany).

Modelling

Conceptual modelling

Most cases of Botrytis infection take place via floral parts of the senescing bloom. After successful flower infection the fungus remains quiescent in green strawberries (Bristow et al., 1986). Proanthocyanidins (PA), dimers and higher oligomers of flavan-3-ols (Haskins and Gorz, 1996), are unspecific and colourless enzyme inhibitors that are thought to govern the quiescence (Jersch et al., 1989). PA are end products of the flavonoid biosynthesis pathway and so are the anthocyanins (Strack and Wray, 1993; Boss et al., 1996). Quantification of PA is commonly done by depolymerisation in HCl/BuOH (Porter

et al., 1986) and the vanillin assay (Sun et al., 1998). Both methods, however, have shortcomings (side reactions and absence of standards), as well as being destructive.

Anthocyanins are responsible for the red colour in strawberries and start to appear during the white stage of fruit development (Hancock, 1999), with synthesis continuing after harvest (Holcraft and Kader, 1999). Leucoanthocyanidins are the direct precursors of both the anthocyanidins and the flavan-3-ols, but many more precursors exist in the flavonoid pathway. Figure 6.2, adapted from Strack and Wray (1993) and Boss et al. (1996), shows the last part of the flavonoid pathway. The anthocyanidins, also red coloured, are not found in strawberries (Bakker et al., 1994, Gil et al., 1997) and are reported to be less stable than anthocyanins (Francis, 1989). It might be assumed that the rate constant for anthocyanin formation is very high.

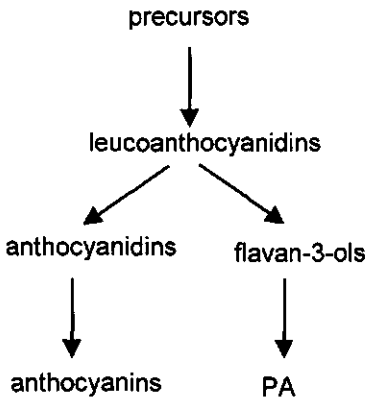

Fig. 6.2. Scheme of the last part of the flavonoid pathway.

Large differences have been shown between flavan-3-ol concentrations between harvests, but not for PA, in seeds and skins of different white grape cultivars (De Freitas and Glories, 1999). An explanation might be that the reaction rate constant for flavan-3-ol formation is limited compared to the reaction rate constant for the formation of PA from the flavan-3-ols (Fig 6.2). According to Walton et al. (1983), flavan-3-ols do not precipitate proteins as PA do, so flavan-3-ols are probably not active as Botrytis inhibiting agents.

The keeping quality may depend solely on the Botrytis pressure and the ability to form sufficient PA to inhibit spreading of the Botrytis infection in strawberries. Light treatment was able to overcome poor red colour and 'white shoulders' in two cultivars while also diminishing fruit rot at the same time (Saks et al., 1996). This indicates that a light inducible precursor of both the colour compounds and the PA compounds is still sufficiently available to influence keeping quality for harvested strawberries. An assumption is that the amount of precursor available at the moment of harvest solely

governs the keeping quality of strawberries in case of constant Botrytis pressure. In other words, synthesis of the precursor stops at harvest and the amount of precursor available at harvest will govern the colour and the resistance against botrytis.

If the assumption is correct that the amount of PA at the moment of harvest, next to the Botrytis pressure, is governing the keeping quality of strawberries then it might be possible to assess this by measuring the initial colour at harvest. Both colour (anthocyanins) and antifungal (PA) components are linked in the flavonoid pathway (Fig. 6.2) by their common precursor. To show if the initial colour assessment is indicative for spoilage behaviour, two independent models, both estimating the common precursor on a batch level, may be used. If a relation between both estimates exists, then the initial colour might be used to assess the expected keeping quality of a batch. The first model describes the colour development in time, using only colour data and temperature, to estimate the amount of precursor at harvest per batch. The second, already existing, model describes the spoilage development in time, using only spoilage data and temperature, to estimate the initial spoilage per batch. Since we assume that spoilage is primarily caused by the absence of PA, initial spoilage may be an indicator of the amount of precursor at harvest per batch.

Botrytis is known to produce a number of pectin degrading enzymes (Kamoen, 1997) that can enhance the naturally occurring softening that is due to ripening. Our assumption is that once insufficient PA is available to inhibit Botrytis growth in harvested strawberries, the maturation process will have decreased cell wall thickness enough to readily allow penetration of Botrytis hyphal tips. Compared to unripe strawberries, cell walls of ripe strawberries decline to 40% of the original thickness (Redgwell et al., 1997).

Mathematical models

Spoilage

Spoilage modelling was done according to Hertog et al. (1999). Herein the behaviour of a batch of strawberries in terms of percentage affected is described by the differential equation for a sigmoidal change in time (Eq. 6.1):

$$\frac{dN}{dt} = k_{bot} \cdot N \cdot \frac{(N_{max} - N)}{N_{max}} \qquad (6.1)$$

beginning with N_0, the initial spoilage, N being the percentage of strawberries affected. The maximum spoilage (N_{max}) is 100%. The progress of spoilage is solely determined by the spoilage rate constant k_{bot} (day^{-1}). The initial spoilage is regarded as a batch

dependent parameter. The spoilage rate is assumed to depend on temperature according to Arrhenius' law (Eq. 6.2):

$$k = k_{ref} \cdot e^{\frac{Ea}{R_{gas}} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} \qquad (6.2)$$

where R_{gas} is the gas constant (8.314 J $mol^{-1}K^{-1}$), k_{ref} represents the rate constant at reference temperature T_{ref} (K) and Ea (J mol^{-1}) expresses the dependence of the rate constant on product temperature (K).

Colour development

Colour development is dependent on the amount of precursor and the proportions transformed into anthocyanins and to flavan-3-ols, these being the rate determining steps of the process described in Fig. 6.2. The set of differential equations belonging to these processes (Eq. 6.3 and Eq. 6.4) can be solved analytically to describe the red (anthocyanin) formation in time (Eq. 6.5.)

$$\frac{\partial prec}{\partial t} = -k_m \cdot prec - k_r \cdot prec \qquad (6.3)$$

$$\frac{\partial red}{\partial t} = k_r \cdot prec \qquad (6.4)$$

$$red(t) = red_0 + \frac{k_r}{k_m + k_r} \cdot prec_0 \cdot (1 - e^{-(k_m + k_r) \cdot t}) \qquad (6.5)$$

where k_m and k_r are the reaction rate constants for the formation of the flavan-3-ols and the formation of the anthocyanins, respectively. Both reaction rate constants are temperature dependent via the Arrhenius equation (Eq. 6.2). The initial concentrations of anthocyanin and the initial precursor concentration are expressed as red_0 and $prec_0$.

Statistical analysis

Experimental data on spoilage and colour development were analysed statistically using the non-linear regression routine of the statistical package Genstat 5 (release 3.2, Lawes

Agricultural Trust, Rothamsted Experimental Station, UK). The equations and mathematical description of the colour model were developed using Maple V (release 4, Waterloo Maple Software, Waterloo, Canada). The spoilage data were analysed using the model formulation of Eq. 6.1 together with the temperature dependence according to Arrhenius (Eq. 6.2). The spoilage data were analysed simultaneously using temperature and time as independent variables to acquire N_0 for each batch. Colour data were analysed using the model formulation of Eq. 6.5 together with the temperature dependence according to Arrhenius' law (Eq. 6.2). The colour data were analysed simultaneously using temperature and time as independent variables. The reference temperature for the Arrhenius equation (T_{ref}) was in all cases 283 K (10°C).

Analysis of variance (ANOVA) was used for the comparisons of the Pg3-gl concentration as function of storage time ($P<0.05$).

Results

Spoilage

Analysis of the spoilage data from all batches is shown in Table 6.1. To account for the differences between batches, the initial spoilage, N_0, was estimated for each of the twelve batches. The differences in N_0 cannot be assessed visually at harvest with any certainty as all values vary between 0.10 and 0.86%, which equates to 0.13 and 1.1 affected strawberries with an average batch size of 130. Using the estimated parameters the keeping quality can be calculated at 10 °C to compare batches. High EC batches generally have a higher keeping quality than low EC batches but exceptions exist. Furthermore there seems to be no clear relation between keeping quality and plant age, which is indicated by the batch number. The batch number is higher with each subsequent harvest. The estimated parameters presented in this study are probably more accurate because of larger batch size (130 versus 25) and because secondary infections were explicitly prevented by the storage method used, but they closely resemble those reported by Hertog et al. (1999). Fig. 6.3 shows the experimental development of spoilage for all batches against the expected values according to the spoilage model.

Firmness development

Firmness measurements in time from the same strawberry showed generally a scattered and quick decay. But, contrary to expectation, firmness did not always decrease. Strawberries stored at 16 °C and grown at high EC especially showed an increase in

firmness until day 3. There was no relationship found between the amount of initial spoilage, N_0, and the initial firmness measurements at harvest per batch. Cell wall thickness might be unimportant with regards to the defence against Botrytis, at least in this cultivar. Another explanation is that changes in turgor were measured rather than changes in cell wall thickness. Turgor might rise when strawberries are brought from a greenhouse with a relatively low RH to a storage facility with a high RH. Furthermore, a slow loss of turgor may take place during storage, despite constant VPD, because of fast air movement (~1 m/s) in the storage rooms.

process parameters					
$k_{bot,ref}$	0.5009	s.e.	0.0111	n_{obs}	321
Ea	68811	s.e.	4752	R^2_{adj}	97.2%

batch parameters					
batch	EC (dS m^{-1})	storage temperature (°C)	N_0	s.e. N_0	KQ at 10°C (days)
4	high	16	0.098	0.044	7.9
8	high	10	0.099	0.024	7.9
7	low	16	0.106	0.047	7.8
9	low	5	0.195	0.05	6.6
1	high	5	0.266	0.066	6.0
5	high	5	0.301	0.073	5.7
10	high	16	0.434	0.158	5.0
2	high	10	0.465	0.095	4.8
6	low	10	0.513	0.104	4.6
12	low	16	0.678	0.141	4.0
3	low	5	0.841	0.173	3.6
11	low	10	0.859	0.162	3.6

Table 6.1. Parameter estimates and their standard error (s.e.) for the analysis of the spoilage data of 'Elsanta' strawberry batches.

Colour development

Due to memory limitations of Genstat only 5 fruit from each batch could be analysed simultaneously to obtain the reaction rate constants (k_m and k_r) over all fruit and the initial precursor concentration, $prec_0$, per fruit. Consequently, fruit longest in storage were chosen for inclusion in the analysis. Only the R-values from the RGB values were used, expressed as (1/R)*100 to get an increase in colour for ageing strawberries, for red(t). For red_0, the initial colour measurement was used in Eq. 6.5. Initial estimates for the process parameters were found on the condition that all precursor available is spent on red formation, k_m=0. The percentage variance accounted for (R^2_{adj}) was high, 90%. Then the colour data were fitted with initial estimates from the earlier optimisation to obtain the process parameters for the colour model (Table 6.2). Fig. 6.4 shows the experimental colour development for 5 arbitrary strawberries per temperature against the expected values according to the colour model.

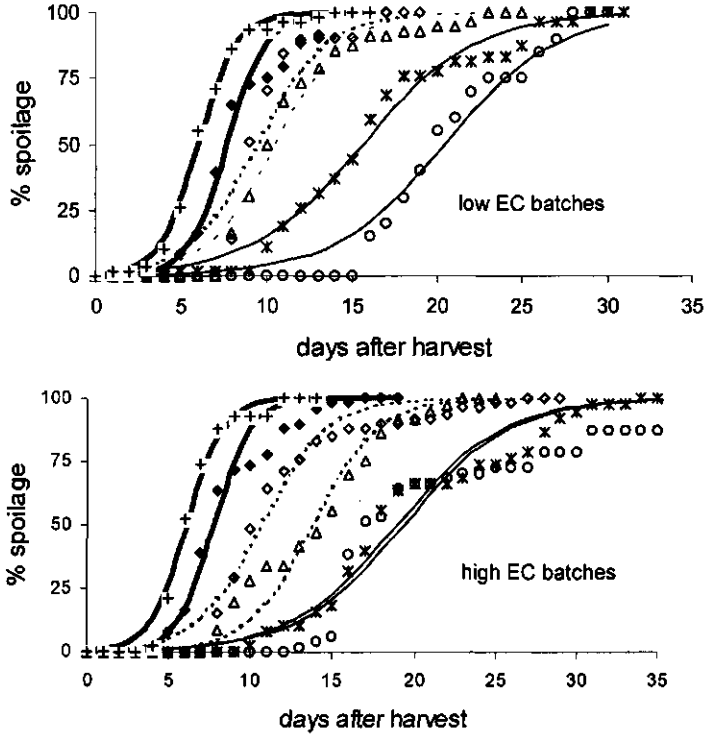

Fig. 6.3. Fitted spoilage of 'Elsanta' strawberry batches stored at 16 °C (—), 10°C (---) and 5 °C (——) as a function of time. Experimental development of spoilage is indicated at 16 °C (+, □), 10 °C (◊,Δ) and 5 °C (*,○). Upper and lower figures show the spoilage behaviour of the low and the high EC batches.

process parameters						
$k_{m,ref}$	0.1155	s.e. 0.0041	$k_{r,ref}$	0.01505	s.e.	0.00032
E_m	1456	s.e. 595	E_r	5542	s.e.	335
n_{obs}	569					
R^2_{adj}	96.7%					

Table 6.2. Parameter estimates and their standard error (s.e.) for the analysis of the colour data of 'Elsanta' strawberries.

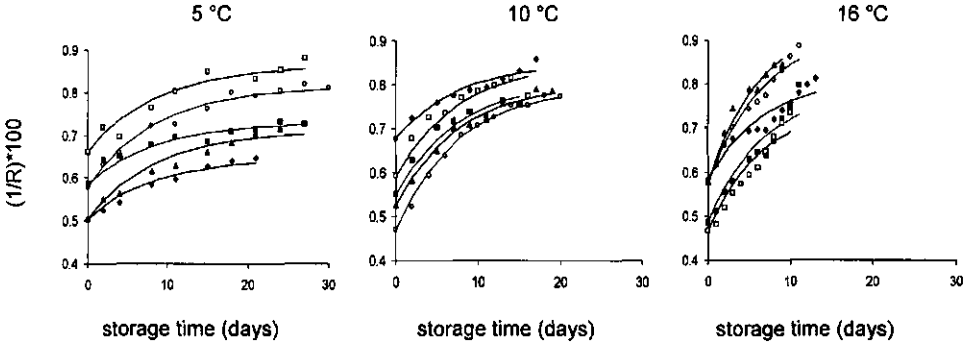

Fig. 6.4. Fitted and experimental colour development of 'Elsanta' strawberries for 5 arbitrary strawberries at 5, 10 and 16 °C as a function of time.

The colour process parameters show that the ratio k_m/k_r decreases from 9.9 (5°C) to 4.8 (16°C) with increasing temperature (Table 6.2). This indicates that higher storage temperature primarily results in higher production of red colour and not so much in a higher production of Botrytis inhibiting compounds. When combined with the process parameters from the spoilage data where the temperature dependence for Botrytis growth is very high (Table 6.1) the following picture emerges with regards to the biological background of spoilage in strawberries. At low temperatures, 2-5 °C, the production of Botrytis inhibiting compounds may parallel the growth rate of Botrytis. For higher temperatures the emphasis may not be in producing sufficient Botrytis inhibiting compounds but more in enhancing the red colour, so that strawberries will be attractive for animals to eat and spreading of the strawberry seeds can be achieved before spoilage occurs.

Batch colour

On the basis of the earlier acquired reaction rate constants, the initial precursor concentration, $prec_0$, per strawberry was fitted for all strawberries in all batches in a separate fitting procedure.

If the assumption that the initial precursor concentration governs the keeping quality is valid then it is expected that a relation exists between the estimates of the common precursor from the independent colour and spoilage models. A linear relationship is found between the average initial precursor concentration, $prec_0$, per batch (colour model) and the initial spoilage per batch, N_0, (spoilage model) for the low EC batches (Fig. 6.5, R^2_{adj} = 86%). The relationship is less clear for the high EC batches (Fig. 6.5, R^2_{adj} = 71%).

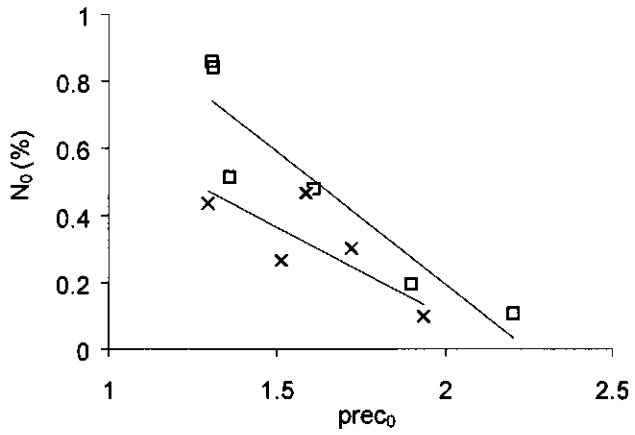

Fig. 6.5. Plot of the average fitted amount of precursor for low (□) and high (x) EC batches versus the initial spoilage, N_0, per batch.

When the precursor concentration at harvest is determining the keeping quality then the colour distribution at harvest might also contain this information. Colour distributions of four low EC batches are shown in Fig 6.6. With increasing initial spoilage, N_0, an increase in the skewness is observed for the colour distributions fitted with a standard gamma function. The gamma function is characterised by values for the maximum height, the median, the standard deviation and the skewness. For the low EC batches skewness of the initial colour distributions is indicative for the keeping quality (R^2_{adj} = 90%, Fig. 6.7). Unfortunately, this is not the case for the high EC batches.

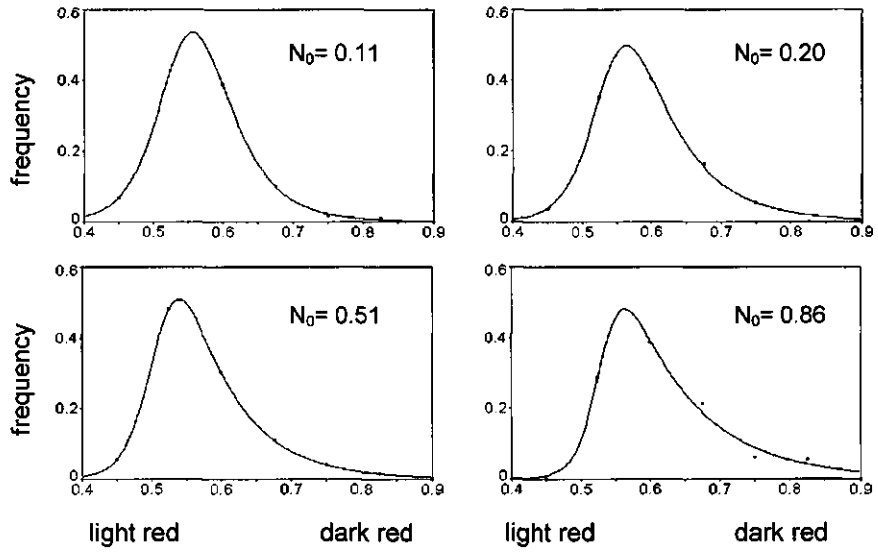

Fig. 6.6. Low EC colour distributions. Values on the x-axis represent colour values expressed as (1/R)*100. On the y-axis the relative frequency is depicted. The distributions are fitted with the gamma function.

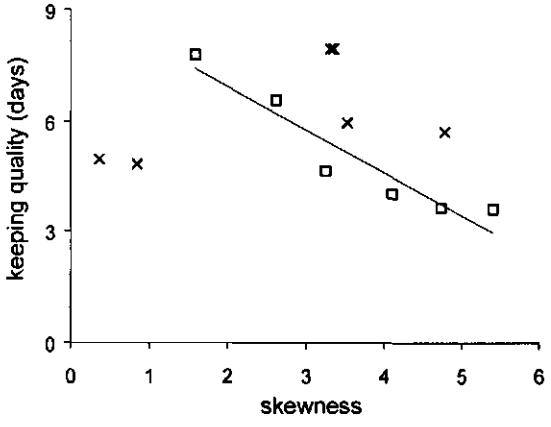

Fig. 6.7. Plot of the skewness of the gamma function, which was used to fit the colour distributions for the low EC (□) and the high EC (x) batches, versus keeping quality. Only the linear regression line for the low EC batches is shown.

In Fig. 6.8 colour development of strawberries grown under low EC growing conditions are depicted, one with strawberries with a high and one with a low keeping quality. In both cases a 90% confidence interval is shown indicating that all but the 5% whitest and the 5% most red strawberries are not shown. Strawberries with a high keeping quality will have a considerable amount of precursor at harvest left and these strawberries will generally be much more red at $t=\infty$, when all precursor is spent, than strawberries with a low keeping quality. Harvesting will commence when enough strawberries are within the harvesting window (Fig. 6.8). For the strawberries with a low keeping quality almost all could be selected for harvesting save only a small amount that might be considered too red. For the strawberries with a high keeping quality only about half of the available strawberries could be selected. Because only a small part of the available strawberries with a low keeping quality is too red with regards to the harvesting window, these strawberries will also be harvested and belong therefore to the low keeping quality batch. This is not the case for the strawberries with a high keeping quality, where only half of the available strawberries *can* be harvested because the other half has already been taken during an earlier harvest. So here a batch consists of about half the available strawberries. This results in a colour distribution for the low keeping quality batch that is skewed because also some very red strawberries have been harvested (T2, Fig 6.8) and a normal distribution (T1, Fig. 6.8) for the high keeping quality batch that consists of strawberries which are all almost linearly increasing in red colour. Thus, the skewness in the colour distributions is caused by the limitation of the available strawberries in a batch during harvest.

Harvest of the low keeping quality batch takes place when all available strawberries are in an advanced stage of development. All strawberries are near their maximum red colour. This is not the case for the strawberries belonging to the high keeping quality batch that are only about halfway their colour development. The time between harvest dates might shorten if growing strawberries until halfway coloured takes fewer days compared to growing strawberries until they have their maximum colour development. For low EC batches this seems to be the case (R^2_{adj} = 91%, Fig. 6.9), but, again, not for high EC batches.

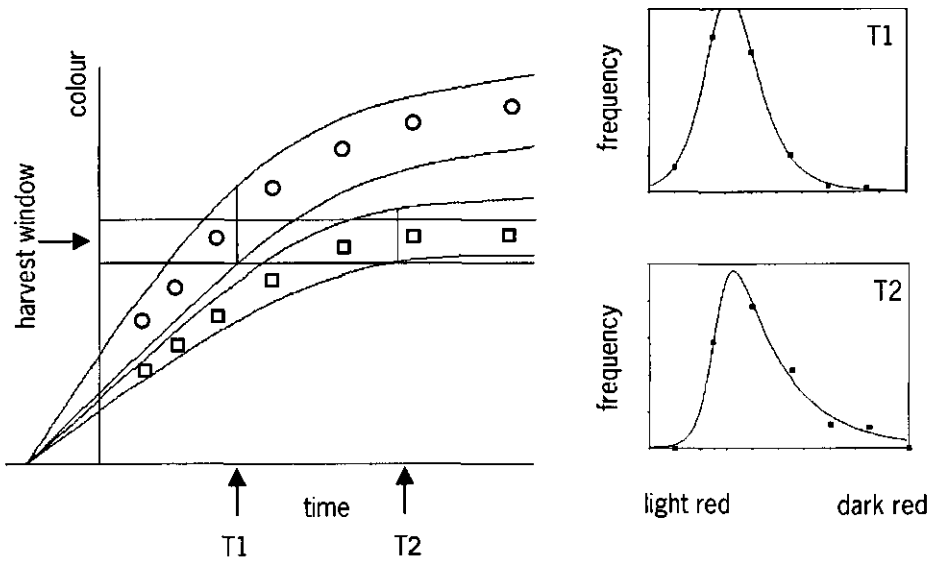

Fig. 6.8. Left-hand side graph depicts a high keeping quality batch (o) and a low keeping quality batch (□) and the harvest window. Harvest takes place at T1 for the high keeping quality batch and at T2 for the low keeping batch. The colour distributions at T1 and T2 are depicted in the right-hand side graph.

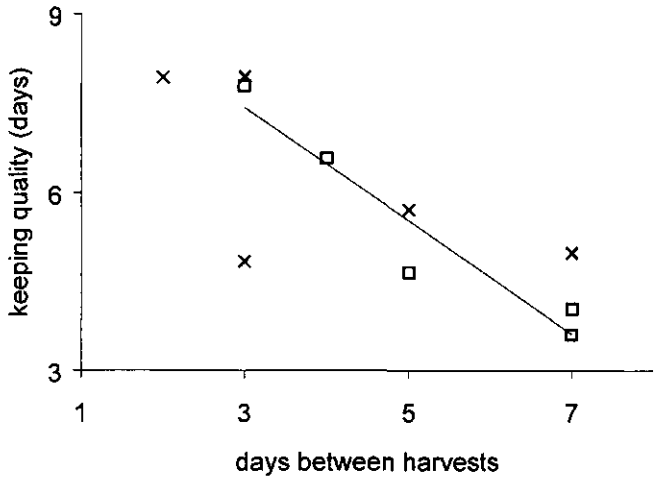

Fig. 6.9. Plot of the number of days between the previous and the current harvest for the low EC (□) and the high EC (x) batches, versus keeping quality. Only 10 data points are depicted instead of 12 because the first low and high EC batches have no previous harvest. Only the linear regression line for the low EC batches is shown.

It is well known that for very acidic solutions all the anthocyanin will be in its red flavylium form, but with increasing pH the colour intensity decreases because more anthocyanin will be converted into the colourless form (Brouillard and Delaporte, 1978). Preliminary experiments showed unripe strawberries having a very low pH, around 2, and ripe strawberries having a higher pH, around 4. This would mean that, during storage, the pH of the strawberries would change and that the colour intensity as measured by the video colour camera is not a measure of the pigment concentration. To investigate why the colour model apparently works well, at least for the low EC batches, destructive measurements were used. First the composition of the colour compounds in 'Elsanta' strawberries was investigated. Two colour compounds were found, in varying amounts. The main colour compound was identified as Pg-3-gl by comparison with the authentic standard. The smaller peak was identified as an acylated form of Pg-3-gl by alkaline hydrolysis. This composition of colour compounds for 'Elsanta' strawberries is contrary to an earlier report, identifying the smaller peak as cyanidin-3-glucoside (Bakker et al., 1994). During ageing the concentration of both colour compounds increased for all measured batches, however not all results were significantly different ($P<0.05$). The largest increase in Pg-3-gl was found for batches stored at 16°. For those batches the concentration of Pg-3-gl increased from 261 and 275 μg/g at day 0, to 423 and 463 μg/g at day 8 and to 464 and 524 μg/g at day 11 at low EC and high EC respectively. Least significant difference was 59 μg/g with 74 repetitions for the low EC and 66 μg/g with 64 repetitions for the high EC treatment.

It was observed that older sub-batches belonging to the same batch generally had a decreased ratio of Pg-3-gl / acylated Pg-3-gl and a higher pH (Fig. 6.10). As the presence of acylating groups in anthocyanins has been correlated with pigment stability (Giusti et al., 1999), it might be expected that a lower ratio in Pg-3-gl / acylated Pg-3-gl is related to the general higher pH in older high EC batches in order to protect the colour intensity. This process of getting acylated Pg-3-gl converted from Pg-3-gl as the pH rises to protect the redness of the strawberry may well be the reason why the colour model works without actually incorporating the pH in its formulation, at least for the low EC batches.

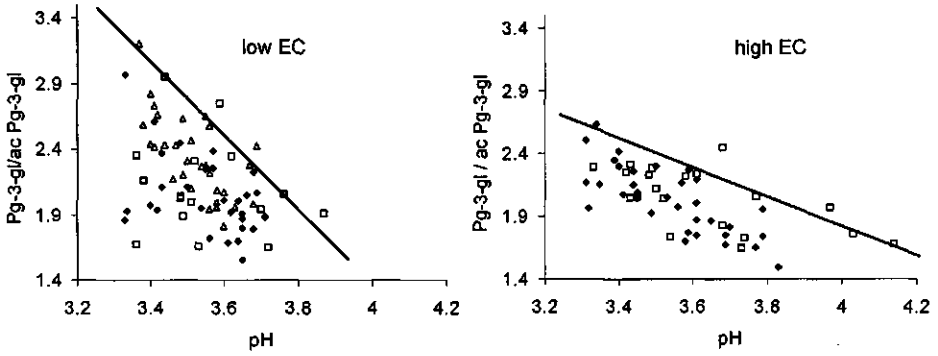

Fig. 6.10. Plot of the pH values for sub-batches from a low EC (left) and a high EC (right) batch, both stored at 16 °C, against the ratio Pg-3-gl / acylated Pg-3-gl. Sub-batches were taken at day 0 (Δ), day 6 (♦) and day 8 (□). The sub-batch taken at day 0 for the high EC batch was not available.

Discussion

Consumer expectations on quality of food products are high and tend to increase with time. A strawberry grower might react by growing strawberries at a higher EC. It is known that a higher EC will benefit taste and texture for tomatoes (Chretien et al., 2000). However, the predictability of the keeping quality of 'Elsanta' strawberries grown at higher EC levels seems to be very poor. The reason is unclear but might be related to co-pigmentation issues. Co-pigmentation is the well documented phenomenon between anthocyanin and co-pigment molecules resulting in increased colour intensity interaction (Davies and Mazza, 1993; Figueiredo et al., 1996). If different levels of co-pigmentation occur in high EC batches compared to low EC batches it is expected that the correlation between the colour intensity, measured by the video camera, and the colour concentration, measured by HPLC, is poor. The correlation between the colour intensity measured and the concentration for three sub-batches measured at day 0, day 6 and day 8 and stored at 16 °C is better for the low EC batch (R^2_{adj}=77%) than for the high EC batch (R^2_{adj}=55%). This might indicate that for the low EC batches the colour intensity is indeed a measure of colour concentration, but less so for the high EC batches. The general low correlation found is at least partly caused by HPLC measurements which are indicative for the whole strawberry while video camera measurements are only indicative for the surface side. From Fig. 6.10 it can be found that for high EC batches during the first part of the storage the ratio Pg-3-gl / acylated Pg-3-gl is smaller than for low EC batches. At the same time the summation of the Pg-3-gl and acylated Pg-3-gl

concentrations are generally larger (data not shown) for the high EC batches. If the assumption is correct that Pg-3-gl and acylated Pg-3-gl form a couple to protect the strawberry from colour intensity changes due to pH then this might indicate that for the high EC batches more Pg-3-gl interacts with unknown co-pigments.

In practise 'Elsanta' strawberries are grown at EC levels varying from 0.5 –1.5. This is close to the level for the low EC batches (Fig. 6.1). To get an indication of the keeping quality for those batches two methods can be used according to this study. The skewness of the colour distribution of the harvested strawberries can be measured with a sophisticated colour measuring system. Or, much more practically, the number of days between harvest dates can be counted, although then the keeping quality of the first batch cannot be estimated. However, actual predictions are not feasible yet because no validation of both methods has been performed. Also, no information on the possibility to use these methods with other cultivars is available. Furthermore, the effect of different Botrytis pressures is unknown. The next step should be a repetition of the experiments in different greenhouses using more cultivars to validate the proposed methods and to expand them for the different Botrytis pressures in each greenhouse.

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7

Batch variability and cultivar keeping quality of cucumber and strawberry

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Abstract

A batch model is presented for the interpretation of batch behaviour in terms of current maturity, biological variation and maximal maturity towards keeping quality of cucumbers and strawberries. The batch model describes the influence of one source of biological variation, here assumed to be variation in light conditions during the preharvest period, on the distribution of precursor concentrations. The batch model consists of a kinetic part, describing the behaviour of the precursor for individuals, and a stochastic part, describing the precursor distribution. The stochastic part is generic, but the kinetic part depends on the processes that determine keeping quality.

The batch model is tested using postharvest colour data of cucumber batches from three cultivars ('Volcan', 'Beluga' and 'Borja') and two growing seasons. Colour data were gathered consisting of repeated colour measurements on the same individuals in a batch during storage. Applying a previously developed cucumber colour model, the precursor concentration determining the keeping quality at harvest was estimated (Chapter 5). The amount of colour precursor at maximal maturity could be estimated in common for batches of the same cultivar and was defined as cultivar keeping quality.

The batch model was further validated using colour data of cucumber batches from twelve experimental cultivars and colour data of strawberry batches. Cucumbers from twelve experimental cultivars were harvested from either the stem or the vine part of cucumber plants. Cucumber batches from the same cultivar could be analysed together, whether harvested from stem or vine parts. Six batches of strawberries (cultivar 'Elsanta') were harvested from two flights from the same plants. Colour data were gathered consisting of repeated colour measurements on the same individuals in a batch during storage. Applying a previously developed strawberry colour model, the keeping quality

determining precursor concentration at harvest was estimated (Chapter 6). Applying the batch model, the distribution of strawberry colour precursor at harvest of multiple batches was analysed.

The batch model enables to estimate cultivar specific information. It thereby takes *advantage* of the biological variation present in all biological batches, instead of treating it as a nuisance.

Introduction

Daily quality control routines applied at auctions and wholesale markets aim at quickly estimating the acceptability of batches. A batch may be defined as all individuals sharing the same harvest date, grower and cultivar (Chapter 4), implying a common growth history. If all individuals in a batch were identical and stored under the same conditions, they would all reach the quality limit, the end of keeping quality, at the same time. Deciding whether a batch is acceptable would then only require the examination of one individual per batch. However, because of the presence of biological variation, this is not the case. Quality controllers are therefore required to inspect a representative part of a batch.

Batch variation can be described as the composite of biological properties that differentiate individual units of a batch (adapted from Tijskens and Konapacki, 2003). One of those properties may be maturity. Maturity at harvest has often a decisive influence on the behaviour of the product after harvest (Chapter 3, Hertog et al., 1997). For external quality attributes like colour and texture, differences in maturity within a batch and between batches are hard to observe and to evaluate. For internal quality attributes like vitamin or sugar content it is even harder.

Mathematical tools to deal with batch variation are scarce. Recently, Hertog (2002) outlined a mathematical approach to interpret batch behaviour for shrivelling of apples and colour change of avocados. In this paper, a similar mathematical approach will be shown to describe batch behaviour of colour change in cucumbers and of spoilage by *Botrytis cinerea* (grey mould) in strawberries. The aim of this research is to develop mathematical tools to describe batch variation in terms of maturity and cultivar influences.

Material and methods

Cucumber batches

For the development of the batch model six batches of 80-100 cucumbers, each belonging to either cultivar 'Volcan', 'Borja' or 'Beluga', were used. Three batches were harvested September 1998 (autumn season) and three batches were harvested June 1999 (spring season) in the Almeria region of Spain. These Spanish cucumbers were grown under equal, commercial growing conditions and were of marketable size and colour (Chapter 5).

For the validation of the batch model cucumbers from twelve experimental cultivars (isogenic lines) were used, cultivars a to l. For each cultivar twelve cucumber plants were grown at two randomly assigned plots in a greenhouse in the west of The Netherlands. These Dutch cucumbers were harvested from the stem or the vine part of the cucumber plants and were of marketable size and colour. Stem and vine cucumbers were harvested in June and July 2002, respectively. A total of 48 batches were harvested with on average 24 cucumbers per batch. 40 batches were used for the validation of the batch model. For eight batches the minimal amount of 20 cucumbers per batch, was not reached.

Upon arrival at the measuring facility, all cucumbers were individually tagged on the lightest side and stored in the dark at 20 °C and 100% RH.

Strawberry batches

Strawberry plants (cv. 'Elsanta') were planted in a greenhouse compartment in July 2000. A fairly constant nutrient solution was applied by drip irrigation. Fruits were harvested when about 130 strawberries without white patches were available. Six batches were harvested in two flights of the same plants; only regularly shaped fruits without signs of *Botrytis cinerea* infection were used. Strawberries were individually tagged and stored in the dark at 5, 10 or 16 °C with a controlled constant vapour deficit of 100 Pa (Chapter 6).

Cucumber colour measurements

Colour was measured on individual cucumbers twice a week using an image analysis system (Chapter 3). Colour was expressed as the ratio of the blue/red (B/R) values from the separate intensities of the blue and red values of the RGB colour scale. Colour

measurements for the cucumbers started one day after arrival at the measuring facility and ended when yellowing was complete or when decay of the cucumber was imminent (Chapter 5).

Strawberry colour measurements

Colour was measured on individual strawberries using an image analysis system. Measurements were carried out daily, every two or every three days for batches stored at 16, 10 or 5 °C, respectively and ended when the strawberry showed the first signs of *Botrytis cinerea* infection. Affected strawberries were removed and the number of affected strawberries over time was recorded per batch. By using software that recognises colour differences, strawberry images could be separated in flesh, seeds, calyx, clay (used to immobilise the fruit) and background colour (ATO, Wageningen). Strawberry flesh colour measurements were expressed as the inverse of the red (1/R) values from the RGB colour scale to get an increase in colour for ageing strawberries (Chapter 6).

Statistical analysis

Precursor concentrations at harvest were estimated for the Spanish and Dutch cucumber colour data set ($Pchl_0$) and for the strawberry colour dataset ($prec_0$) applying the calibrated colour models (Chapters 5 and 6). Precursor concentrations of all batches were analysed statistically to obtain t^b_m and σ per batch and $Pchl_{minvar}$ (cucumbers) or $prec_{minvar}$ (strawberries) per cultivar applying Eq. 13 (see Table 7.1 for description of symbols). The value for the class size (q_b-q_a), expressed as Pchl value, was chosen to avoid having more than 40% of all precursor values in the most crowded class. The class-size was chosen as 0.45 and 0.30 for the Spanish and Dutch cucumbers, respectively and 0.30 for the strawberries.

For the statistical analysis, the non-linear regression routine of Genstat 5 (release 3.2, Lawes Agricultural Trust, Rothamsted Experimental Station, UK) was used. The equations and mathematical description of the batch variation model were developed using Maple V (release 4, Waterloo Maple Software, Waterloo, Canada).

Modelling

Legend of symbols and abbreviations used in the modelling section is shown in Table 7.1 for reference purposes.

chl	chlorophyllide concentration
CHL	chlorophyll concentration
k_f	reaction rate constant for the formation of colour componentsl
k_t	reaction rate constant for the formation of Pchl from TPchl
light[b]	variation in light conditions on a batch level
PA	proanthocyanidin concentration
Pchl	protochlorophyllide concentration
$Pchl_0$	protochlorophyllide concentration at harvest
$Pchl_{minvar}$	protochlorophyllide concentration at minimal variation and cultivar specific keeping quality parameter
Pr	probability
prec	strawberry precursor concentration
$prec_0$	strawberry precursor concentration at harvest
$prec_{minvar}$	strawberry precursor concentration at minimal variation
q_a,q_b	class border a or b, expressed as $Pchl_0$
t_m	maturity of an individual
t^b_m	maturity for a batch
t_{minvar}	time after start growth when minimal variation in Pchl or prec occurs
T-Pchl	Pchl concentration stored in a the ternary complex
T-$Pchl_s$	Pchl concentration stored in a the ternary complex at start cucumber growth

Table 7.1. Legend and abbreviations.

Batch keeping quality for cucumbers

A colour model was developed describing the change in concentration of the compounds with green colour (chlorophyll (CHL) and chlorophyllide (chl)) and their precursor (protochlorophyllide (Pchl)) over time. It was assumed that no precursor would be synthesised after harvest. Colour is defined as the sum of CHL and chl (Chapter 5). The mechanism is shown in Fig. 7.1, together with an indication of the colour behaviour in time for three hypothetical cucumbers. These cucumbers differ only in $Pchl_0$, with $Pchl_0$ being the concentration of Pchl present at harvest time. The colour model is generic and does not contain or need cultivar factors (Chapter 5).

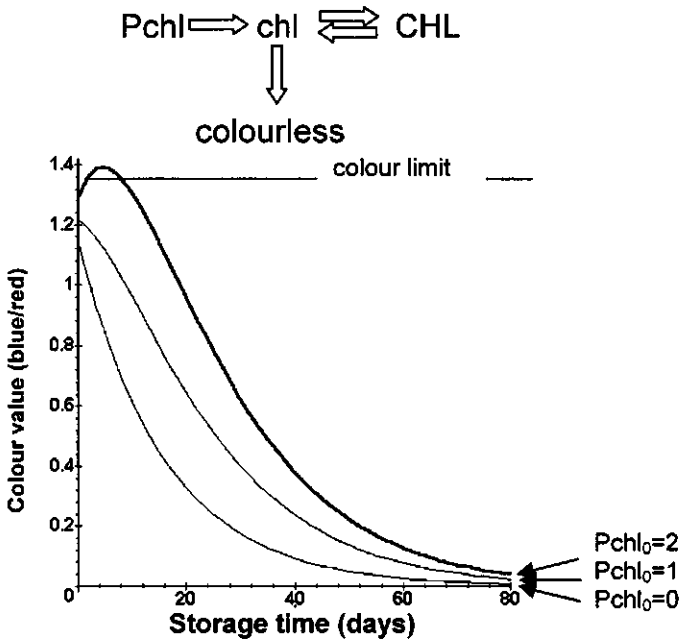

Fig. 7.1. Colour behaviour during postharvest dark storage at 20 °C for three hypothetical cucumbers. Indicated is the acceptance colour limit. The cucumber colour model is shown in a schematic form. Arrows indicate synthesis and catabolism of colour components (chlorphyllide (chl), chlorophyll (CHL) and their colourless precursor (Pchl)).

The batch keeping quality was defined as the time it takes until 5% of all cucumbers in a batch reach the colour limit (Chapter 4). Batch keeping quality can be obtained by estimating the time it takes for every cucumber in a batch to cross the colour limit and applying a simple linear interpolation procedure to estimate when 5% of the individuals in a batch crossed the colour limit. To do this, the colour development in time needs to be assessed by repeatedly measuring all individual cucumbers in the batch. When these colour data were analysed with the colour model it was found that the *average* concentration of $Pchl_0$ per batch was closely related to the batch keeping quality (Chapter 5).

Batch keeping quality for strawberries

Batch keeping quality for strawberries can be defined as the time it takes until 5% of all strawberries in a batch is spoiled by *Botrytis cinerea* (Hertog et al., 1999). Keeping quality may depend on the ability to sufficiently produce anti-fungal compounds to keep botrytis in a quiescent state. A colour model was developed describing the change in

concentration of compounds responsible for keeping botrytis quiescent (proanthocyanidins (PA)), the colour compounds (anthocyanins) and their common precursor during dark storage. It was assumed that no precursor would be synthesised after harvest (Chapter 6). The mechanism is shown in Fig. 7.2, together with an indication of the colour behaviour in time for three hypothetical strawberries. These strawberries differ only in $prec_0$, with $prec_0$ being the concentration of precursor present at harvest time.

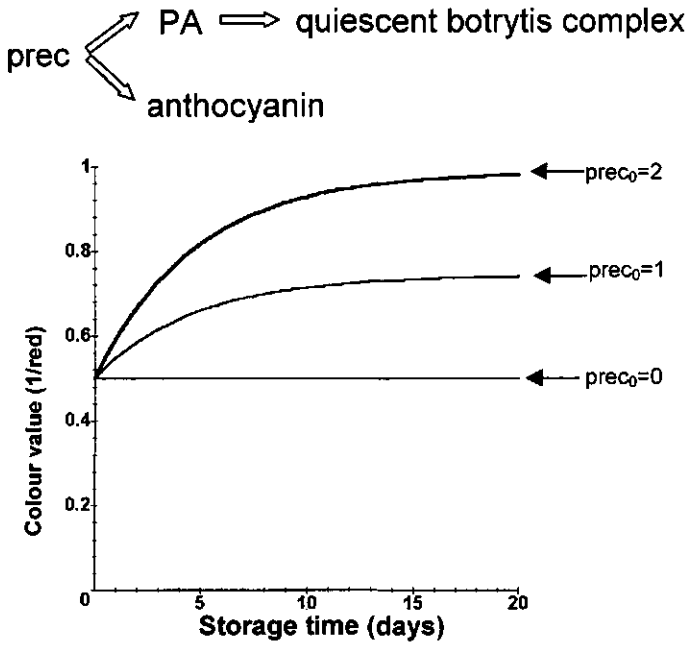

Fig. 7.2. Colour behaviour during postharvest dark storage at 20 °C for three hypothetical strawberries. The strawberry colour model is shown in a schematic form. The precursor (prec) of both colour compounds (anthocyanin) and the anti-fungal compounds (PA) next to the quiescent botrytis complex (PA-botrytis) are shown.

To obtain the batch keeping quality, the percentage spoilage over time per batch was fitted to a function describing a sigmoidal change in time. The batch keeping quality was established as the time when the sigmoidal function exceeded the 5% spoilage limit. A storage temperature correction was included by assuming that the spoilage rate constant of the sigmoidal function was dependent on temperature according to Arrhenius' Law, enabling the estimation of the keeping quality for each batch at the same temperature. When the colour data per strawberry, obtained by following the colour development in time by repeatedly measuring the same strawberries, were analysed with

the strawberry colour model it was found that the *average* concentration of $prec_0$ per batch was closely related to the batch keeping quality (Chapter 6).

Cucumber precursor behaviour

So, the Pchl concentration at harvest is the determining factor for the batch keeping quality of cucumbers. It is therefore of interest to investigate the behaviour of Pchl in terms of synthesis and decay during the preharvest period. Pchl is stored in a ternary complex (T-Pchl), together with the photo enzyme POR and NADPH, and can be released under the influence of light (Porra, 1997). The conversion of Pchl to chlorophyllide by POR is also known for its requirement for light (Lebedev and Timko, 1998). It is assumed that the concentration of T-Pchl only decreases after start of cucumber fruit growth, implying that T-Pchl synthesis in the cucumber matches the volume increase during the preharvest period but not the transformation into colour components.

The behaviour of Pchl during the pre- and post harvest period can be described as a consecutive reaction where Pchl as part of the ternary complex (T-Pchl) is transformed into Pchl (Eq. 7.1), and the subsequent decay of Pchl (Eq. 7.2) into colour components.

$$\text{TPchl} \xrightarrow{k_t \cdot \text{light}} \text{Pchl} \tag{7.1}$$

$$\text{Pchl} \xrightarrow{k_f \cdot \text{light}} \text{colour components} \tag{7.2}$$

where k_t is the reaction rate constant for the formation of Pchl from TPchl and k_f the reaction rate constant for the formation of colour components. Light conditions affect the transformation from TPchl to Pchl and from Pchl to colour components directly by increasing the apparent reaction rate k*light. From this proposed mechanism, the behaviour of Pchl over time, disregarding the colour components can be extracted using the fundamental rules of chemical kinetics. The set of differential equations is given in Eqs. 7.3-7.4.

$$\frac{d\,\text{TPchl}}{dt} = -k_t \cdot \text{light} \cdot \text{TPchl} \tag{7.3}$$

$$\frac{d\,\text{Pchl}}{dt} = k_t \cdot \text{light} \cdot \text{TPchl} - k_f \cdot \text{light} \cdot \text{Pchl} \tag{7.4}$$

Assuming that no Pchl is available at the start of cucumber growth, as all initial Pchl is immediately absorbed in the ternary complex, the behaviour of Pchl can be expressed as follows (Eq. 7.5).

$$Pchl(t) = \frac{k_t \cdot TPchl_s(e^{-k_f \cdot light \cdot t} - e^{-k_t \cdot light \cdot t})}{k_t - k_f} \qquad (7.5)$$

where $TPchl_s$ is the concentration of TPchl present at the start of the cucumber growth.

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 Fig. 7.3 shows the simulated Pchl concentration after the start of cucumber growth assuming that light conditions vary maximally a factor two between the lowest and highest amount of light. The other left-hand side plots of Fig. 7.3 show the Pchl concentration starting at different values for $TPchl_s$. Regardless of $TPchl_s$, variation in Pchl over time will be minimal at t_{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} (left-hand side plots of Fig. 7.3). t_{minvar} is independent of $TPchl_s$ (left-hand side plots of Fig. 7.3) but will depend on k_t and k_f. Analysing cucumber colour data, the numerical values of all reaction rate constants, including k_f, were very similar for a number of cultivars (Chapter 5). It is therefore logical to assume that, next to k_f, k_t does not show much difference between cultivars. In that case the time after anthesis with minimal variation in Pchl will be at t_{minvar} regardless of cultivar when grown at the same temperature. Interestingly, the behaviour of Pchl over time, starting at t_{minvar}, resembles a s-curve (left-hand side plots of Fig. 7.3).

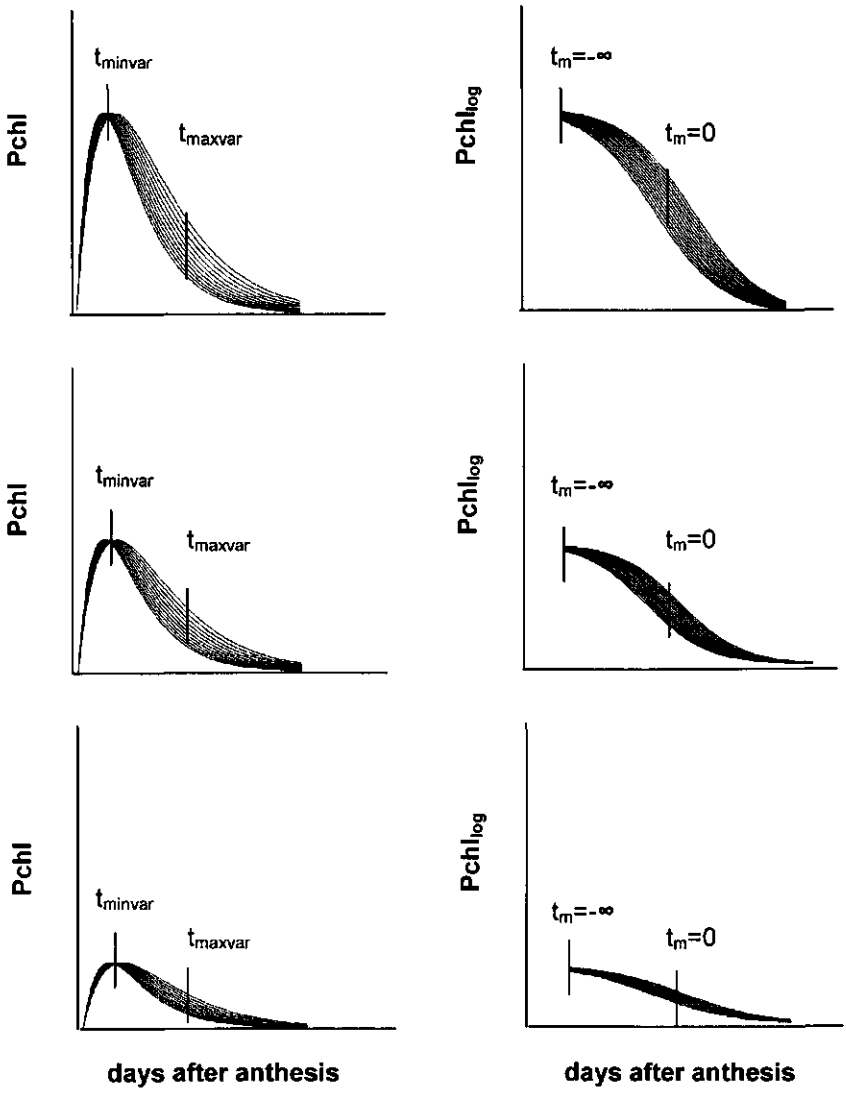

Fig. 7.3. Simulated precursor behaviour over time for individual cucumbers belonging to the same batch. The upper left-hand side plot shows the precursor behaviour (Pchl) assuming that each cucumber is grown at slightly different light conditions, applying Eq. 7.5. The different left-hand side plots show the effect of different initial amounts of TPchl and constant light variation. 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. 7.6. The different right-hand side plots show the effect of different amounts of $Pchl_{minvar}$.

The batch model formulation

The batch model aims to describe the variation in the precursor values encountered at harvest. To do so, we need to invert the precursor function for a mathematical reason described below. With the current representation of the precursor function, Pchl, this is not possible because the Pchl function (Eq. 7.5) is first increasing and later decreasing over time (left-hand plots of Fig. 7.3). So, we need to find a representation of the precursor function that may be inverted. Here, we propose that a decreasing logistic between $Pchl_{minvar}$ and 0 (Eq. 7.6) may be approximated by the Pchl function (Eq. 7.5) when harvest takes place at or after t_{minvar}.

$$Pchl_{log}(t_m) = \frac{Pchl_{minvar}}{1+e^{(Pchl_{minvar} \cdot t_m)}} \qquad \text{with } t_{minvar} \le t_m \qquad (7.6)$$

When variation is assumed on t_m, minimal variation in $Pchl_{log}$ is reached at $t_m=-\infty$. After $t_m=-\infty$ the variation increases, reaching the maximal variation at $t_m=0$, and then decreases again (upper plot of Fig. 7.4). The variation pattern is symmetrical around $t_m=0$. This $Pchl_{log}$ variation pattern is similar to the Pchl variation pattern when t_{minvar} is substituted with $t_m=-\infty$ and t_{maxvar} is substituted with $t_m=0$ in the Pchl equation (Eq. 7.5). Applying different values for $Pchl_{minvar}$ and constant variation in t_m, the variation patterns of Pchl for different values of $TPchl_s$ (left-hand side of Fig. 7.3) can be simulated satisfactorily (right-hand side of Fig 7.3). It seems, therefore, that a decreasing logistic function with variation in t_m, can simulate the variation in Pchl, caused by the variation in light conditions during the preharvest period. So, although the actual behaviour of the Pchl equation (Eq. 7.5) and the logistic function (Eq. 7.6) over time differ, the variation patterns seem to be very similar; variation in light conditions during the preharvest period is represented by variation in t_m.

By using the logistic function (Eq. 7.6) for the Pchl equation (Eq. 7.5), a time transformation is introduced. In Eq. 7.5, t stands for the time from the anthesis, but in Eq. 7.6 it is replaced by t_m, the maturity with regard to keeping quality. Normally, maturity is used in terms of fitness for harvest or in terms of fitness for human consumption during the pre- and postharvest period, respectively. Neither description of maturity is suitable with regard to the keeping quality, and we propose that t_m can be regarded as an index of maturity towards keeping quality. At $t_m=-\infty$ the maximal maturity and at $t_m=+\infty$ the minimal maturity is defined for an individual cucumber. Harvest at $t_m=-\infty$ will result in a maximal amount of Pchl, and therefore also the maximal keeping quality for this cucumber. At

t_m=+∞ no Pchl is left and harvest at this point will result in the cucumber losing colour quickly, in spite of the initial colour ($Pchl_0$=0 in Fig. 7.1).

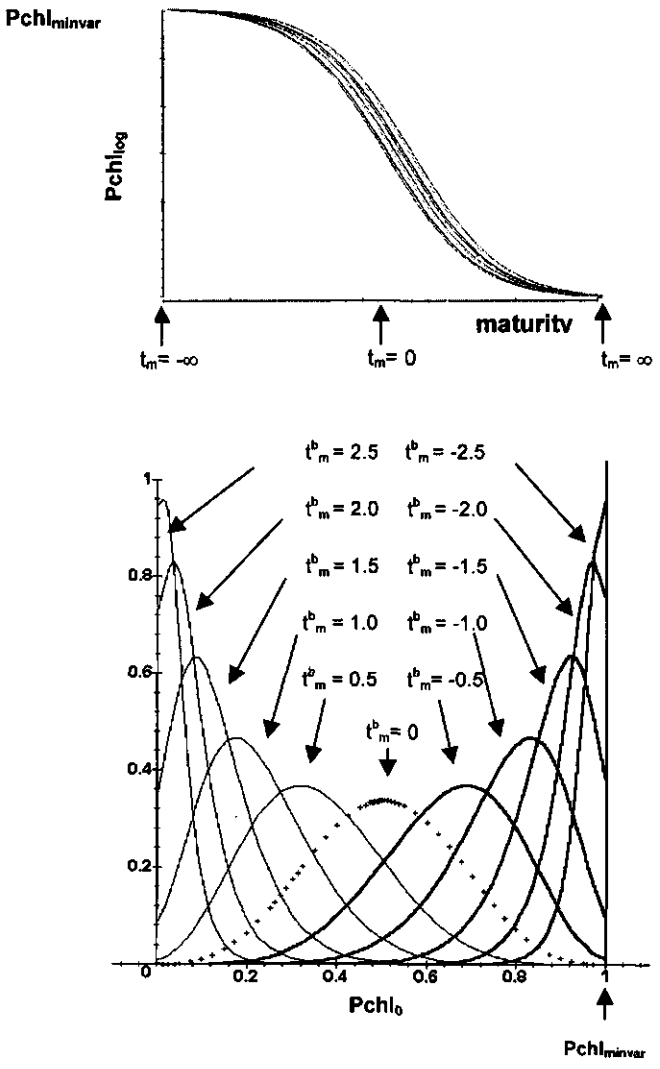

Fig. 7.4. Simulation of the Pchl distribution of one batch as function of maturity, indicated by t_m (upper plot). Simulation of Pchl distributions for one batch harvested at different maturity stages, indicated by t^b_m. Simulation was carried out using $Pchl_{minvar}$=1 and σ=0.5, applying Eq. 7.13 (lower plot).

The batch model aims to describe the variation in $Pchl_0$ values of cucumbers in terms of light variation during the preharvest period. Mathematically this can be expressed in terms of the probability (Pr) that $Pchl_0$ values belong to a class $(q_a,q_b]$, which includes class border q_b and excludes class border q_a. The probability that $Pchl_0$ values are part of the class $(q_a,q_b]$, (left-hand side of Eq. 7.7) can be described as the probability that $Pchl_0$ values are smaller than or equal to q_b minus the probability that $Pchl_0$ values are smaller than or equal to q_a (right-hand side of Eq. 7.7) (section 3.4 of McClave and Sincich, 2003).

$$Pr(Pchl_0(t) \in (q_a,q_b]) = Pr(Pchl_0(t) \le q_b) - Pr(Pchl_0(t) \le q_a) \tag{7.7}$$

The $Pchl_0$ variation pattern may be approximated using a logistic function and assuming variation in t_m (Eq. 7.6). Now we can transform the probability that $Pchl_0$ values are smaller than general class border q (right-hand side of Eq. 7.7) to Eq. 7.8 (section 3.3 of McClave and Sincich, 2003).

$$Pr(Pchl_0(t) \le q) \approx Pr(Pchl_{log}(t_m) \le q) \tag{7.8}$$

If we assume that t_m is a random variable with continuous distribution function ψ we can rewrite the right-hand side of Eq. 7.8 into Eq 7.9, with $Pchl_{log}^{-1}$ being the inverse of $Pchl_{log}$.

$$Pr(Pchl_{log}(t_m) \le q) = Pr\left(t_m \ge Pchl_{log}^{-1}(q)\right) = 1 - \psi\left(Pchl_{log}^{-1}(q)\right) \tag{7.9}$$

As the logistic function (Eq. 7.6) can be inverted (Eq. 7.10), it is now possible to rewrite the stochastic batch model (left-hand side of Eq. 7.7) to describe the variation in $Pchl_0$ values of cucumbers at harvest in terms of variation in t_m (Eq. 7.11)) (section 5.3 of McClave and Sincich, 2003). The ≈ sign in Eq. 7.11 refers to the approximation shown in Eq. 7.8.

$$Pchl_{log}^{-1}(q) = \frac{\ln\left(-\frac{q - Pchl_{minvar}}{q}\right)}{Pchl_{minvar}} \tag{7.10}$$

$$Pr(Pchl_0(t) \in (q_a,q_b] \approx \psi\left(\frac{\ln\left(-\frac{q_a - Pchl_{minvar}}{q_a}\right)}{Pchl_{minvar}}\right) - \psi\left(\frac{\ln\left(-\frac{q_b - Pchl_{minvar}}{q_b}\right)}{Pchl_{minvar}}\right) \tag{7.11}$$

The last step in the formulation of the batch model is the choice for the distribution function ψ. If we assume, out of convenience, t_m to be normally distributed, with mean t^b_m (batch maturity) and standard deviation σ (biological variation), then ψ can be expressed as Eq. 7.12.

$$\Psi(t_m) = \Phi\left(\frac{t_m - t^b_m}{\sigma}\right) \tag{7.12}$$

with Φ the cumulative standard normal distribution function. Φ is often a standard function in statistical packages. Hence, the batch model formulation can now be expressed in its final formulation (Eq. 7.13).

$$Pr(Pchl_0(t) \in (q_a, q_b]) \approx \Phi\left(\frac{\ln\left(-\frac{q_a - Pchl_{minvar}}{q_a}\right)}{\frac{Pchl_{minvar}}{\sigma}} - t^b_m\right) - \Phi\left(\frac{\ln\left(-\frac{q_b - Pchl_{minvar}}{q_b}\right)}{\frac{Pchl_{minvar}}{\sigma}} - t^b_m\right) \tag{7.13}$$

An indication of the batch behaviour for one batch varying in maturity (having $Pchl_{minvar} = 1$), is shown in the lower plot of Fig. 7.4. Variation in $Pchl_0$ at harvest can now be described on a batch level in terms of maturity (t^b_m), biological variation (σ) and $Pchl_{minvar}$.

Results & Discussion

Spanish cucumber batches

The $Pchl_0$ distributions for the autumn batches of 'Beluga' and 'Volcan' have the skewed shape indicative for batches with a low $Pchl_0$ concentration (square symbols in the bottom and top plots of Fig. 7.5). The shape of the $Pchl_0$ distributions of the spring batches of 'Volcan', ' Borja' and 'Beluga' is similar to the normal, symmetrical, distribution (shown as diamond symbols in Fig.7.5).

Applying the batch model formulation of Eq. 7.13 during initial analysis resulted in the estimation of the parameters t^b_m, σ and $Pchl_{minvar}$ 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 7.2). From these parameters the $Pchl_0$ probability was calculated (shown as lines in Fig. 7.5). Remarkable is that all autumn batches have a positive and all spring batches have a negative value of t^b_m. This indicates that all cucumbers belonging to the spring batches have a maturity closer to $t_m=-\infty$, the maximal maturity. In other words, the spring batches may be considered younger than the autumn batches. Furthermore, a three-fold difference in the biological variation (σ) is encountered between the spring batch of 'Borja' (0.157) and the autumn batch of 'Volcan' (0.519). This indicates that, even though batches were grown under equal conditions and all cucumbers were of marketable size, large biological variation is present. Remarkable is that the spring batch of 'Borja' shares the highest value of $Pchl_{minvar}$, a maturity closest to the maximal maturity ($t_m=-\infty$) and the smallest biological variation, resulting in a batch keeping quality of more than 13 days (Table 7.2).

$Pchl_{minvar}$, is largest for 'Borja' followed by 'Beluga' and finally 'Volcan'. This apparently cultivar dependent parameter may be defined as the cultivar keeping quality, the keeping quality at maximal maturity for batches of the same cultivar. As a relation is present between the level of $Pchl_{minvar}$ and $TPchl_s$ (left-hand side plots of Fig. 7.3) it might be hypothesised that the initial amount of TPchl at the start of the cucumber growth shows very little variation for cucumbers of the same cultivar. It might therefore be a determining factor for the cultivar keeping quality.

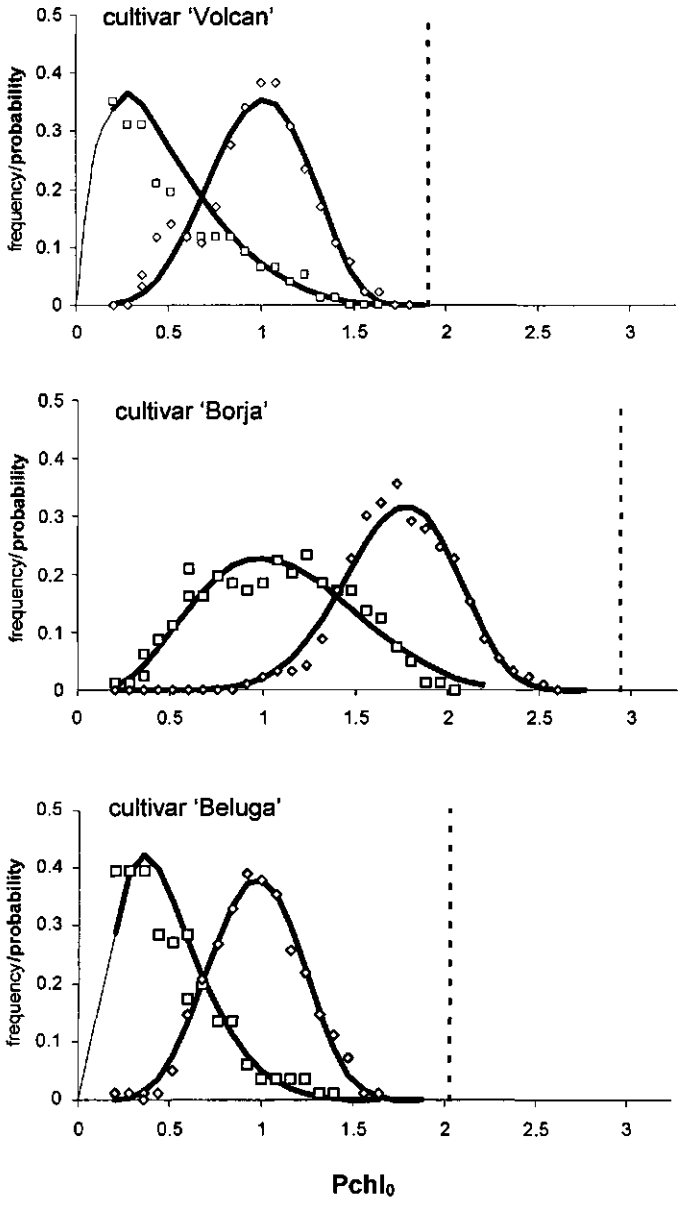

Fig. 7.5. Distribution of cucumber precursor concentrations at harvest ($Pchl_0$) for two Spanish batches per cultivar. One batch was harvested in the autumn season (□) or the spring season (◊) per cultivar. Symbols indicate the $Pchl_0$ batch distribution obtained from colour data and the lines the distribution from batch model estimations. $Pchl_{minvar}$ is indicated per cultivar by the dashed line.

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	0.051		
'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							

Table 7.2. Overview of parameter estimates and their standard error (s.e.) belonging to the six cucumber batches.

Dutch cucumber batches

$Pchl_0$ distributions for the batches belonging to three experimental cultivars are shown as symbols in Fig. 7.6 as an example. For cultivar I, the shape of the distributions varies between two limits, in which vicinity they are skewed. On the other hand, for cultivar c distributions are encountered which have an almost normal shape. Applying the batch model resulted in estimations of t^b_m, σ per batch and $Pchl_{minvar}$ per cultivar (Table 7.3 and lines in Fig. 7.6). All cultivars in Table 7.3 are listed with decreasing $Pchl_{minvar}$ value. Differences in $Pchl_{minvar}$, the cultivar keeping quality, are considerable among the twelve experimental cultivars. Variation in σ, the biological variation, between the batches is even larger, up to a factor 20 (Table 7.3). The average value for σ of batches belonging to the same cultivar is correlated with $Pchl_{minvar}$ (R^2_{adj}=75% (not shown)), indicating that the amount of biological variation might be cultivar dependent.

A common value of $Pchl_{minvar}$ was estimated for batches harvested from stem or vine parts of the same cucumber plants. This may indicate that, cultivar induced effects on keeping quality remain unchanged for cucumbers, regardless of changing sink-source relations in the ageing cucumber plant. Of the batches belonging to experimental cultivars a to f, ten batches have positive values for t^b_m. This is encountered for only five batches of the cultivars g to l (boxed in Table 7.3). So, batches belonging to cultivars with a high value for $Pchl_{minvar}$ tend to have positive values of t^b_m and vice versa. This may be related to the harvest criterion. Cucumbers are primarily harvested when sufficient weight is accumulated (Marcelis, 1998), colour is of secondary concern. A batch with a positive t^b_m value consists of cucumbers that have reached their harvest weights relatively late compared to a batch of the same cultivar with a negative value of t^b_m. This implies that batches from cultivars with a low value for $Pchl_{minvar}$ are generally better synchronized with

the harvest criterion with regard to a high keeping quality. Better batch keeping quality may be achieved by applying a cultivar with a high value of $Pchl_{minvar}$ and then harvesting a few days before the weight criterion has been reached. These batches may be labelled as having a somewhat lower harvest weight and a high batch keeping quality intended for export markets. The extra revenue generated from the high batch keeping quality should be compared to the smaller revenue due to the lower batch harvest weight.

Strawberry batches

The precursor distributions at both the first and second harvest (symbols in Fig. 7.7) show similar behaviour as the cucumber distributions. Applying the logistical batch model formulation (Eq. 7.13) for $prec_{0,}$ instead of $Pchl_0$, resulted in parameters t^b_m, σ and $prec_{minvar}$ (instead of $Pchl_{minvar}$) per batch. The value for $prec_{minvar}$ was similar for all batches. So, $prec_{minvar}$ was taken in common for all batches and the batch parameters were estimated (Table 7.4). From these parameters the $prec_0$ probability was calculated (lines in Fig. 7.7). Remarkable is that, contrary to the cucumber batches, there is little variation in the biological variation (σ) among the strawberry batches. So, most differences between the $prec_0$ distributions could be assigned to differences in maturity (t^b_m).

Obviously, the behaviour of the strawberry precursor ($prec_0$) could be treated similar to the cucumber precursor ($Pchl_0$) on the batch level. There is not much known about the nature of the strawberry precursor. It is known that precursor release is stimulated by postharvest light treatment. Poor red colour and 'white shoulders' could be overcome while at the same time diminishing fruit rot (Saks et al., 1996). Recently, the role of two enzymes at the end of the flavonoid pathway was investigated to explain the difference in light sensitivity of red coloration between two Japanese strawberry cultivars (Li et al., 2002). Perhaps a strawberry precursor complex exists, including a photo enzyme, that is stimulated by light to release the precursor, much in the same way as described for the cucumber precursor (see earlier in this chapter). If variation in light conditions during the preharvest period is the main source of variation, similar precursor behaviour at harvest might be expected for batches of cucumbers and strawberries. Unfortunately, strawberry batches of only one cultivar were available, so whether different values for the cultivar keeping quality ($prec_{minvar}$) exist remains to be established.

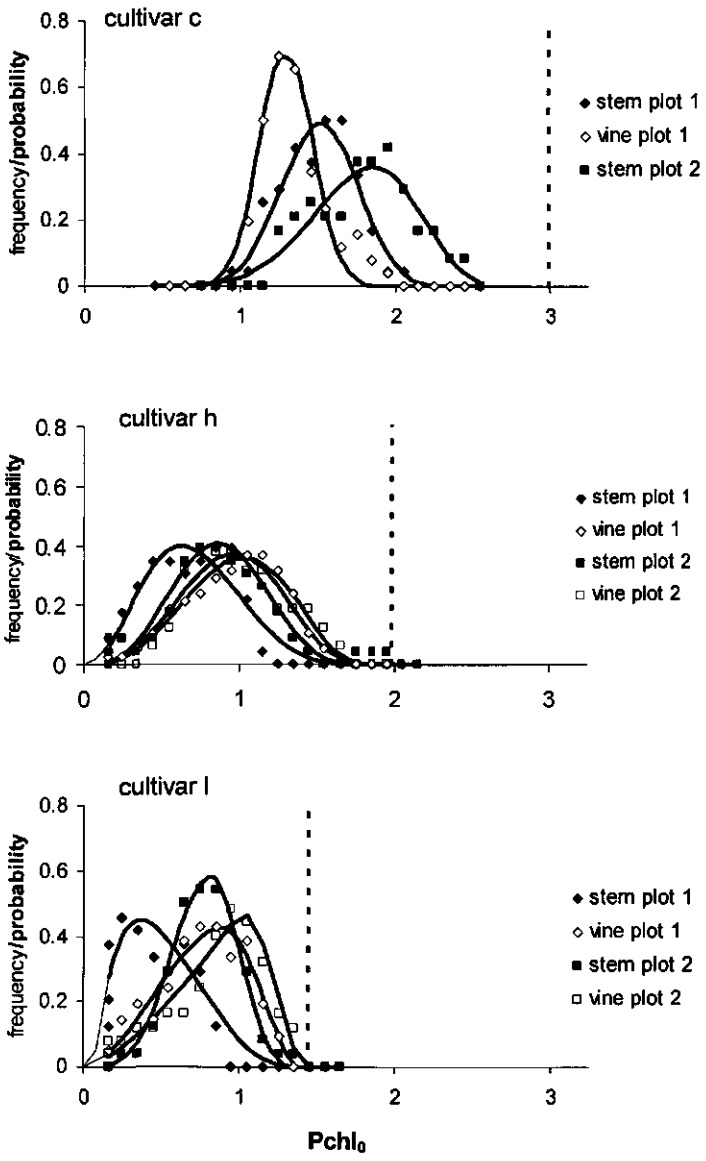

Fig. 7.6. Distribution of cucumber precursor concentrations at harvest ($Pchl_0$) for three or four Dutch batches per cultivar. Batches were harvested from either different plots (plot 1 or plot 2) and from different parts of the cucumber plants (stem or vine). Symbols indicate the $Pchl_0$ batch distribution obtained from colour data and the lines the distribution from batch model estimations. $Pchl_{minvar}$ is indicated per cultivar by the dashed line.

cultivar	stem/vine	t^b_m		σ		$Pchl_{minvar}$	
		estimate	s.e.	estimate	s.e.	estimate	s.e.
a	stem	-0.031	0.086	0.073	0.027		
a	vine	0.048	0.141	0.097	0.036	3.25	0.20
a	stem	0.134	0.058	0.098	0.036		
b	stem	0.070	0.153	0.095	0.046		
b	vine	0.099	0.139	0.086	0.040	3.00	0.77
b	stem	0.190	0.103	0.042	0.047		
b	vine	0.040	0.169	0.061	0.030		
c	stem	-0.044	0.091	0.114	0.029		
c	vine	0.064	0.067	0.072	0.016	3.00	0.33
c	stem	-0.195	0.130	0.174	0.057		
d	stem	0.255	0.058	0.097	0.056		
d	vine	-0.092	0.131	0.133	0.039	2.54	0.31
d	stem	0.025	0.100	0.154	0.039		
d	vine	-0.081	0.128	0.120	0.034		
e	stem	-0.195	0.116	0.169	0.039		
e	vine	0.218	0.121	0.151	0.036	2.41	0.20
e	stem	-0.187	0.114	0.171	0.039		
f	stem	-0.392	0.100	0.201	0.037		
f	vine	-0.074	0.056	0.100	0.013	2.35	0.12
f	stem	-0.471	0.114	0.249	0.053		
g	stem	-0.115	0.030	0.342	0.021		
g	vine	-0.509	0.053	0.208	0.018	1.91	0.04
g	stem	-0.645	0.064	0.406	0.046		
h	stem	0.282	0.042	0.378	0.042		
h	vine	-0.101	0.083	0.394	0.061	1.98	0.12
h	stem	0.054	0.064	0.339	0.045		
h	vine	-0.045	0.076	0.373	0.054		
i	stem	-0.120	0.086	0.263	0.036		
i	vine	-0.402	0.129	0.236	0.043	1.83	0.10
i	stem	-0.242	0.103	0.233	0.036		
j	stem	0.239	0.048	0.444	0.042		
j	vine	-0.577	0.131	0.533	0.095	1.65	0.07
j	stem	-0.100	0.076	0.487	0.057		
k	stem	0.411	0.048	0.671	0.070		
k	vine	-0.800	0.211	0.394	0.099	1.61	0.08
k	stem	-0.313	0.119	0.292	0.046		
l	stem	0.470	0.040	0.714	0.049		
l	vine	-0.377	0.076	0.727	0.066	1.44	0.04
l	stem	-0.346	0.068	0.484	0.043		
l	vine	-0.690	0.102	0.846	0.099		

Table 7.3. Overview of parameter estimates and their standard error (s.e.) of 40 cucumber batches from twelve experimental cultivars sorted on $Pchl_{minvar}$.

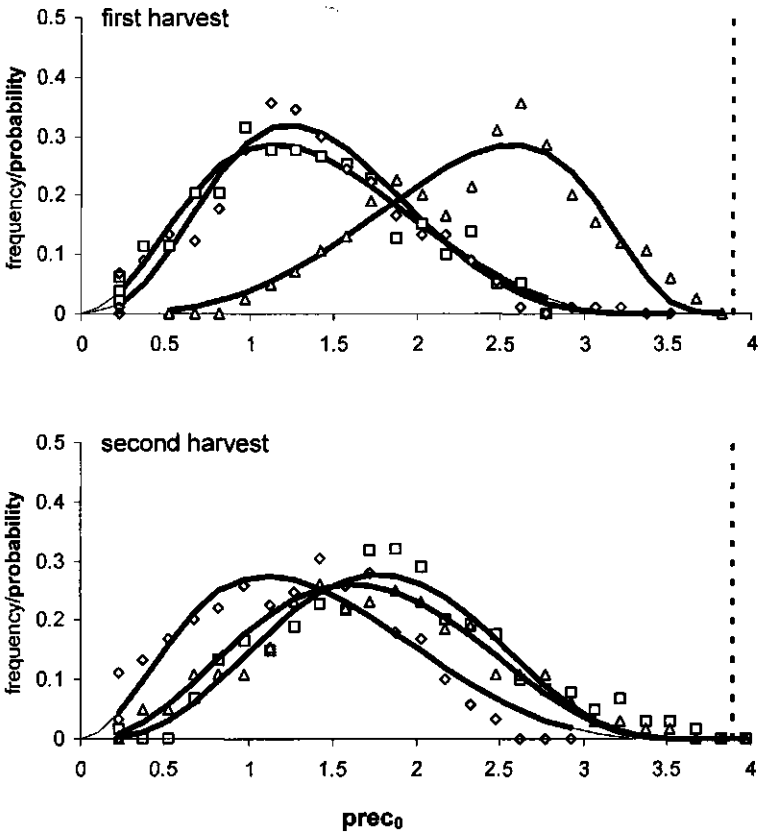

Fig. 7.7. Distribution of strawberry precursor concentrations at harvest ($prec_0$) for three batches per harvest. Symbols indicate the $prec_0$ batch distributions obtained from colour data and the lines the distribution from batch model estimations. $prec_{minvar}$ is indicated by the dashed line.

batch keeping quality (days)	t^b_m estimate	s.e.	σ estimate	s.e.	$prec_{minvar}$ estimate	s.e.
3.6	0.157	0.017	0.205	0.018		
4.6	0.147	0.017	0.179	0.016		
7.8	-0.174	0.044	0.211	0.033	3.890	0.168
6.6	0.011	0.026	0.190	0.020		
3.6	0.161	0.018	0.215	0.019		
4.0	0.046	0.024	0.204	0.021		
R^2_{adj} (%)	87.8					
N	159					

Table 7.4. Overview of parameter estimates and their standard error (s.e.) belonging to the six strawberry batches of cultivar 'Elsanta'.

Batch model considerations

The batch model may be divided in two parts: the formulation of the precursor behaviour (the kinetics, Eqs. 7.1-7.6), and the development of the stochastics (Eqs. 7.7-7.13). In the kinetics part the precursor behaviour for cucumber was deduced. Other products may have keeping quality determining factors that show completely different behaviour. Nonetheless, if a function for the keeping quality determining factor can be deduced, a batch model can be formulated as the stochastic part of the batch model is generic. The stochastic part of the batch model shows similarity with the approach proposed by Hertog (2002) who outlines a mathematical approach to interpret batch behaviour in terms of shelf life for shrivelling of apples and colour change of avocados. Prerequisite for this approach is the availability of a quality function that describes the behaviour over time for individuals and a product property as the main source of biological variation. In the current approach, however, the main source of biological variation is not a product property, but the variation in light conditions during the preharvest period. Second step in Hertog's approach is to invert the quality function, transforming the product property distribution into a distribution of shelf lives. This step is similar to our approach. The transformation of the product property function into a cumulative distribution function is, however, quite different. First, the need for the product property to be normally distributed is not necessary in our approach as the distribution function Ψ (Eq. 7.12) can be any distribution function. The choice for the normal distribution function was based on convenience only, and more research should be done to support or reject this choice. Secondly, the cumulative normal distribution used in both approaches has indeed no analytical solution, but statistical packages like Genstat have no problem analysing data with an error function like Φ (Eq. 7.13). The use of the 'logistic binomial' function by Hertog is therefore unnecessary. Also, when a comparison is made between the cumulative normal distribution and the 'logistic binomial' function, such as shown by Hertog (2002), the most deviating part is at low (and high) distribution values. A number of products have a batch acceptance limit at 5%, like cucumber (colour), strawberries (spoilage), apples and pears (weight loss). Therefore, by using the 'logistic binomial' a methodological error is introduced.

Traditionally, biological variation is treated like an ever-present nuisance which should be minimised as much as possible. The most commonly used technique to deal with biological variation is sorting and grading on external quality attributes (Tijskens et al., 2003). Colour sorting based on averages is, however, not sufficient for cucumber and strawberry with regard to keeping quality (Chapters 5 and 6). Building in biological variation on the level of t_m for the precursor behaviour seems to be a step towards

understanding biological variation and linking postharvest methodology to preharvest information.

Variation in precursor at harvest for cucumber batches from the same cultivar is assumed to be caused by variation in light conditions during the preharvest period. While variation in light conditions is probably an important source of biological variation, it is surely not the only source. A number of preharvest factors, including water stress, pest management and insufficient availability of micronutrients may have an influence on the variation in precursor concentration encountered at harvest. The correct description and inclusion of multiple sources of biological variation is one of the challenges for future work on batch model formulations.

An application of the batch model might be the determination of cultivar keeping quality as a test tool for the development op new cultivars by cucumber-, and possibly strawberry-, breeding companies. However, also suitability for long term storage and optimal harvest date for, e.g., batches of apples and pears might be assessed, provided a function for the (keeping) quality determining factor is available.

Conclusions

A batch model is presented to assess keeping quality precursor distributions in terms of maturity and biological variation for cucumber and strawberry batches of the same cultivar. The model describes the influence of one source of biological variation, here assumed to be variation in light conditions during the preharvest period, on the distribution of precursor concentrations at harvest. Estimations on cucumber batches grown in different seasons and cultivars revealed that next to light variation, also cultivar is a source of biological variation.

The batch model consists of kinetic part, describing the behaviour of the precursor for individuals, and a stochastic part, describing the precursor distribution. The stochastic part is generic, but the kinetic part depends on processes determining the keeping quality. This means that the application of the batch model is limited only to the availability of fundamental, kinetic, models that describe individual quality determining behaviour.

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8

Discussion

The gap

Traditionally, the horticultural production chain is separated in a preharvest and a postharvest part. In the preharvest part, growers generally try to find an optimum between quantity and quality and in the postharvest part quality retention is maintained as good as possible by a quick and cold transfer towards the consumer. In the Netherlands, both separate parts of the horticultural production chain are well organised, but hardly interact with each other. Growers can often not perceive what the keeping quality of products will be at harvest time. The same can happen at wholesale organisations and auctions when products are judged by quality inspectors which can assess current quality attributes, but not the expected keeping quality.

Nowadays, there is a strong trend that growers need to be certified according to e.g. EUREPGAP (http://www.eurep.org/) and HACCP standards. Included in these certification protocols is the necessity to trace and track batches on their way through the chain. This is currently one way communication, e.g. to track the origin of health hazards for consumers back towards growers. However, the same infrastructure may also be used to transfer preharvest information further down the horticultural production chain. Keeping quality is produced during preharvest. It has been shown that the EC level of the nutrient solution for cucumbers (Chapter 4) and for strawberries (Chapter 6) has a clear effect on keeping quality. Furthermore, keeping quality is produced batch-wise (Chapter 5). For instance, a two-fold difference in keeping quality between batches of strawberries of the same cultivar and grower was established within a two-week harvest window (Table 6.1). This kind of preharvest information is not communicated towards other participants in the horticultural chain. This is mainly because it is not known which information should be gathered and how it should be used. Sending this information on a batch level would link the preharvest and the postharvest trajectories tightly, creating an improved performance for the horticultural production chain as a whole.

Keeping quality protocol

Cucumber growers might extract keeping quality information for harvested batches by using the *practical application* section of Chapter 5 and send this information further down the horticultural production chain. Strawberry growers could also use this check-list and ignore only point 1 or, alternatively use the time between harvests method to gather batch keeping quality information (Chapter 6). Although cucumber and strawberry may be considered either an important bulk- or an important high value- product, they consist of only a minor part of all products in the horticultural production chain. It would be

interesting to find out whether protocols, like provided in the checklist, may be created for other important products. Such a protocol can be used to predict the keeping quality, provided (i) the quality attributes that limit the keeping quality are known, (ii) relevant non-destructive measuring techniques are used and (iii) a physiological kinetic model is available.

(i) Tijskens and Polderdijk (1996) provided a list of about sixty products whose keeping quality could be described assuming a single limiting quality attribute, without actually stating the quality attribute. This finding may occur strange as e.g. apples and tomatoes have colour or firmness as a limiting quality attribute, depending on the storage temperature. In fact, cucumber-like (green beans, Brussels sprouts) and strawberry-like (raspberry, blue berry) products may be exceptions as these products show a single limiting quality attribute over a considerable temperature range. However, as long as it is known which quality attributes may be limiting under circumstances that occur in the horticultural production chain, for each of them a quality limit should be defined. Once defined, the keeping quality may be described after development of a physiological kinetic model that incorporates the behaviour of all limiting quality attributes.

(ii) Already quite a number of measuring techniques are non-destructive. However, the technique needs also sufficient accuracy. For instance, current existing colour measurement techniques are often based on the Lab colour space. It is doubtful whether this technique is sufficiently accurate to e.g. measure the colour bump, characteristic for long keeping quality cucumbers. For cucumber and strawberry the expected keeping quality can be predicted on the basis of colour measurements. The interpretation of these colour measurements into batch predictions comprises the estimation of the precursor concentration. Measuring the concentration of the chemical compound would have been much easier, however in both cases the precursor is colourless. The development of non-destructive techniques (e.g. NIR, photochemical techniques) that directly measure the keeping quality limiting compound(s) have the advantage that the complex interpretation of colour data can be omitted.

(iii) A kinetic model is a mathematical representation of a number of parallel processes occurring in the same time frame. In the current set-up, the mathematical representation needs to have an analytical solution as the model development software (Maple, Waterloo Maple Software, Waterloo, Canada) is separated from the statistical analysis software (Genstat, Lawes Agricultural Trust, Rothamsted Experimental Station, UK). An analytical solution will exist when it is possible to invert a matrix representation of the kinetic model.

Linear algebra states that often these matrices cannot be inverted, especially those representing more complicated processes. In fact, it was surprising that an analytical solution for the cucumber colour model (Chapter 5) could be generated, even without the applied steady state assumption. So, when trying to formulate new physiological kinetic models, luck comes into play whether analytical solutions exist. Using a series expansion, such as the Taylor expansion, may generate analytical solutions. However, whether a series expansion is sufficiently mimicking the actual solution depends on the order of the series expansion. A sufficient order is dependent on the kinetic parameters of the model which are unknown at the time of model development. A rather new development is the application of a software package (Athena, Stewart and Associates Engineering Software, Madison, USA) that enables combined model development and calibration on the basis of coupled partial differential equations. These differential equations can be extracted regardless of the number and complexity of the processes, opening the door to build new physiological kinetic models.

Manipulating keeping quality

During the growing period the expected batch keeping quality may be estimated under the condition of immediate harvest. Cucumbers are primarily harvested when sufficient weight is accumulated. Volume measurements (e.g. by immersion in a water bucket) can be used as a non-destructive way to measure weight as the density is almost constant (Marcelis, 1992). When sufficient harvest weight is reached, the grower might decide whether to harvest or to suspend the harvest procedure. Suspending the harvest procedure might be interesting when colour measurements over the growing period indicate an increasing batch keeping quality. This will be the case especially when a cultivar is used with a high value of $Pchl_{minvar}$ (Chapter 7). Interestingly, IMAG is developing a harvest robot (http://www.imag.dlo.nl/PDF/Publications%20Harvesting%20Robot.pdf) that can measure the volume of growing cucumbers, applying near infrared cameras. This robot is also equipped with a video camera that is able to measure colour. Based on colour and volume measurements a grower would be able to decide whether exchanging keeping quality for weight is economically desirable.

The presented physiological kinetic models are predictive in nature because of the dark storage after harvest. During dark storage precursor synthesis is inhibited and only breakdown of the accumulated pool of precursor is possible (Chapter 5, Chapter 6). The understanding that dark storage has a profound effect on the physiology of cucumbers

and strawberries opened the door to describe precursor behaviour not only during postharvest but also during preharvest. This led to a description of the precursor distribution at harvest affected by the variation in light conditions and cultivar for cucumbers (Chapter 7). Interestingly, the total amount of solar radiation can be calculated for the Spanish cucumber batches (Chapter 5). The batches were grown at the Almeria region (latitude 36.8 °). According to http://leu.irnase.csic.es/microlei/manual2/pdfs/cmbm%20eng.pdf, the solar radiation from space is on average 40.84 MJ m^{-2} d^{-1} in May-June (spring season) and 25.83 MJ m^{-2} d^{-1}in September-October (autumn season) at a latitude of 40 °. Total solar radiation received per batch was calculated by multiplying the seasonal solar radiation with the growing period (Table 8.1). More total solar radiation was received in the spring season than in the autumn season, despite the somewhat longer growing period in the autumn season (Table 8.1). Negative t^b_m values encountered in spring, indicative for batches closer to the maximal batch maturity (Chapter 7), might be related to higher amounts of total solar radiation and vice versa (Table 8.1). A combination of total received solar radiation over the growing period together with the cultivar keeping quality ($Pchl_{minvar}$, Chapter 7) might serve as an indicator of batch keeping quality. When solar radiation is measured at the greenhouse level, growing days might be exchanged for batch keeping quality.

season	cultivar	t^b_m	duration (days) anthesis-harvest	total solar radiation for Almeria (MJ/m^2)
autumn	'Volcan'	0.666	8	206.6
autumn	'Borja'	0.175	9	232.5
autumn	'Beluga'	0.638	10	258.3
spring	'Volcan'	-0.131	7	285.9
spring	'Borja'	-0.170	8	326.7
spring	'Beluga'	-0.022	9	367.6

Table 8.1 Batch characteristics, growth duration and total solar radiation for six batches of Spanish cucumbers.

Extended manipulation of batch keeping quality as function of weight development and solar radiation may be achieved when knowledge is gathered regarding the generation of keeping quality during preharvest period. Such a combined keeping quality-growth model might be build on the basis of colour and volume (weight) measurements during the whole trajectory from anthesis to harvest. Actually, a cucumber growth model already exists. Marcelis and Gijzen (1998) developed a mechanistic model to predict weekly fresh

weight yield at harvest based on modules on assimilate partitioning, greenhouse light transmission, light interception and leaf and canopy photosynthesis. Unfortunately, the assimilate partitioning module is based on the ad-hoc linking of empirical growth factors that may be calibrated satisfactorily for a batch of cucumbers, but that is unlikely applicable over batches (Schouten et al., 2002). Still, such a model would be an excellent information source to expand the keeping quality model to a combined preharvest keeping quality-growth model.

Biological variation

Biological variation was defined as the composite of biological properties that differentiate individual units of a batch and a batch was considered all individuals with a common growth history. Consequently, biological variation was expressed as being present between individuals, not within individuals (Chapter 1). Fig. 8.1 shows both yellow and green patches of a cucumber during dark storage. This is indicative of colour precursor variation within one cucumber. Patchiness is likely also present within one of the colour patches when observed with a microscope. So, the appearance of biological variation is connected to the aggregation/observation level applied, and biological variation is present on all levels.

In Chapter 7 a batch model was presented for the interpretation of batch behaviour due to one source of biological variation and cultivar. This source, the variation in preharvest light conditions is likely an important source of biological variation as nutrient and temperature profiles are often more or less constant in a greenhouse. However, there are certainly more sources of biological variation. For products which are grown in the open field, nutritional variation will be a source of biological variation that might be as important as light variation. Another source of biological variation is the position in the truss for e.g. bananas and truss tomatoes (Tijskens et al., 2003).

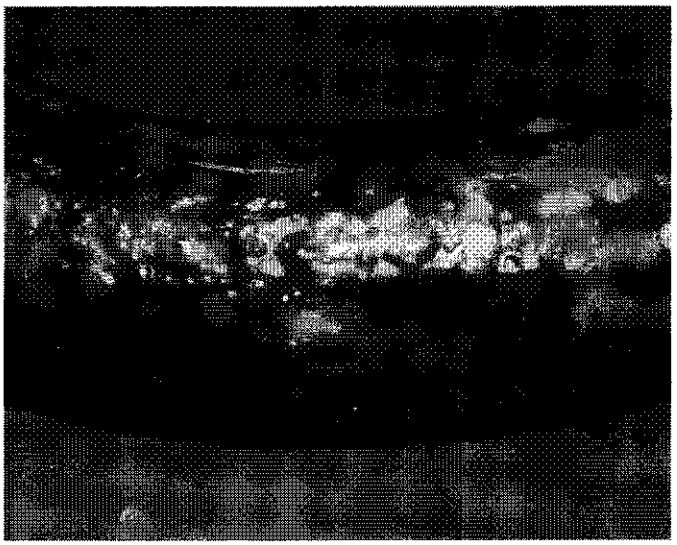

Fig. 8.1 Colour patchiness in a cucumber

An interesting question is whether a batch will have one maturity for each source of biological variation. Konapacki et al. (2003) measured SSC (Soluble Solids Content, important for a favourable taste) and firmness of cherries over time and described the observed sigmoid behaviour with a logistic function, resulting in SSC and firmness distributions similar to the ones depicted in the lower plot of Fig. 7.4. The SSC distribution changes from a skewed distribution to a distribution similar to the normal, symmetrical, distribution when batches are harvested in a 35 days window. On the other hand, the firmness distribution changes from a distribution similar to the normal, symmetrical, distribution to a skewed distribution during the same harvest window. So, these distributions look different whether the SSC or firmness distributions are considered. This may point to a batch having multiple maturities but perhaps also to a batch with only one maturity. This last option may happen when a common compound is found that is governing both firmness decay and SSC synthesis, similar to the strawberry precursor of both colour and Botrytis inhibiting compounds (Chapter 6). This means that, when the derived physiological model can be transformed into a batch model and the SSC and firmness distributions are measured at harvest, future development of both can be predicted. Multiple maturities for one batch may indicate a lack of synchronicity in the development of quality attributes. This might be the case for tropical fruit (e.g. mango) batches for which colour development is often unrelated to ripeness in terms of firmness.

Next to different sources of biological variation, there are also factors that influence batch behaviour, such as the cultivar specific parameter $Pchl_{minvar}$ for cucumbers and $prec_{minvar}$ for strawberries. This factor modifies the range of precursor concentrations

(lower plot of Fig. 7.4) for each cultivar. $Pchl_{minvar}$ could be estimated in common for stem and vine batches of the same cultivar, indicating that this factor is probably not influenced by the changing source sink ratios in the ageing cucumber plants (Chapter 7) and probably under genetic control. So, biological variation is not only determined by preharvest light conditions but also by cultivar.

Future development

Batch variation methodology was developed to solve keeping quality issues for cucumber batches. In an early stage of development it was realised that the physiological model may be product specific, but that the general approach is generic. To prove this, strawberry keeping quality issues were investigated and treated to with the same approach. Chapter 7 shows a generic approach for the formulation of batch models. The presented approaches may open up a complete new practical scientific field. Practical, because in today's world the economic emphasis is not on individual products, but on batches. Being able to supply the horticultural chain with preharvest information should increase their performance considerably in terms of achieving more revenue from guaranteed high keeping quality batches and avoiding a long horticultural production chain for short keeping quality batches. Whether batch variation methodology will achieve the actual new scientific field status depends on three issues: (i) application of current batch models to create a general practical awareness that this methodology will solve problems that cannot be solved by traditional grading and sorting, (ii) building of physiological kinetic models and (iii) solve the current mathematical limitations surrounding physiological and stochastic models and produce a generic *batch model maker*.

(i) Over 2.000.000 cucumbers per day may be sorted and graded into six weight classes and two uniformity classes at a large Dutch auction. Adding batch keeping quality information is a major logistical operation even when colour measurement equipment and a calibrated batch model can be added to existing sort and grade equipment. It would mean that weight classification is abolished and replaced by (keeping) quality classification per batch. Revenue for the participants in the chain would not be governed by general supply and demand but by supply and demand per batch keeping quality class. Currently, the change from a quantity driven cucumber chain to a quality driven chain will probably be too costly, especially when quality guidelines are not part of e.g. EUREPGAP certification. On the other hand, guaranteed keeping quality for batches of cucumbers may be an ideal marketing tool for grower associations that want to increase or protect their market share in an increasingly customer driven world. For strawberries the outlook to introduce batch keeping quality information looks

promising as one extra day of keeping quality can often be decisive whether batches are sold or rejected.

(ii) The lack of non-destructive methods or the lack of knowledge to interpret non-destructive methods is an important reason that hinders the formation of additional physiological kinetic models. Another reason is that in literature often the only applied statistical procedure is ANOVA. This statistical procedure is based on the assumption that distributions are normal, while the results presented in this thesis show that this is often not the case. The consequence of applying ANOVA is that batch information is destroyed. The main reason, however, is a mental one. Both physiologists and geneticists are focussed to measure all kinds of quality attributes without realising they are trying to interpret very complex behaviour which is actually often a combination of very simple processes. One last reason is that when physiological models are developed for predictions on batch behaviour, this should be reflected in the experimental set-up. For instance, a batch should consist of a large number of individuals. Also, perhaps as much as ten batches should be measured from different growing conditions and cultivars to create a variation in batch keeping quality. Such an experimental set-up will consist of much more measurements than currently is deemed appropriate. The big advantage, however, is that such an experimental set-up does not have to be repeated for every new growing condition and cultivar, as the kinetic parameters are reaction rate constants.

(iii) Currently, there are mathematical limitations to both the physiological kinetic part and the stochastic part of the batch model. The mathematical representation of the kinetic part needs to have an analytical solution as it needs to be inverted to be incorporated into the stochastic model (Chapter 7). Not all kinetic models will have an analytical solution and not all kinetic models with analytical solutions can be inverted. When analytical solutions exist which cannot be inverted, like the equation for the effect of light variation on the cucumber precursor concentration (Eq. 7.5), it may be approximated by a function that describes the variation patterns properly (Eq. 7.6). Another, more generic solution would be to check whether intervals exist for which the analytical solution is strictly decreasing or increasing. A separate inverse may be generated for each interval that is of practical importance. A more permanent solution would be to apply the Athena software package (see section Keeping quality protocol) to build a procedure called *batch model maker*. This would eliminate the analytical solution condition as the physiological kinetic model could be expressed as coupled partial differential equations. Whether the invertability condition can be circumvented when the batch model is generated on the partial differential equation level is unknown. Generating a *batch model maker* will be dependent on the development of physiological kinetic models, but also on solving some problems of a highly mathematical nature.

Trying to set-up a new scientific field is perhaps above all limited by funding. The funding for the application of current batch models in practise is probably not very difficult as this is a clear example of 'technology push' and often specific money sources can be tapped for this purpose. Receiving funding to build physiological models and overcome the mathematical limitations that prevent the formulation of a generic *batch model maker* is more problematic. This will be the next big challenge.

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Summary

Introduction/Overview (Chapter 1). Agricultural products show variation in quality attributes between individuals and batches. Examples of biological variation are well known but fundamental research on biological variation methodology is almost non-existent. Almost, because this thesis tries to show some key issues for the development of biological variation methodology with regard to the keeping quality of batches. Keeping quality is the time until the product attribute drops below the acceptance limit. The development is shown with regard to the batch keeping quality of strawberry and cucumber. Key issues are:

(i) repeated non-destructive measurements on individuals over time are essential to generate accurate knowledge concerning the development of a quality related property (Chapter 3, 5 and 6).

(ii) building physiological kinetic models. Kinetic modelling is a useful technique in relation to quality changes as they represent (interactions with) biochemical or physical reactions that proceed at a fixed rate and with certain kinetics (Chapter 5 and 6).

(iii) building of a batch model, capable of combining product specific kinetic models and generic stochastics. The development of these mathematical tools might enable the use of the hidden information, the biological variation, for characterisation of e.g. the keeping quality at the batch level (Chapter 7).

Literature overview (Chapter 2). In this thesis colour measurements of both cucumbers (colour retention) and strawberries (rot incidence) are linked to quality issues. Colour is perhaps the most ideal property to measure at the moment: it can be measured very accurately and the measurement is non-destructive, enabling repeated measurements over time. An overview is presented of colour measurements, colour processes, recent colour research and the bottlenecks when trying to convert physiological knowledge into colour applications. Solutions for the bottlenecks, such as

improved colour measurements, development of colour models that incorporate physiological knowledge and the batch concept are introduced here and expanded in later chapters.

First steps (Chapter 3) In the absence of defects, colour is one of the few practical criteria for assessing cucumber quality. However, cucumbers with the same colour at harvest can exhibit large differences in quality upon reaching the consumer. Photochemical and colour measurements were combined with a logistic model to test if the keeping quality could be predicted. Unfortunately, it seemed that either the colour measurements or the colour model was insufficiently developed, as the predictive power of the approach was limited.

The batch approach (Chapter 4) A batch is considered to be all individuals with a common growth history, in practise all individuals from one grower, one cultivar and one harvest. The shape of cucumbers colour distributions at harvest, obtained by measuring the colour of all cucumbers in a batch was different for batches differing in growing conditions (nutrient density and plant density). An indication for the maturity was found by observing the skewness of the colour distribution. The hypothesis was developed that characterisation of the cucumber colour distribution provides sufficient information to specify the batch keeping quality. The batch keeping quality is the number of days for which 95% of individuals in a batch exceed the acceptance limit.

Batch keeping quality of cucumbers (Chapter 5) The keeping quality for a cucumber depends on the state of the chlorophyll metabolism. In other words, whether there is still sufficient colour generation (chlorophyllide and chlorophyll) to counter the chlorophyllide breakdown. A physiological model was build that describes the postharvest colour behaviour in time and temperature for individual cucumbers, irrespective of growing conditions and cultivar on the basis of colour measurements only. For six batches, from three cultivars over two growing seasons, the batch keeping quality, was obtained. It was shown that initial colour measurements could be used to estimate the average precursor concentration per batch, which is indicative for the batch keeping quality. As the basis of the batch keeping quality predictions is a generic model, it might be possible to predict batch keeping quality for many other products which have green colour as the limiting quality attribute.

Batch keeping quality of strawberries (Chapter 6) Postharvest life of strawberries is largely limited by *Botrytis cinerea* infection. A colour model is presented that describes the development of red colour and anti-fungal function of individual strawberries over time. For twelve batches, differing in the nutrient solution concentration and flights, the batch keeping quality, was obtained. The spoilage per batch at harvest was found to relate to the precursor concentration of both the colour and the anti-fungal compounds at harvest. Batch keeping quality could be derived from the initial batch colour distributions. Also, batch keeping quality correlated highly with the time between harvest dates. For a practical implementation additional research is needed, especially regarding different Botrytis pressures between greenhouses and different strawberry cultivars.

The batch model (Chapter 7) A batch model is developed that describes the influence of one source of biological variation, the variation in light conditions during the preharvest period, on precursor distributions of cucumber and strawberry. The batch model consists of a kinetic part, describing the behaviour of the precursor for individual cucumbers and strawberries, and a stochastic part, describing the precursor distribution. The stochastic part is generic, but the kinetic part depends on the processes that determine keeping quality (Chapter 5 for cucumbers and chapter 6 for strawberries). Interestingly, cucumber batches from twelve experimental cultivars, harvested from either the stem or the vine part of cucumber plants, could be analysed together. The existsence of a cultivar specific keeping quality could be assessed.

Discussion (Chapter 8) The developed biological variation methodology may open up a complete new scientific field in agriculture. Whether this methodology will achieve this status depends on three issues: (i) application of batch models to create a general practical awareness that this methodology will solve problems that cannot be solved by traditional grading and sorting, (ii) building of physiological kinetic models and (iii) solve the current mathematical limitations surrounding physiological and stochastic models and produce a generic batch model maker.

Samenvatting

Introductie/Overzicht (Hoofdstuk 1). Landbouwproducten vertonen variatie in kwaliteitseigenschappen, zowel tussen individuen als partijen. Voorbeelden van biologische variatie zijn welbekend, maar fundamenteel onderzoek is nagenoeg afwezig. Dit proefschrift probeert dit te veranderen door de ontwikkeling van een biologische variatie methodologie te presenteren die gericht is op de houdbaarheid van partijen aardbeien en aardbeien. Houdbaarheid is de tijdsduur totdat bepaalde producteigenschappen onder de acceptatiegrens zakken. Hoofdonderwerpen zijn:

(i) herhaalde, non-destructieve metingen op individuen gedurende een bepaalde periode zijn essentieel om kennis op te doen over de ontwikkeling van een kwaliteitsgerelateerde eigenschap (Hoofdstuk 3, 5 en 6).

(ii) bouwen van fysiologische kinetische modellen. Kinetisch modelleren is een techniek die voor het modeleren van kwaliteitsverandering heel nuttig is aangezien de achterliggende interacties tussen biochemische of fysische processen kunnen worden weergegeven.

(iii) bouwen van een partijmodel dat in staat is om productspecifieke kinetische modellen en generieke stochastiek te combineren. De ontwikkeling van dit soort wiskundig gereedschap maakt het mogelijk om verborgen informatie, de biologische variatie, voor de karakterisering van bijvoorbeeld de houdbaarheid op partij niveau te gebruiken (Hoofdstuk 7).

Literatuur overzicht (Hoofdstuk 2). In dit proefschrift worden kleurmetingen van zowel komkommer (kleurbehoud) als aardbeien (rot frequentie) gekoppeld aan kwaliteits-eigenschappen. Kleur is op het ogenblik misschien wel de meest ideale eigenschap om te meten: het kan erg nauwkeurig gemeten worden en de meting is non-destructief, waardoor metingen door de tijd heen herhaald kunnen worden. Er wordt een overzicht gegeven van kleurmetingen, kleurprocessen, recent kleuronderzoek en de knelpunten bij het converteren van fysiologische kennis naar kleurtoepassingen. Oplossingen voor de

knelpunten, zoals verbeterde kleurmetingen, ontwikkeling van kleurmodellen die fysiologische kennis bevatten en het partijconcept worden hier geïntroduceerd en verder uitgewerkt in latere hoofdstukken.

Eerste stappen (Hoofdstuk 3) Kleur is een van de weinige praktische criteria om de komkommer kwaliteit te beoordelen. Echter, komkommers met dezelfde kleur bij de oogst kunnen grote kwaliteitsverschillen vertonen als ze bij de consument aankomen. Fotochemische metingen en kleurmetingen werden gecombineerd met een logistisch model om te testen of de houdbaarheid voorspeld kan worden. Helaas waren of de kleurmetingen of het kleurmodel onvoldoende, aangezien de voorspellende waarde van deze aanpak beperkt was.

De partij benadering (Hoofdstuk 4) Een partij bestaat uit individuen met een gezamenlijke groeigeschiedenis, in de praktijk individuen van één teler, één cultivar en één oogst. De vorm van de kleurdistributie van de komkommers bij de oogst, verkregen door meting van de kleur van alle komkommers in een partij was verschillend voor partijen met verschillende groeicondities (voedingsdichtheid en plant dichtheid). Een indicatie voor de rijpheid werd gevonden door te kijken naar de scheefheid van de kleurdistributie. De hypothese werd ontwikkeld dat karakterisering van de komkommer kleurdistributie voldoende informatie geeft om partijhoudbaarheid vast te stellen. Partijhoudbaarheid is het aantal dagen dat 95% van de partij boven de kwaliteitslimiet zit.

Partijhoudbaarheid van komkommers (Hoofdstuk 5) De houdbaarheid van een komkommer hangt af van de toestand het chlorofyl metabolisme. Met andere woorden, of er nog genoeg kleuraanmaak (chlorofyllide en chlorofyl) is om de chlorofyllide afbraak in evenwicht te houden. Een fysiologisch model werd gebouwd dat het kleurgedrag tijdens de vooroogst als functie van bewaartijd en bewaartemperatuur voor individuele komkommers beschrijft, ongeacht de groeicondities en cultivar op basis van kleurmetingen alleen. Van zes partijen, van drie cultivars over twee groeiseizoenen, werd de partijhoudbaarheid verkregen. Er werd aangetoond dat initiële kleurmetingen gebruikt konden worden om de gemiddelde precursor concentratie per partij te schatten, hetgeen een indicatie is voor de partijhoudbaarheid. Aangezien de basis van de houdbaarheidsvoorspellingen een generiek model is, is het wellicht mogelijk om de partijhoudbaarheid te voorspellen van andere producten, mits groene kleur de beperkende kwaliteitseigenschap is.

Partijhoudbaarheid van aardbeien (Hoofdstuk 6) Na de oogst worden aardbeien vroeger of later rot. Een kleurenmodel is gepresenteerd die de ontwikkeling van de rode kleur en de fungistatische stoffen in individuele aardbeien beschrijft tijdens bewaring. Voor twaalf partijen, afkomstig van aardbeienplanten die groeiden bij een verschillende voedseldichtheid en van verschillende oogsten, werd de partijhoudbaarheid bepaald. The rot per partij bij oogst was gerelateerd aan de precursor concentratie van zowel de kleur als de fungistatische verbindingen. Partijhoudbaarheid kon worden afgeleid uit de initiële kleur distributies. Daarnaast bleek dat de partijhoudbaarheid gerelateerd was aan de tijd tussen twee oogsten. Voor een praktische implementatie is nog additioneel onderzoek nodig, speciaal met betrekking tot verschillende Botrytis drukken en verschillende cultivars.

Het partij model (Hoofdstuk 7) Een partijmodel is ontwikkeld die de invloed van een bron van biologische variatie, de variatie in lichtomstandigheden tijdens de groeiperiode, beschrijft op precursor concentraties van aardbeien en komkommers. Het partijmodel bestaat uit een kinetisch deel die het gedrag beschrijft van de precursor voor individuele komkommers en aardbeien, en een stochastisch deel die de precursor distributie beschrijft. Het stochastische deel is generiek, maar het kinetische deel hangt af van de onderliggende processen die de houdbaarheid bepalen (Hoofdstuk 5 voorkomkommers en Hoofdstuk 6 voor aardbeien). Het was mogelijk om met behulp van het partij model komkommer partijen van twaalf experimentele cultivars, geoogst van de stam of de rank van komkommerplanten te analyseren. Het bestaan van een cultivar specifieke houdbaarheid is vastgesteld.

Discussie (Hoofdstuk 8) De biologische variatie methodologie is mogelijk een compleet nieuw onderzoeksveld in de landbouw. Of deze methodologie ook echt deze status zal bereiken hangt af van drie zaken: (i) kan de toepassing van partij modellen de bewustwording genereren dat sommige problemen niet opgelost kunnen worden met bijvoorbeeld traditionele sorteertechnieken, (ii) het bouwen van fysiologische kinetische modellen en (iii) het oplossen van de huidige wiskundige beperkingen omtrent fysiologische en stochastische modellen zodat een generiek partij model kan worden opgesteld.

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Curriculum Vitae

Robbert Eduard Schouten is geboren op 12 juli 1968 in Arnhem. Na het VWO in Zetten (Heldring college, nu Pierson college) is hij gestart met een studie scheikunde aan de KUN, met specialisaties in biochemie en biofysica. Vanaf halverwege 1995 heeft hij bij de groep naoogstkwaliteit van verse producten van de ATO gewerkt. Vanaf 2000 is hij verbonden aan de leerstoelgroep Tuinbouwproductieketens van Wageningen Universiteit. Naast het promotieonderzoek heeft hij zich beziggehouden met o.a. het leren modeleren, studentbegeleiding, commerciële rapportages en het geven van college algebra.

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