External Quality Measurements Reveal Internal Processes

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Abstract

With the present developments in CA technology it becomes possible to fine tune the storage conditions to the specific needs of the product. This generates the need to know the exact quality conditions of the product before storage starts. By measuring the initial quality we can determine these conditions optimally. At present the most likely candidates to assess the initial quality with fast and non-destructive measurements are colour, chlorophyll fluorescence, and maybe NIR spectroscopy. Two examples are presented where initial colour measurements on all products in a batch can be shown to be indicative for the keeping quality of that batch. The first example focuses on how initial colour measurements using a 3CCD video camera can be utilised to predict the keeping quality of a batch of cucumbers where colour itself is regarded as the most important quality attribute. The second example focuses on how colour measurements can be used to predict the keeping quality of a batch of strawberries where the ability to suppress a *Botrytis cinerea* infection is the most important quality attribute. Furthermore, attention is given to the use of modulated chlorophyll fluorescence imaging as a possible initial quality indicator for rose leafy stem cuttings. The level of inhomogeneity in the quantum yield of photochmistry od PSII of leaves of rose cuttings may be an indictor of the capability of the cutting to recover from severance, and to form roots and generate regrowth.

INTRODUCTION

Nowadays CA recommendations are available (CD CA Bibliography (1981-2000) and CA recommendations 2001) which provide storage conditions for a host of horticultural perishables. Although the applied regimes generally have some safety margins to allow for variations in initial quality no special care or use is taken of the inherent biological variance available in each stored batch; a batch can be defined as all products from a single cultivar, grower and harvest. When the initial quality is known batch specific optimal storage conditions may be applied resulting in better retention of quality traits. Two approaches may be viable: start with a general storage condition and iteratively adapt it until optimal storage conditions are reached for the stored batch or measuring the initial quality fast and non-destructively enabling the storage of the batch at the optimal storage conditions almost immediately.

The first approach is used for a new development in CA storage, the ATO patented DCS system, which uses the ethanol response of a stored batch to iteratively change the storage conditions just above the onset of fermentation. The DCS system and its benefits are described by Veltman et al., submitted.

For the second approach no applications for CA storage yet exist because of the inherent difficulty to assess the initial quality of a batch. Colour, chlorophyll fluorescence, and maybe NIR spectroscopy are the only options for non-destructive and fast measurements to probe the initial quality of a batch. Besides the constraints of the measurements, the interpretation of batch data is often illusive. Here we want to report examples of horticultural produce where the initial colour measurements could be used to predict the keeping quality of a batch. The interpretation of the initial measurements we use is based on published information about the biochemical pathways of the quality related processes. As the reaction rate constants belonging to the different processes in the

pathway are assumedly not affected by genotype or phenotype and depend only on temperature, they need to be assessed once per produce. As the reaction rate constants are fixed per temperature the behaviour of the produce is governed by the amount of a certain precursor, which is assumed to be turning over during growth and only breaking down after harvest. Predictions of quality can then be translated into the assessment of the available amount of precursor. We will show how we apply this concept for batches of cucumbers, where we look at colour itself as the quality attribute, and for batches of strawberries, where we use colour measurements to determine the capability to suppress a *Botrytis cinerea* infection. We also report briefly on a new development using modulated chlorophyll fluorescence imaging as possible initial quality indicator for rose cuttings.

COLOUR AS INDICATOR OF INITIAL CUCUMBER BATCH QUALITY

Measurements

Image analysis was used for colour measurements. The system consists of a colour 3-CCD video camera in a container with a light controlled environment connected to a PC. In the container two mirrors along the long side of the cucumber enable the flat-3D picture of the skin of the whole cucumber. After measuring the light intensities the red, green and blue values are separately averaged over all pixels belonging to the cucumber (Fig. 1). Colour values are expressed as RGB values.

Interpretation

1. Pathway. The main quality attribute of a batch of cucumbers at the Dutch auctions, provided there is no mechanical damage or visible infection, is the green colour of the skin (Schouten et al, 1997). According to Porra (1997) and Heaton and Marangoni (1996) the last part of the chlorophyll pathway can be shown in Fig 2. Pchl stands for protochlorophyllide, chl for chlorophyllide and CHL for chlorophyll. The colour, that can be detected as green in the skin, is determined by the concentration of both chlorophyll and chlorophyllide as they have almost identical spectra and all other pigments involved are colourless or colour stable during senescence. We assume Pchl to be the precursor which builds up while the fruit is still attached to the plant. After severance we assume that the amount of Pchl is not replenished anymore.

2. Data analysis. As the reaction rate constants of the different reactions described in Fig. 2. are supposedly only dependant on temperature according to Arrhenius' law, experiments with repeated colour measurements over time varying in temperature disclosed the values of the reaction rate constants. We expressed the reaction rate constants in Red (R) as well as in Green (G) and Blue (B) values of the RGB measurements.

By using these reaction rate constants it is possible to estimate the size of the initial pool of Pchl from time series of colour measurements. This has been done for six batches, consisting of about 100 cucumbers varying in growing season and cultivars measured 2-3 times per week until yellowing stopped or decay was imminent. The average size of the estimated initial pool of Pchl per batch was related to the keeping quality per batch (the time 95% of the batch has an acceptable colour) as shown in Fig. 3a (Schouten et al, 2002a).

The reaction rate constants of the initial red intensity (R) appear to favour the synthesis of chlorophyll out of Pchl while the initial green intensity (G) favours the chlorophyll catabolism (Fig. 1). The ratio of these intensities (R/G) turned out to be measure of the initial pool of Pchl. Therefore, the batch keeping quality could be predicted when the ratio of the initial red (R) and the green (G) intensities were determined as an average for all cucumbers per batch (Fig. 3b). The physiological basis for this different behaviour of the red (R) and green (G) intensities of the RGB colour measurement are probably based in the different spectral characteristics of chlorophyll a

and chlorophyll b (Schouten et al, 2002b).

COLOUR AS INDICATOR OF INITIAL STRAWBERRY BATCH QUALITY

Measurements

The same setup for the strawberry colour measurements was used as for the cucumber measurements. Exceptions were that no mirrors were used and that discs with 24 fruits were placed on a turntable to measure each fruit individually. Colour learning software was used to separate the images into flesh, seeds and calyx (Fig 4). Light intensities of the red, green and blue values are separately averaged over all pixels belonging to the flesh part.

Interpretation

1. Pathway. Most cases of Botrytis infection occur via floral parts of the senescing flower. After successful flower infection the fungus remains quiescent in green strawberries (Bristow et al., 1986). Proanthocyanidins (PA) are unspecific and colourless enzyme inhibitors thought to govern the quiescence (Jersch et al. 1989). PA are end products of the flavonoid biosynthesis pathway and so are the anthocyanins. Anthocyanins are responsible for the red colour in strawberries. An assumption is that the amount of precursor, leucoanthocynidins (LA), available at harvest time will govern solely the colour development and the resistance against botrytis (Fig 5). A higher amount of initial LA will result in a strawberry which is eventually more red and more resistant to botrytis.

2. Data Analysis. As the reaction rate constants of the different reactions described in Fig. 5. are supposedly only dependant on temperature according to Arrhenius' law, experiments with repeated colour measurements over time varying in temperature disclosed the values of the reaction rate constants. We expressed the reaction rate constants in the red (R) value of the RGB measurements.

By using these constant reaction rates it is possible to estimate the size of the initial pool of LA from time series of colour measurements. This has been accomplished for six batches, consisting of about 130 strawberries measured 2-3 times per week until botrytis was detected. The estimated average size of the initial pool of LA per batch was related to the keeping quality per batch (the time 95% of the batch is not spoiled by botrytis) as shown in Fig. 6a. The keeping quality was determined per batch by daily observation of the amount of spoilage as percentage of the total amount of strawberries per batch (Schouten et al, 2002a).

Normally, strawberries are harvested when they have a sufficiently red colour. For strawberries with a low amount of PA only after considerable time the colour development will be sufficient to start harvesting. Almost all the strawberries will be selected for harvesting. As part of the strawberries in this batch have already reached their maximal red colour and part are still accumulating anthocyanins, the colour distribution will be skewed. On the other hand, for strawberries with a high amount of PA a considerable amount of strawberries will reach the harvest limit quickly and will be harvested, leaving the rest for another harvest date. As this batch is still accumulating anthocyanins, the colour distribution of this batch will be normally distributed. Therefore the skewness of the colour distribution is an indicator of keeping quality per batch (Fig 6b), (Schouten et al., 2002a).

CHLOROPHYLL FLUORESCENCE IMAGING AS INDICATOR OF INITIAL ROSE CUTTING QUALITY

Availability of chlorophyll fluorescence (CF) imaging equipment commercially, makes it possible to look at the distribution of damage in the peel of fruits and vegetables. Such possibility may also be extended to other horticultural produces like cuttings used for propagation purposes. In fact, it was shown via modulated CF that the quantum yield of photochemistry of PSII (Φ_{PSII}) was an important quality attribute of cuttings submitted

to storage (Van Kooten and Peppelenbos, 1993). An early indication of future growth of cuttings could be also obtained by using modulated CF imaging to analyse the photosynthetic characteristics of leaves and its response to severance and propagation. In Fig. 7, the Φ_{PSII} of a leaf of a rose cuttings is shown before (Fig. 7a) and after (Fig. 7b) severance by using the false colour CF imaging. Stress imposed by severance increased inhomogeinity of the Φ_{PSII}. The leaf is able to recover from severance in few hours (Fig. 7c) but after 14 days large inhomogeinity is again encountered (Fig. 7d) which may be an indication of water stress or modified source-sink relations (sink limitation) (Costa et al., 2001). By making stress visible and using spatial statistics to quantify the effects of severance or pre-severance treatments (e.g. storage) on Φ_{PSII} of cuttings it should be possible to predict rooting and growth of cuttings. More research will be performed to develop this technique as an useful tool for quality prediction on leafy stem cuttings.

ACKNOWLEDGEMENTS

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Figures

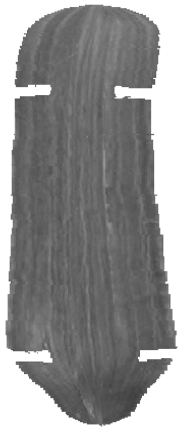

Fig. 1. Flat-3D picture of the skin of a cucumber. Four small pieces are missing because of the two hooks which support the cucumber.

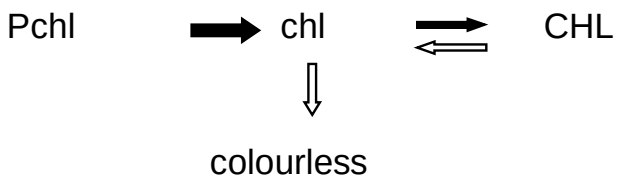

Fig. 2. Last part of the chlorophyll pathway. Closed arrows are used to indicate synthesis and open arrows are used for the catabolism

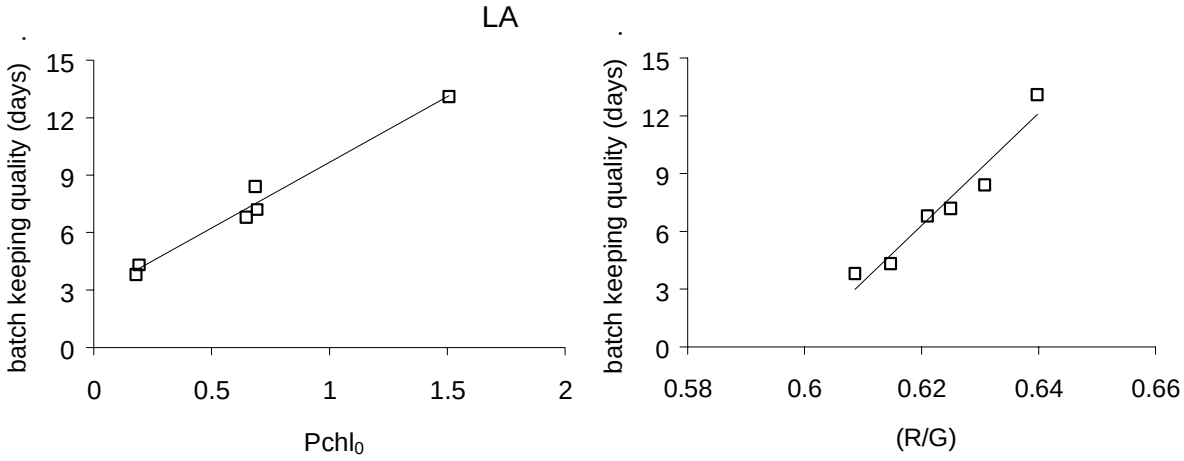

Fig. 3. The amount of the average initial precursor ($Pchl_0$) per batch (left picture, Fig. 3a) and the ratio of red and green intensities (right picture, Fig. 3b) against the keeping quality per batch. The amount of Phl is established by analysing time series of colour data while the ratio R/G is derived from the initial colour measurements at harvest.

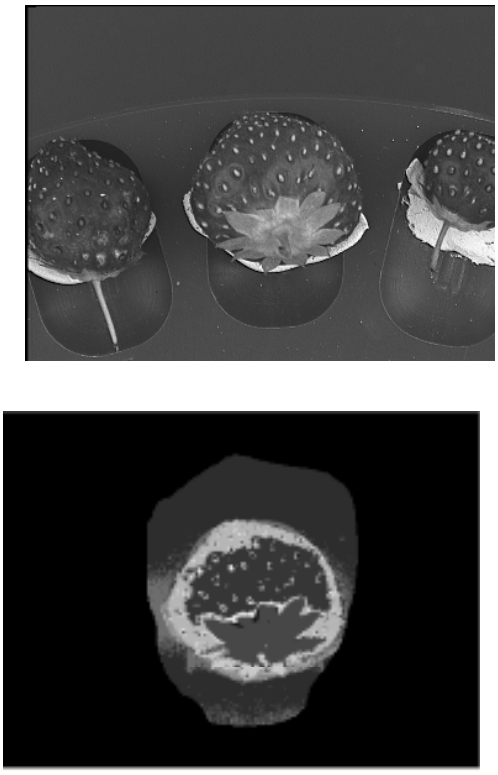

Fig. 4. Upper picture shows part of the turntable with 3 strawberries in holes supported by a clay roll. Lower picture shows the colour separation into calyx, seeds, clay roll and two flesh parts.

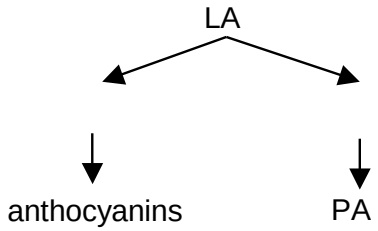

Fig. 5. Simplified scheme of the last part of the flavonoid pathway.

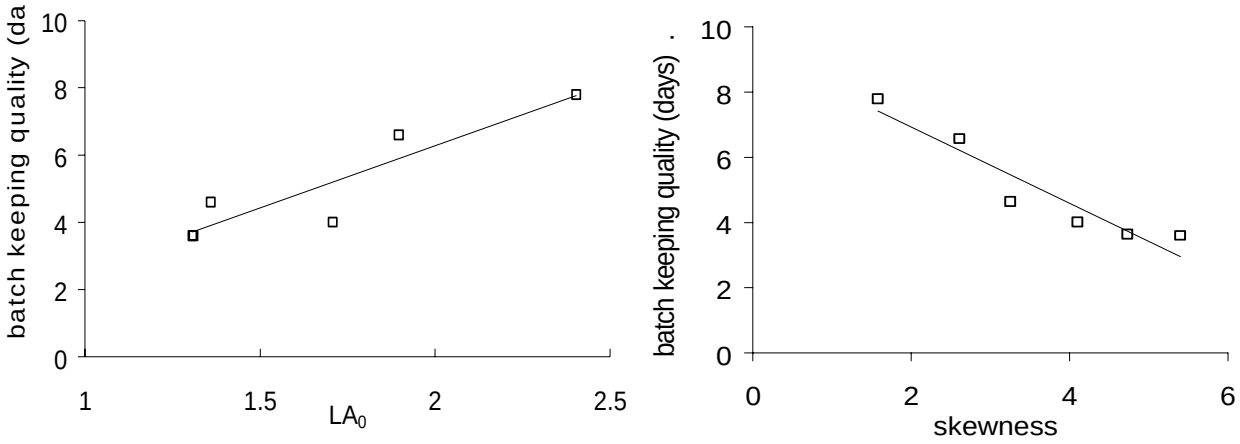

Fig. 6. The amount of average initial precursor (LA_0) per batch (left picture, Fig. 6a) and the skewness of the colour distribution per batch (right picture, Fig. 6b) against the keeping quality per batch. The amount of LA is established by analysing time series of colour data while the skewness of the colour distribution is from the initial colour measurements at harvest.

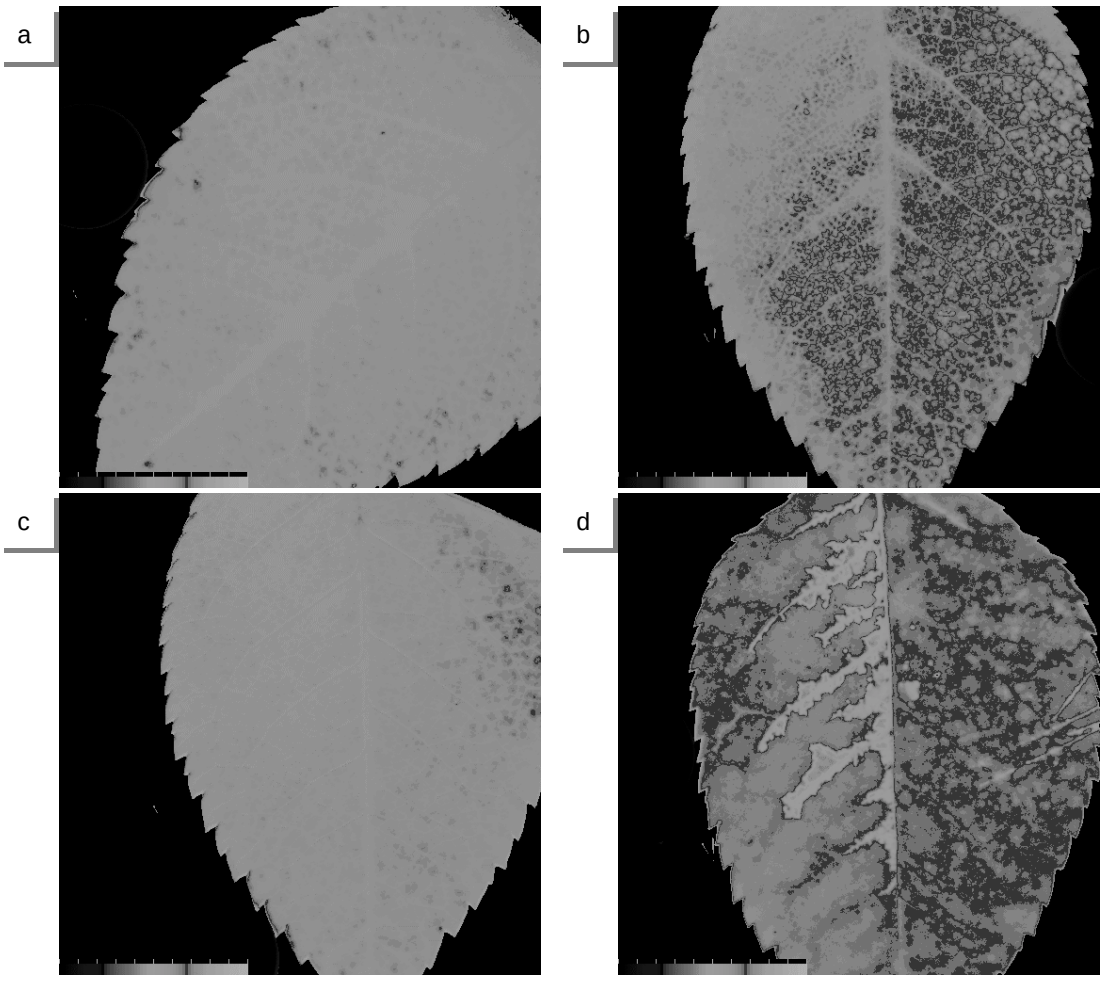

Fig. 7. CF imaging using false colours to indicate different levels of Φ_{PSII} values. Pictures indicate the CF image before severance (Fig 7a), immediately after severance (Fig 7b), 2 hours after severance (Fig. 7c) and after 14 days (Fig. 7d). The colour scale on the left bottom side of the pictures is indicative for the difference in Φ_{PSII} values .