Keeping Quality of Strawberry Batches

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ABSTRACT

Post-harvest 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 here. A 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 batches from the same growing conditions stored per batch at 5, 10 or 16 °C after harvest. Batches are considered to be all strawberries from one harvest. Spoilage per batch was recorded during storage. The fitted initial spoilage per batch was found to relate to the fitted amount of precursor of both the colour and the anti-fungal compounds. Batch keeping quality could be predicted from the initial batch colour distributions. An explanation for the predictive ability of the model without having to use a term describing the pH is presented from pigment HPLC measurements.

INTRODUCTION

Known for their delicious taste and nutritional value, strawberries are also very perishable. Spoilage by the fungus *Botrytis cinerea* is the major limiting quality attribute for strawberries (Saks et al., 1996). Main criterion is whether the strawberries are affected or not, rather than the degree of decay. Quality losses can be expresses in terms of batch keeping quality and can be defined as the time it takes when one strawberry in a consumer package of 20 strawberries becomes rotten (Hertog et al., 1999). The aim of this study is to develop a model predicting the keeping quality for 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. Non-destructive colour measurements are used for repetitive measurements on the same strawberry.

MATERIAL AND METHODS

Strawberries

Frigo strawberry plants (*Fragaria x ananassa* Duchesne cv. 'Elsanta') were purchased from a local supplier at the 3rd of July and planted on the 5th of July 2000 in a greenhouse compartment. Growth conditions used in practise were applied. A batch was defined as all strawberries harvested on the same day. Fruits were harvested when the predetermined amount of about 130 strawberries with no white patches had been reached. A total of 6 batches were harvested from 25th of August to 21st of September 2000. After arrival at the measuring facility only regular shaped fruits without signs of botrytis were placed on plastic discs (Ø 28 cm) with 24 holes (3.5 x 2.2 x 0.4 cm).

Storage and Rot Assessment

Strawberries were stored per batch at 5, 10 or 16 °C in climate chambers. The quality was visually assessed by counting the number of strawberries per batch affected by botrytis on a daily basis. Affected strawberries were removed. No discrimination was made on the basis of the level of decay.

Non-destructive Measurements

Colour measurements were carried out in a controlled light environment with a 3CCD video camera. Discs with 24 fruits 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 in flesh, seeds, calyx, clay and background colour. Light intensities for the red, green and blue (RGB) values are separately averaged over all pixels that belong to the different parts of the strawberry.

Destructive Measurements

For pigment extraction purposes strawberries were placed overnight at 5 °C to defrost and in the morning the each fruit was blended and a sample of 1 g per fruit was used for the pigment extraction and HPLC analysis according to Gil et al, 1997. Pigment compounds were characterised by chromatographic comparison with authentic standards. The acylated form of Pg-3-gl was quantified using Pg-3-gl as standard.

MODEL DEVELOPMENT

Conceptual Model

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, are unspecific 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 (Boss et al., 1996).

Anthocyanins are responsible for the red colour in strawberries and start to appear during the white stage of fruit development and its synthesis continues after harvest (Perez et al., 1998). Part of the process described is shown in Fig 1. Leucoanthocyanidins are the direct precursors of the anthocyanidins and the flavan-3-ols. The anthocyanidins, also red coloured, are not found in strawberries (Gil et al., 1997) and less stable than anthocyanins (Francis, 1989). It might be assumed that the rate constant for anthocyanin formation is very high.

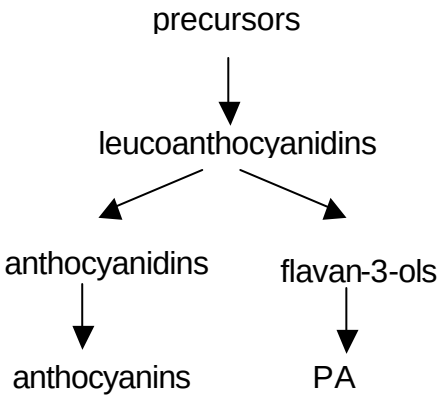

Fig. 1 Scheme of the last part of the flavonoid pathway.

Big differences have been shown between flavon-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 rate constant for favon-3-ol

formation is rate limiting compared to rate constant for the formation of PA from the flavon3-ols. According to Walton et al., 1983, flavon-3-ols do not precipitate proteins as PA do, so flavon-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.

Mathematical model

1. Spoilage. Spoilage modelling is done according to Hertog et al., 1999. Herein the behaviour of a batch strawberries in terms of percentage affected is described by the differential equation for a sigmoidal change in time (Eq. 1):

$$\frac{dN}{dt} = k_{bot} \cdot N \cdot \frac{(N_{max} - N)}{N_{max}} \quad (1)$$

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 will be regarded as a batch dependant parameter. The spoilage rate is assumed to depend on temperature according to Arrhenius' law (Eq. 2):

$$k = k_{ref} \cdot e^{\frac{Ea_{bot}}{R_{gas}} \cdot (\frac{1}{T_{ref}} - \frac{1}{T})} \quad (2)$$

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).

2. Colour Development. Colour development is dependent on the amount of precursor and the ratio it will be transformed to anthocyanins and to flavon-3-ols, being the rate determining steps of the process described in Fig. 1. The set of differential equations belonging to these processes (Eq. 3 and Eq. 4) can be solved analytically to describe the red (anthocyanin) formation in time (Eq. 5).

$$\frac{\partial prec}{\partial t} = -k_m \cdot prec - k_r \cdot prec \quad (3)$$

$$\frac{\partial red}{\partial t} = -k_r \cdot prec \quad (4)$$

$$red(t) = red_0 + \frac{k_r}{k_m + k_r} \cdot prec_0 \cdot (1 - e^{-(k_m + k_r) \cdot t}) \quad (5)$$

Where k_m and k_r are the reaction rates for the formation of the flavon-3-ols and the formation of the anthocyanins, respectively. Both reaction rate are temperature dependent

via the Arrhenius equation (Eq. 2). The initial concentrations of anthocyanin and the initial precursor concentration are expressed as red_0 and $prec_0$.

3. 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. 1 together with the temperature dependence according to Arrhenius (Eq. 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. 5 together with the temperature dependence according to Arrhenius' law (Eq.2) for the reaction rates. The colour data were analysed simultaneously using temperature and time as independent variables to acquire $prec_0$ for each strawberry. The reference temperature for the Arrhenius equation (T_{ref}) was in all cases 283 K (10 °C).

Table 1. Parameter estimates and their standard errror for the analysis of the spoilage data of 'Elsanta' strawberry batches.

process parameters:

$k_{bot,ref}$	0.5011	SE 0.0115		n_{obs}	321
Ea	7797	SE 572		R_{adj}^2	97.2%

batch parameters:

Batch	N_0	SE	KQ (10°C)
3	0.106	0.047	7.8
4	0.195	0.050	6.6
6	0.478	0.171	4.8
2	0.513	0.104	4.6
1	0.841	0.173	3.6
5	0.859	0.162	3.6

RESULTS & DISCUSSION

Spoilage

Analysis of the spoilage data from all batches is shown in Table 1. To account for the differences between batches, the initial spoilage (N_0) was estimated for each of the twelve batches. Using the estimated parameters the keeping quality can be calculated at 10 °C to compare batches.

Colour Development

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. 5. The process parameters for the model were obtained by

Table 2. Parameter estimates and their standard errror for the analysis of the colour data of 'Elsanta' strawberries.

process parameters

$k_{m,ref}$	0.1155	SE 0.0041	$k_{r,ref}$	0.01505	SE 0.00032	R_{adj}^2 = 96.7%
E_m	1456	SE 595	E_r	5542	SE 335	n_{obs} = 569

fitting the colour data (Table 2). If the assumption that the amount of precursor at harvest governs the keeping quality, then it is expected that a relation exists between N_0, the initial spoilage per batch, and the average initial amount of precursor, $prec_0$, per batch. An relation between $\ln(N_0)$ and the average amount of $prec_0$ was found. (R^2_{adj} = 93%). When the amount of precursor for colour formation at harvest is determining the keeping quality then the colour distribution at harvest itself might also contain this information. With increasing initial spoilage, N_0, an increase in the skewness is observed for the colour distributions fitted with a standard gamma function.

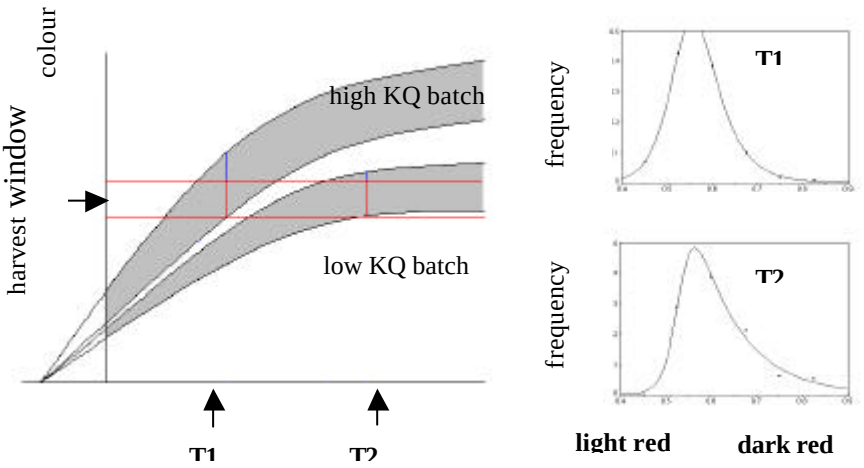

Fig. 2 The left picture depicts two batches with different keeping quality and the harvesting window. Harvest takes place at T1 for the high keeping quality batch and at T2 for the low keeping quality batch. The colour distributions at T1 and T2 are depicted on the right side.

In Fig. 2 colour development of two strawberry batches are depicted, one 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 from the high keeping quality batch will have a considerable amount of precursor left at harvest and these will generally be much more red at t=∞, when all precursor is spent, than strawberries from the low keeping quality batch. Harvesting will commence when enough strawberries are within the harvesting window (Fig. 2). For the strawberries with a low keeping quality almost all could be selected for harvest save only a small amount which 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 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 consist of

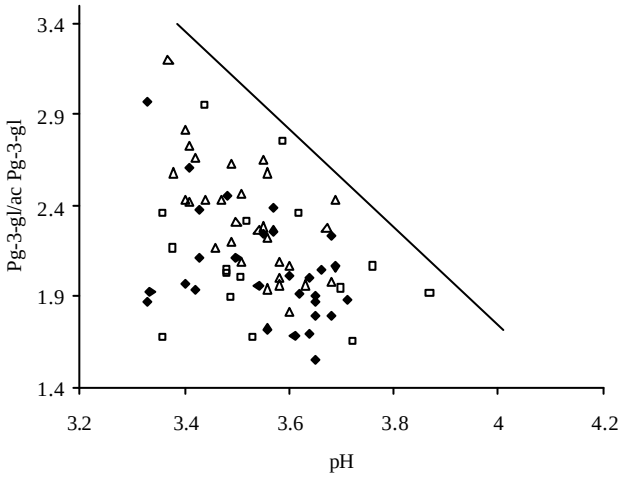

Fig. 3 Scatterplot of the pH values for sub-batches from the same batch taken at day 0 (Δ), day 6 (♦) and day 8 (?) stored at 16 °C, against the ratio Pg-3-gl/acylated Pg-3-

about half the available strawberries. This results in a colour distribution for the low keeping quality batch which is skewed because also some very red strawberries are harvested (T2, Fig 2.) and a normal distribution (T1, Fig. 2) for the high keeping quality batch which exists of strawberries which are all almost linearly increasing in red colour.

It is well known that for very acidic solutions all the anthocyanin will be in its red flavylium form but that with increasing pH the colour intensity decreases because more anthocyanin will converted into the colourless form (Brouillard and Delaporte, 1978). Prelimenary 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 colour concentration. To investigate why the colour model apparently works well 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. The smaller peak was identified as an acylated form of Pg-3-gl by alkaline hydrolysis. It was observed that older sub-batches belonging to the same batch generally have a decreasing ratio of Pg-3-gl/acylated Pg-3-gl and a higher pH (Fig. 3). As the presence of acylating groups in anthocyanins have 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 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 having to use a term describing the pH in its formulation.

CONCLUSIONS

Destructive and non-destructive measurements were used to support and build a model that links the initial amount of spoilage in 'Elsanta' strawberries to the initial amount of common precursor of the colour and the botrytis inhibiting compounds. As a consequence batch keeping quality predictions may be feasible by non-destructive measurements, such as colour measurements.

REFERENCES

Boss, P.K., Davies C. and Robinson. S.P. 1996. Expression of anthocyanin biosynthesis pathway genes in red and white grapes. Plant Molecular Biology. 32, 565-569.

Bristow, P.R., McNicol, R.J. and Williamson, B., 1986. Infection of strawberry flowers by *Botrytis cinera* and its relevance to grey mould development. An. Appl. Biol. 109, 545-554.

Brouillard, R. and Delaporte, B.,1978. Etude par relaxation chimique des equilibres de transfert de proto, d'hydratation et tautomerie prototropique de l'anthocyane majoritaire de Vitis vinifera, la glucoside-3-malvidine. In: Laszlo, P. ed. Protons and Ions involved in Fast Dynamic Phenomena. 403-412.

De Freitas, V.A.P. and Glories, Y., 1999. Concentration and compositional changes of proanthocyanidines in grape seeds and skin of white Vitus vinifera varieties. J. Sci. Food Agric. 79, 1601-1606.

Francis, F.J., 1989. Food colorants: Anthocyanins. Critical Reviews of Food Science and Nutrition. Boca Raton, Fla: CRC Press. 28, 273-314.

Gil, M.I., Holcroft D.M. and Kader A.A., 1997. Changes in strawberry anthocyanins and other polyphenols in response to carbone dioxide treatments. Journal of Agriculture and Food Chemistry. 45, 1662-1667.

Giusti, M.M, Rodriguez-Saona, L.U. and Wrolstad, R.E., 1999. Molar absorptivity and color characteristiscs of acylated and non-acylated pelargonidin-based anthocyanins.

Hertog, M.L.A.T.M., Boerrigter H.A.M., van de Boogaard G.J.P.M. and Schaik A.C.R., 1999. Predicting keeping quality of strawberries (cv. 'Elsanta') packed under modified atmospheres: an integrated model approach. Postharvest Biol. Technol. 15, 1-12.

Jersch, S., Scherer, C., Huth, G. and Schlosser E., 1989. Proanthocyanidins as basis for quiescence of Botrytis cinerea in immature strawberry fruits. Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz. 96, 365-378.

Perez, A.G., Olias, R., Olias, J.M. and Sanz, C., 1998 Strawberry quality as a function of the 'high pressure fast cooling' design. Food Chemistry. 62, 161-168.

Saks, Y., Copel, A. and Barkai-Gola, R., 1996. Improvement of harvested strawberry quality by illumination: colour and Botrytis infection. Postharvest Biol. Technol. 8, 19-27.

Walton, M.F., Haskins, F.A. and Gorz H.J. 1983. False positive results in the Vanillin-HCl Assay of tannins in sorghum forage. Crop Science. 23, 197-200.