Effects of CA Treatment and Temperature on Broccoli Colour Development

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Abstract

Broccoli combines high contents of vitamins, fibres and glucosinolates with a low calorie count and is sometimes referred to as the 'crown jewel of nutrition'. Colour is one of the most important quality attributes of broccoli, and yellowing due to senescence of broccoli florets is the main external quality problem in the broccoli supply chain. Controlled Atmosphere (CA) is a very effective method to maintain broccoli quality but the effects of CA on colour retention have not been studied extensively. The aim of this paper is to characterise the colour behaviour (measured by RGB colour image analysis) of broccoli as affected by CA and temperature. Data on colour behaviour and gas exchange were gathered for broccoli heads that were stored in containers at three temperatures and subjected to four levels of O_2 and three levels of CO_2. Gas conditions and temperature have a clear effect on the colour change of broccoli especially at low O_2 in combination with high CO_2. An integrated colour model is proposed that combines a colour model with a standard gas exchange model. The colour model is based on three differential equations describing the formation of (blue/green) chlorophyllide from the colourless precursor, the bidirectional conversion of chlorophyllide into (blue/green) chlorophyll, and the decay of chlorophyllide. During the first step of building the integrated model, gas exchange data were analysed simultaneously using multi response regression analysis. No fermentation was encountered for this batch of broccoli. During the second step it was found that only one of the reactions of the colour model, the decay of chlorophyllide, is affected by the gas conditions. In the final step, a multi-response approach was applied where gas exchange parameters were estimated using the gas exchange model, the colour parameters were estimated using the colour model with both models linked via the reaction rate constant affected by the gas conditions. Such a calibrated, integrated, model could be used as a tool for predicting colour change in the postharvest chain.

INTRODUCTION

Broccoli combines high contents of vitamins, carotenes, fibres, glucosinolates and other phytochemicals. Broccoli is a highly perishable vegetable with a shelf-life of only a few days. Colour is the main external quality attribute of broccoli. Yellowing due to senescence of broccoli florets is the main external quality problem in the broccoli supply chain. Controlled Atmosphere (CA) is known to be a very effective method to maintain broccoli quality; best retention of the green colour is obtained when 1-2% O_2 is combined with 5-10% CO_2 at temperatures between 0 and 5°C (Jones et al., 2006). However, the effects of CA on the dynamics of colour retention have not been studied extensively. The aim of this paper is to characterise the colour behaviour of broccoli as affected by CA and temperature. Data on colour behaviour and gas exchange were gathered for broccoli heads stored at three temperatures subjected to four concentrations of O_2 and three concentrations of CO_2. An existing colour model based on physiological processes was adapted and combined with a gas exchange model into an integrated model. Such a calibrated, integrated model describes the combined effects of O_2, CO_2, temperature and time on the colour change of broccoli. It may be used as a tool to predict colour change in the postharvest chain.

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MATERIAL AND METHODS

Broccoli

About 250 broccoli heads (cv. '1997'), healthy and of marketable size, were freshly harvested in one batch. All broccoli heads were labelled at the stem and randomly assigned to one of the 12 CA treatments. Broccoli heads were stored in three dark, temperature controlled rooms (at 5, 10 and 18°C) each with four 65 litres CA containers. Each CA container was filled up as much as possible without damaging the heads with on average twenty broccoli heads per container. CA containers were connected to a flow-through system flushing humidified and constant gas mixtures at a flow rate between 400 and 500 ml min^{-1} throughout the duration of the experiment. Broccoli heads were subjected to four levels of O_2 (1.5, 3, 10 and 21 kPa) and three levels of CO_2 (0, 6 and 15 kPa) (Table 1).

Gas Exchange Measurements

Gas exchange measurements were conducted using a mobile GC analyzer. During the storage period, daily GC measurements were carried out at one of the temperature controlled rooms. After measuring the gas exchange of all four containers at one room, the GC was moved immediately into one of the other temperature controlled rooms to ensure that the GC was adjusted to the higher or lower temperature for the measurements the next day. After calibration, the flow-through system was closed to let the gases accumulate and the first GC measurement was carried out. The second measurement was carried out at the end of the accumulation period. The difference in gas partial pressure between the first and second GC measurements was converted into gas exchange rates. The accumulation periods were 4, 2 and 1h for the rooms at 5, 10 and 18°C respectively. GC measurements were carried out approximately every 3 d for the broccoli stored at 5 and 10°C and approximately every 2 d (Fig. 2) for the broccoli stored at 18°C.

Colour Measurements

Image analysis was used for the RGB colour measurements. Colour was measured on individual broccoli heads using a colour video camera in a controlled light environment (Schouten et al., 2002). Broccoli stems were placed in a small plastic vase (Ø 3 cm) to keep the broccoli upright during measurement. A circular mask (Ø 5 cm) was used during measurement to select the central floret of each broccoli head. The effects of handling damage that occurs at the edges, such as browning or the light green colour of damaged florets at the edges, will be minimal when the colour of only the central part of the broccoli head is measured.

RESULTS AND DISCUSSION

Gas Exchange

Nowadays the Michaelis-Menten gas exchange models proposed by e.g., Hertog et al. (1998) are commonly used to describe the O_2 consumption and CO_2 production as function of the applied gas conditions. It was established that no significant fermentation is present in this broccoli batch (respiration quotient between 0.6 and 1.4) and that a small preference was found for the uncompetitive type of CO_2 inhibition, resulting in the following description of the O_2 consumption (Eq. 1) and CO_2 production (Eq. 2).

$$V_{O_2} = \frac{Vm_{O_2} O_2}{Km_{O_2} + O_2 \left(1 + \frac{CO_2}{Kmu_{CO_2}}\right)} \tag{1}$$

$$V_{CO_2} = RQ_{ox} V_{O_2} \tag{2}$$

with VmO_2 the maximum O_2 consumption rate (nmol kg^{-1} s^{-1}), KmO_2 the Michaelis constant for O_2 consumption (kPa), Kmu_{CO2}, the Michaelis constant for the uncompetitive CO_2 inhibition of O_2 consumption (kPa). RQ_{ox} represents the respiration quotient for oxidative respiration. VmO_2 is assumed to depend on temperature according to Arrhenius' law. Gas exchange data were analysed using the gas conditions (O_2 and CO_2) simultaneously as independent variables and O_2 consumption and CO_2 production rates as dependent variables (multi response regression analysis (Table 2)).

Colour Model

Schouten et al. (2002) proposed that, from a modelling viewpoint, only compounds with green colour (chlorophyll, chlorophyllide, pheophytin and pheophorbide) and their precursors (protochlorophyllide) are of interest for products that have green colour as the limiting quality attribute. Figure 1 shows the proposed mechanism during the synthesis and breakdown of chlorophyll (CHL). Chlorophyllide (chl) holds a central position as it is an intermediate in both synthesis and breakdown. The initial level of Pchl (as part of the ternary complex) is depicted as crucial and governs the colour behaviour. The colour model is based on a key assumption: the level of Pchl in the ternary complex formed during preharvest is restrictive for the amount of chlorophyllide produced during (dark) postharvest storage. From the mechanism, shown in Figure 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. 3-5.

$$\frac{d\,Pchl}{dt} = -k_f\ Pchl \tag{3}$$

$$\frac{d\,CHL}{dt} = k_{fw}\ chl - k_{bw}\ CHL \tag{4}$$

$$\frac{d\,chl}{dt} = k_f\ Pchl - k_{fw}\ chl + k_{bw}\ CHL - k_d\ chl \tag{5}$$

with k_f, k_{fw}, k_{bw} and k_d the reaction rate constants for the formation of chl, the formation of CHL, the decay of CHL and the decay of chl, respectively. The reaction rate constants are each assumed to depend on temperature according to Arrhenius' law. This set of differential equations can be solved for constant external conditions under the assumption that chl is in steady state with Pchl and CHL (analytical solution not shown).

Gas conditions have a clear effect on the colour change of broccoli florets especially at high temperature. At 18°C, after 10 d of storage at 1.5 kPa O_2/15 kPa CO_2 broccoli heads were still green, but those stored at other CA conditions turned yellow. Broccoli heads stored for 10 d at 10 kPa O_2/0 kPa CO_2 showed fungal decay. Broccoli florets retained their colour better at low O_2 when combined with high CO_2, indicating that the rate of colour change depends on the CA conditions via the energy provided by respiration.

To describe the colour development over time as function of the gas conditions two steps are necessary. The first step is to link the reaction rate constant of the quality change process to the relative metabolic rate describing the aerobic respiration process: the relative respiration (e.g., Hertog et al., 2001) (Eq. 6).

$$k^{CA} = k^{RA}\ \frac{V_{O_2}{}^{CA}}{V_{O_2}{}^{RA}} \tag{6}$$

with k^{CA} the reaction rate constant and $VO_2{}^{CA}$ O_2 consumption rate constant for a certain CA treatment and k^{RA} and $VO_2{}^{RA}$ are the rate constants at regular atmosphere (RA).

During the second step it has to be examined which of the reaction rate constants are linked to the CA treatments. The whole colour data set was used to estimate the kinetic parameters and the initial conditions (CHL_0 and $Pchl_0$) of the colour model allowing (in succession) only one of the three reaction rate constants (k_{bw}, k_d and k_f) to vary with the CA treatments. This was accomplished by subsequently replacing one of the reaction rate constants by 12 estimated factors, one for each CA treatment. Only when k_d was replaced, values for the 12 factors were estimated with small standard errors (data not shown). These k_d replacement factors showed a very good relation with the O_2 consumption rates, indicating that this reaction rate constant is affected by the gas conditions. The link between the gas exchange model and colour model was provided by Eq. 6, only for k_d. The whole colour data set was subsequently analysed estimating the kinetic parameters of the colour model in common and the initial conditions (CHL_0 and $Pchl_0$) individually. The percentage variance accounted for applying a multi-response where both gas exchange and colour parameters were estimated was high, 92% (Table 2).

For a number of broccoli heads, colour synthesis was larger than the colour decay during the storage period, especially at 5°C (Fig. 2). Low temperature is more important than low respiration conditions to retain a dark green colour. However, at higher temperatures (e.g., at the retail market and at home) there is a distinct benefit of a low O_2 and a high CO_2 to increase the period of colour retention. Although currently MAP packaging of broccoli is not (yet) used in practise, the application of MAP will have a strong impact on colour retention of broccoli especially when the retention of the health promoting glucosinolates (Schouten et al., 2008) at low O_2 and high CO_2 is taken into account.

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Tables

Table 1. Overview of the CA conditions for each of the 12 CA containers.

CA treatment	O_2 (kPa)	CO_2 (kPa)	T (°C)
1	1.5	0	5
2	1.5	6	5
3	10	15	5
4	21	15	5
5	3	6	10
6	3	15	10
7	10	6	10
8	21	0	10
9	1.5	15	18
10	3	0	18
11	10	0	18
12	21	6	18

Table 2. Overview of parameter estimates and their standard error of estimates (s.e.) for the integrated colour model. $COLOUR_\infty$ describes the colour of the broccoli when all green colour components have vanished. $COLOUR_\infty$ was fixed on 2 (lowest experimentally observed value).

	Units	Estimate	s.e.
$VmO_{2,ref}$	nmol kg^{-1} s^{-1}	1570.1	63.1
$EVmO_2$	kJ mol^{-1}	117.9	3.6
KmO_2	kPa	7.78	0.47
$Kmcco_2$	kPa	∞	fixed
$Kmuco_2$	kPa	10.12	0.82
RQ_{ox}	-	0.834	0.018
$k_{bw,ref}$	d^{-1}	0.208	0.003
$k_{d,ref}$	d^{-1}	0.900	0.029
$k_{f,ref}$	d^{-1}	0.527	0.026
E_{bw}	kJ mol^{-1}	0	fixed
E_d	kJ mol^{-1}	142.2	1.9
E_f	kJ mol^{-1}	124.4	3.5
$COLOUR_\infty$	1000/R	2	fixed
CHL_o	1000/R	7.00	1.72
$Pchl_0$	1000/R	0.97	1.25
T_{ref}	18°C		
N	1397		
R^2_{adj}	92.1%		

Figures

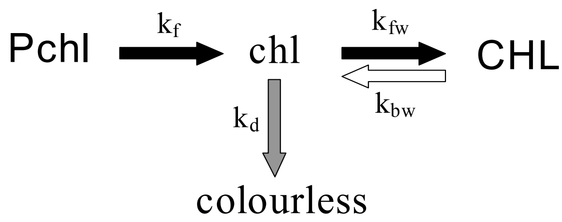

Fig. 1. Model of the last part of the chlorophyll pathway during dark storage. Closed arrows are used to indicate chlorophyll synthesis and open arrows are used for the chlorophyll catabolism. The gray arrow indicates the reaction rate constant affected by CA.

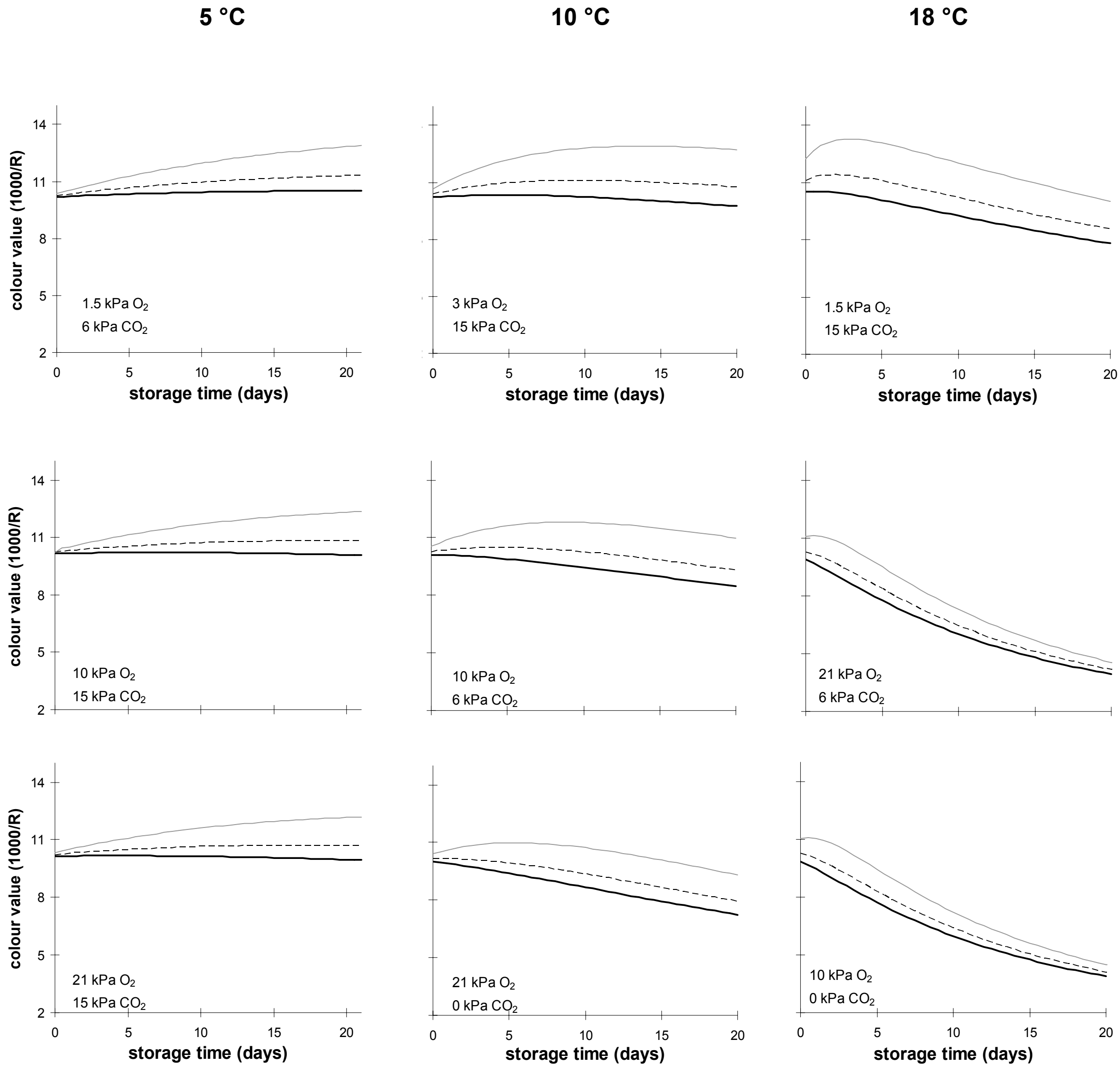

Fig. 2. Plots of colour change for three simulated broccoli heads during dark storage as function of temperature and gas conditions (indicated in the lower left hand side of each plot). The three broccoli heads per plot differ only in $Pchl_0$ value, either 1 (bold line), 2 (dotted line) or 4 (gray line). For CHL_0, the average value for this batch is used.