The Propagation of Variation in Glucosinolate Levels as Effected by Controlled Atmosphere and Temperature in a Broccoli Batch

R.E. Schouten*, X. Zhang, L.M.M. Tijskens and O. van Kooten
Horticultural Supply Chains
Wageningen University
the Netherlands
* E-mail: Rob.Schouten@wur.nl

Keywords: respiration, gas conditions, HPLC, integrated modelling, biological variation

Abstract

Broccoli combines high levels of vitamins, fibres and glucosinolates (GLS) with a low calorie count. GLS are precursors for the characteristic broccoli flavour and have anti-carcinogenic properties. This study describes the effect of controlled atmosphere (CA) and temperature on GLS concentrations in broccoli. Data on GLS behaviour and gas exchange were gathered for broccoli heads that were stored at three temperatures and subjected to four levels of O_2 and three levels of CO_2. The GLS behaviour of three GLS (raphanin, GB and neo-GB) was examined that showed exponential decrease over time, possibly representing the GLS interaction with myrosinase. The most striking feature is the large variation in GLS concentrations at harvest. The propagation of the variation in GLS over time is clearly affected by CA and temperature. Variation in GLS concentrations over time at the same gas conditions and temperature was interpreted with the moment of harvest as main cause of random variation. Assuming that this random variation is normally distributed, the exponential function over time can be transformed into a batch model that describes the changes of variation over time (Schouten et al., 2004, Hertog et al. 2004). The effect of the CA was modelled using the standard gas exchange model. This calibrated gas exchange model was then linked via the reaction rate constant to the batch model to create an integrated batch model. This integrated batch model was subsequently calibrated to describe the variation of the GLS as function of O_2, CO_2, time, temperature and the batch parameters (average biological age and standard deviation). The percentage variance accounted for was on average 85%. Considering that this modelling effort is based on destructive GLS measurements, this is a rather high value. All GLS species were retained by suitable (low O_2, high CO_2) gas conditions, but remarkably, raphanin was found to be less affected by temperature, indicating that CA storage and low temperature would both retain this GLS species to the same extent, while low temperature storage is a better option for e.g. neo-GB.

INTRODUCTION

Broccoli combines high levels of vitamins, carotenes, fibres, glucosinolates (GLS) 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. One of the main internal quality attributes is the level of GLS. The hydrolysis products of GLS are responsible for the characteristic flavour of brassicaceous vegetables and have anti-microbial and cancer-preventing properties. Controlled Atmosphere (CA) is a very effective method to maintain broccoli quality; retention of the green colour is best obtained when 1-2% O_2 combined with 5-10% CO_2 at temperatures between 0 and 5 °C (Jones et al., 2006). However, the effects of CA on the levels of GLS have not been studied. The aim of this paper is to characterise the levels of some GLS of broccoli as affected by CA and temperature. Data on GLS behaviour and gas exchange were gathered for broccoli heads that were stored at three temperatures and subjected to four levels of O_2 and three levels of CO_2. A plausible model for the change of the GLS levels over time is linked to a gas exchange model into an integrated model. Such an integrated model describes the combined effects of O_2, CO_2 and temperature on the GLS levels of broccoli

heads. The model will be applied to compare the GLS levels of CA stored broccoli at relatively high temperature (18° C) compared to that of broccoli stored at relatively low temperature (10 °C) in RA (regular atmosphere).

MATERIAL AND METHODS

Broccoli

About 800 broccoli heads, 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 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 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 according to de Wild and Peppelenbos (2001). The accumulation period varied from four, two and one hour for the rooms at 5°, 10° and 18° C respectively. GC measurements were carried out approximately every three days for the broccoli stored at 5 and 10 °C and approximately every two days for the broccoli stored at 18°C.

GLS Measurements

The central floret of each broccoli head was flash-frozen in liquid nitrogen. Samples were freeze dried and milled before extracting the GLS. GLS were extracted based on HPLC protocols published by Vallejo et al. (2002) and Verkerk et al. (2001). GLSs were quantified using the internal standard glucotropaeolin and expressed as μmol g^{-1} dry matter. Peaks were identified with the help of Ruud Verkerk (personal communication) and by comparison with data from Minchinton et al. (1982).

RESULTS AND DISCUSSION

Glucosinolates

Due to the destructive nature of the GLS measurements only limited information how GLS levels change over time can be obtained. However, by assuming that about twenty broccoli heads per container are representative of the batch, information about the development of GLS over time can be extracted on the batch level by analysing broccoli heads in ‘replicate’ containers (containers stored at the same temperature and gas conditions). This means that, including the initial GLS measurement just after harvest, for each combination of gas conditions and temperature the GLS level is monitored at four points in time (Table 1). Gas conditions have a clear effect on the level of GLS. Broccoli florets retained GLS better at low levels of O_2 when combined with high levels of CO_2, indicating that the rate of decrease depends on the CA conditions via the energy provided by the gas exchange (Hertog et al., 2001). The most striking feature is the large variation in GLS at harvest that seems to diminish over time due to an apparently exponential

decay over time for raphanin, glucobrassicin (GB) and neo-GB. This behaviour may be explained by myrosinase, the enzyme that will cleave glucose from the GLS (Mithen et al., 2000).

Gas Exchange

Decreasing levels of O_2 inhibited both O_2 consumption and CO_2 production with a respiration quotient between 0.6 and 1.4 (data not shown). That is indicative for aerobic respiration only. The inhibiting effect of CO_2 did not decrease with increasing levels of O_2 which points to an uncompetitive type of CO_2 inhibition. Consequently, gas exchange for this broccoli batch is described according to Eq 1-2.

$$V_{o_2} = \frac{Vm_{O_2} \cdot O_2}{Km_{O_2} + O_2 \cdot \left(1 + \frac{CO_2}{Kmu_{CO_2}}\right)} \quad (1)$$

$$V_{co_2} = RQ_{ox} \cdot V_{o_2} \quad (2)$$

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)).

Integrated Batch Approach

To describe the propagation of the variation of GLS over time, temperature and gas conditions two steps are needed. 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 (e.g. Hertog et al., 2001). This equation (Eq. 3) can be expanded with an anaerobic term, but this was unnecessary as no significant fermentation in this batch of broccoli was encountered.

$$k_{GLS}^{CA} = k_{GLS}^{RA} \frac{V_{O_2}^{CA}}{V_{O_2}^{RA}} \quad (3)$$

with k_{GLS}^{CA} the reaction rate constant and VO_2^{CA} the O_2 consumption rate constant for a certain CA treatment and k_{GLS}^{RA} and VO_2^{RA} are the rate constants at regular atmosphere (RA) at the same temperature. k_{GLS}^{RA} is assumed to depend on temperature according to Arrhenius' law.

The approach described above can be combined with the assumed exponential decrease of the GLS level over time for individual broccoli heads (first part of Eq. 4). GLS_0, the GLS level at harvest, was assumed to be the result of GLS decay during preharvest. The postharvest GLS change model can then be expressed as a function of the storage time after harvest and the biological age at harvest (second part of Eq. 4). GLS_{ref} is an arbitrary reference GLS level, Δt the biological age expressed as the time (in day) needed to change the GLS level from GLS_{ref} to GLS_0. k_{GLSpre} (the reaction rate constant during preharvest period) was assumed to correspond to k_{GLS} at the average growth temperature, assumed 10 °C. Combining with Eq. 3 provides the last part of Eq. 4.

$$GLS(t) = GLS_0 \cdot e^{k_{GLS}^{CA} \cdot t} = GLS_{ref} \cdot e^{-k_{GLS}^{CA} \cdot t - k_{GLSpre} \cdot \Delta t} = GLS_{ref} \cdot e^{-k_{GLS}^{RA} \frac{V_{O_2}^{CA}}{V_{O_2}^{RA}} \cdot t - k_{GLSpre} \cdot \Delta t} \quad (4)$$

For the second step, the finding that the biological age is normally distributed, is incorporated in Eq. 4. Experimental data are put into classes and are represented as relative frequency data. The measured variation is then expressed as the probability that

data belong to a certain class (q_a,q_b) of the quality function Q. Assuming that the biological age (Δt) normally distributed this results in the following batch model formulation (Eq. 5) (Schouten et al., 2004).

$$\Pr(Q(t) \in (q_a, q_b)) = \Pr\left(Q(\Delta t) \le q_b\right) - \Pr\left(Q(\Delta t) \le q_a\right) = \Phi\left(\frac{Q^{-1}(q_a) - \mu}{\sigma}\right) - \Phi\left(\frac{Q^{-1}(q_b) - \mu}{\sigma}\right) \quad (5)$$

with Φ the cumulative standard normal distribution function, μ the mean and σ the standard deviation (both expressed in days) of the biological age distribution at harvest. Q^{-1} in Eq. 5 can be replaced by the inverse of the last part of Eq. 4, resulting in Eq. 6.

$$\Pr(GLS) = \Phi\left(\frac{-\ln\left(\frac{GLS_a}{GLS_{ref}}\right) - k_{GLS}^{RA}\,\frac{V_{O_2}^{CA}}{V_{O_2}^{RA}}\cdot t}{k_{pre}^{GLS}} - \mu_{GLS}}{\sigma_{GLS}}\right) - \Phi\left(\frac{\frac{-\ln\left(\frac{GLS_b}{GLS_{ref}}\right) - k_{GLS}^{RA}\,\frac{V_{O_2}^{CA}}{V_{O_2}^{RA}}\cdot t}{k_{pre}^{GLS}} - \mu_{GLS}}{\sigma_{GLS}}\right) \quad (6)$$

Raphanin, GB and neo-GB data were tabulated and the relative frequency data were analysed using non-linear regression analysis based on the model formulation of Eq. 6 together with the temperature dependence according to Arrhenius' law for k_{GLS}^{RA}. Fig. 1 shows the propagation of the measured raphanin distributions (bars) and simulated probabilities (lines) applying the gas exchange parameters and the kinetic/batch parameters from Table 2. It is clear that the frequency distributions and simulated probabilities move, starting from the skewed initial distribution at harvest towards a more symmetrical, spiked distribution with a speed that increases with storage temperature, a high level of O_2 and a low level of CO_2.

The kinetic parameters from the three GLS are quite comparable (Table 2). Taking the variance present in the data into account, the percentage variance accounted for, R^2_{adj}, is relatively high, around 85%. The high variation in the data is likely due to the low number of broccoli heads, around 20, in each container that was used to represent the whole batch. It appears that a higher value for E_{GLS} coincides with a larger value of σ. This might indicate that neo-GB, having the highest value for E_{GLS}, will be more sensitive for small temperature fluctuations during preharvest, resulting in a higher biological variation at harvest compared to e.g. raphanin. Practical information that may be gained from this modelling attempt is to test the suitability of different storage conditions to retain the different GLS using either low temperature or CA storage. For instance, storage of broccoli for ten days at 10°C and RA is a very effective method to retain more than 90% of the initial level of neo-GB and raphanin, whereas storage at 18 °C only leaves less than 10%. To retain raphanin, CA storage (1.5 kPa O_2, 15 kPa CO_2) at 18°C is as effective as storage at 10°C at RA (data not shown).

ACKNOWLEDGEMENTS

Help during the gas exchange measurements by Els Otma and Jan Verschoor and help with the HPLC measurements by Ruud Verkerk is greatly appreciated.

Literature Cited

De Wild, H.P.J. and Peppelenbos, H.W. 2001. Improving the measurement of gas exchange in closed systems. Postharvest Biol. Technol. 22:111-119.

Hertog, M.L.A.T.M., Nicholson, S.E. and Banks, N.H. 2001. The effect of modified atmospheres on the rate of firmness change in 'Braeburn' apples. Postharvest Biol. Technol. 23:175-184.

Hertog, M.L.A.T.M., Lammertyn, J., Desmet, M., Scheerlinck, N. and Nicolaï, B.M. 2004. The impact of biological variation on postharvest behaviour of tomato fruit. Postharvest Biol. Technol. 34:271-284.
Jones, R.B., Faragher, J.D. and Winkler, S. 2006. A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (*Brassica oleracea* var. *italica*) heads. Postharvest Biol. Technol. 41:1-8.
Minchinton, I., Sang, J., Burke, D. and Truscott, R.J.W. 1982. Separation of desulpho-glucosinolates by reversed-phase high-performance liquid chromatography. J. Chromatography 247:141-148.
Mithen, R.F., Dekker, M., Verkerk, R., Rabot, S., and Johnson, I.T. 2000. The nutritional significance, biosynthesis and bioavailability of glucosinolates in human Foods. J. Sci. Food Agric. 80:967-984.
Schouten, R.E., Jongbloed, G., Tijskens, L.M.M. and van Kooten, O. 2004. Batch variability and cultivar keeping quality of cucumber. Postharvest Biol. Technol. 32:299-310.
Vallejo, F., Tomás-Barberán, F.A. and García-Viguera, C. 2002. Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. J. Sci. Food Agric. 82:1293-1297.
Verkerk R., Dekker, M. and Jongen, W.M.F. 2001. Post-harvest increase of indolyl glucosinolates in response to chopping and storage of *Brassica* vegetables. J. Sci. Food Agric. 81:953-958.

Tables

Table 1. Overview of the CA conditions for each of the 36 CA containers for each temperature. The level of O_2 (kPa) and CO_2 (kPa) is indicated under the container numbers.

5°C											
1	2	3	4	5	6	7	8	9	10	11	12
10/15	10/15	10/15	1.5/0	1.5/0	1.5/0	21/15	21/15	21/15	1.5/6	1.5/6	1.5/6

10 °C											
13	14	15	16	17	18	19	20	21	22	23	24
3/6	3/6	3/6	21/0	21/0	21/0	10/6	10/6	10/6	3/15	3/15	3/15

18 °C											
25	26	27	28	29	30	31	32	33	34	35	36
10/0	10/0	10/0	1.5/15	1.5/15	1.5/15	3/0	3/0	3/0	21/6	21/6	21/6

Table 2. Overview of gas exchange, kinetic and batch parameters for three GLS belonging to one broccoli batch. $EVmO_2$ (kJ/mol) and E_{GLS} (kJ/mol) are the activation energies of VmO_2 and $k_{GLS,}$ respectively.

gas exchange				glucoraphanin		GB		NeoGB	
value	estimate	s.e.		value	s.e.	value	s.e.	value	s.e.
$VmO_{2,ref}$	1119.2	69.2	$k_{GLS,ref}$	0.190	0.004	0.247	0.006	0.1159	0.0176
$EVmO_2$	108.16	3.87	E_{GLS}	107.88	2.64	168.21	5.29	242.8	41.8
KmO_2	4.12	0.49	σ	4.52	0.20	9.06	0.65	60.6	10.4
Kmcc	26.0	6.2							
RQ_{ox}	0.82	0.02							
T_{ref}	18°C								
R^2_{adj} (%)	93.2			84.2		87.7		82.3	

Figures

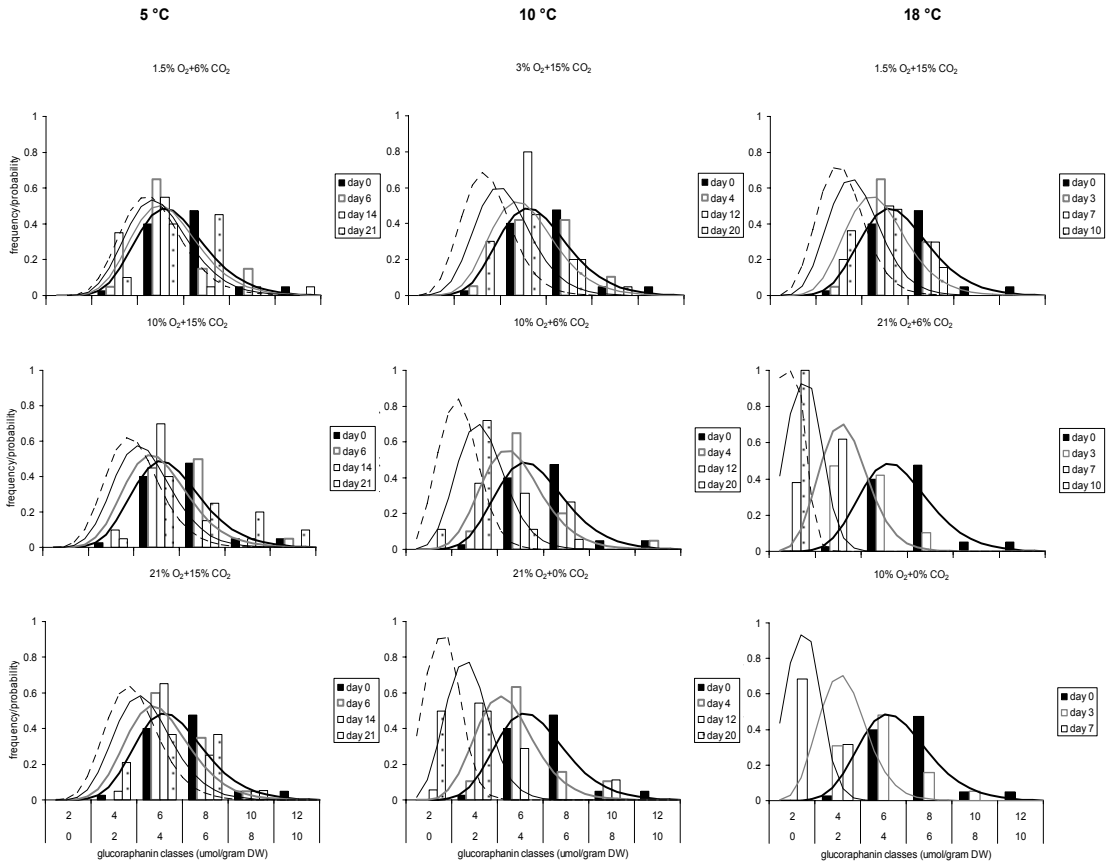

Fig. 1. Raphanin distributions (bars) over time for the broccoli heads stored at three levels of O_2 and three levels of CO_2 (indicated per plot) at three temperatures (indicated per column) together with the simulated batch model (lines).