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Modelling Browning and Brown Spotting of Mushrooms (Agaricus bisporus) Stored in Controlled Environmental Conditions Using Image Analysis

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Modelling browning and brown spotting of mushrooms (Agaricus bisporus) stored in controlled environmental conditions using image analysis

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Abstract

Mushrooms have a short postharvest shelf life compared to most vegetables, due to a very high metabolic activity and high water content, making them prone to microbial spoilage and to exhibit enzymatic browning.

Storage conditions and natural product variability are both important factors that affect the management of mushrooms, and both of them can be managed using monitoring systems. In order to study the effect of the temperature and relative humidity on the whiteness decrease and appearance of brown spotting, an image analysis system was employed. Twenty-five batches of mushrooms were subjected to combinations of three storage temperatures (T) (5 °C, 15 °C and 25 °C) and three storage relative humidities (RH) (70%, 80% and 90%). Further validation experiments were performed at lower and higher temperatures.

The study showed that the kinetics of colour degradation and spotting followed a logistic pattern, and that best storage conditions to delay the onset of browning and spotting could be found at high relative humidities (<90%) and refrigeration temperatures as high as 11 °C without a significant reduction of the whiteness or development of browning during the first two days compared to mushrooms stored at 3 °C. Mushrooms stored at 11 °C for longer than two days, would have quick drop in colour. This findings are in agreement with current practise in retail, where mushrooms normally arrive from the agricultural producer at very low storage temperatures (0–3 °C) and is then retailed at vegetable display conditions (8–11 °C) with a very quick product replacement rate.

Keywords: Image analysis, Mushroom, Storage, Kinetics, Modelling, Non-linear mixed effect

1. Introduction

Mushrooms have a short postharvest shelf life of 3–4 days compared to most vegetables, mainly because they have no cuticle to protect them from physical or microbial attack and water loss. The cultivated mushroom (Agaricus bisporus) is highly susceptible to blemishes caused by a range of bacterial and fungal pathogens, and discoloration induced by bruising, storage and physiological disorders (Vízhányó and Felföldi, 2000). In the same way, they have a very high respiration rate and high water content, making them prone to microbial spoilage. Finally their high tyrosinase and phenolic content makes them very susceptible to enzymatic browning (Brennan et al., 2000).

The main processes which contribute to loss in quality after harvest are (i) discoloration, (ii) browning, (iii) loss of closeness, (iv) weight loss and (v) texture changes (Burton and Noble, 1993; Berendse, 1984). The colour and the shape of the cap is the most important consideration of fresh mushrooms, since it is the first characteristic consumers notice (Brosnan and Sun, 2004). After harvest the mushroom colour gradually changes from white to brown, due to the appearance of browning and possibly bacterial blotching, while the growth of the stipe and the cap continues. The cap growth results in gradual opening of the mushroom cap (Lukkasse and Polderdijk, 2003).

Despite the efforts of agricultural production, classification and packaging, one of the main problems in mushroom production is the uncontrollable effect of the natural product variability. From a retailer point of view different batches of mushrooms arrive at a different maturity stage and inside every batch there is natural product heterogeneity. This variability results in important losses from the retailer–producer point of view and monitoring systems can help to study and manage this natural variability.

Colour can be rapidly analyzed by computerized image analysis techniques. These systems not only offer a methodology for measurement of uneven colouration but can also be applied to the measurement of other attributes of the total appearance (Hutchings, 1999). Computer analysis of camera images of mushroom caps may offer several advantages over visual assessment. It may be
possible to discriminate types of blemish mathematically from the
spectral characteristics. Information about blemishes with known
causes can be stored and compared with new images. The equip-
ment could be operated by a non-specialist, and give an immediate,
objective result (Vizínhony and Fellföldi, 2000).

The agricultural industry uses measurements of colour mainly
due to three reasons: (i) colour serves as instant indicator of prod-
uct quality, (ii) colour measurement has been employed to develop
optimal storage policies with the aim to maintain appealing
form, clear, and fresh) with density of 0.547 ± 0.005 g/cm³, ater
Closed cup mushrooms A. bisporus Sylvan A15 (white, close uni-
(30 °C & 80% RH) to investigate if there were possible departures
representing a typical medium size retail situation. The experi-
ments were carried out over 1 ½ years (April 2004–October 2005).

Accelerated experiments, in order to study the senescence, were
performed in an environmental incubator (MLR-350 HT, SANYO
Electric Biomedical Co. Ltd., Japan) with temperature, relative
humidity and lighting conditions controlled. The study involved
monitoring of the mushrooms at three temperatures (T) levels
(5 °C, 15 °C and 25 °C) and three relative humidity (RH) levels
(70%, 80% and 90%) in a 3×full factorial design for up to 10 days,
based on conditions researched in previous studies (Escriche
et al., 2001; Pai, 2000). Each combination was carried out a mini-
mum of two times using six mushrooms in each experiment.

Two further experiments were performed at lower temperature
(3 °C & 70% RH and 3 °C & 80% RH) and one at a higher temperature
(30 °C & 80% RH) to investigate if there were possible departures
from the model assumptions at lower or higher temperature
ranges that could affect mushroom storage. A total of 25 experi-
ments amounting to 128 individual mushroom kinetics and 3864
experimental measures were taken.

2.2. Image acquisition

The mushroom batches were monitored using inexpensive
webcams under controlled illumination conditions (Logitech©
QuickCam© Express, Logitech Europe S.A, DE). The images were ta-
ken every hour inside an incubator with controlled illumination
conditions. A light source of a fluorescent lamp (40 W) was incor-
porated into the incubator. Six mushrooms were placed at the cen-
tre of the incubator tray covered in non-reflecting black cardboard
and placed on a sample support painted in non-reflecting colour
(black matte). At a sufficient distance not to interfere with the
mushroom image, six non-reflecting coloured cardboard samples
were placed. These cardboard samples were measured at the begin-
ing, the end of the experiment and between experiments with a colorimeter and used to control possible bias between
experiments and drifts in the performance of the camera. These
colour cardboard samples change were not significant during the
experiments.

Camera and mushroom location were fixed in all the experi-
ments. An automated protocol to (i) fix the camera settings and
(ii) automate camera image acquisition was developed using Java
(Sun Microsystems Inc., Santa Clara, CA, USA).

2.3. Image analysis

Image analysis was performed using Image J (NIHM, National
Institute of Mental Health, Bethesda, Maryland, USA). The image
was transformed from the RGB space to an 8-bit greyscale image
using the transformation.

\[ \text{Grey value} = \frac{\text{Red} + \text{Green} + \text{Blue}}{3} \]  (1)

A region of interest (ROI) was selected in a stack of images com-
prising a whole mushroom cap over the storage time.

There are two main colour attributes of mushroom which con-
sumers use to accept or reject the product, the appearance of
brown spots and the general browning of the cap follows in order
of importance. The following image indexes were extracted:

(1) The average greyscale value (grey value) kinetics of the ROI
were employed as a measurement of the whiteness (L*) and
general browning of the mushroom. Under these controlled
illumination situation and range of greyscale for the camera,
a linear relationship between the L* and the greyscale was
found.

(2) The standard deviation (SD) of the ROI was used as a con-
trast measurement to follow the appearance of brown
spotting.

In the case of the local standard deviation the kinetic was associ-
atcd to the onset of brown spotting in the cap of the mushrooms.
The kinetics of this quality index showed the transition from white
to spotted cap.

2.4. Mathematical modelling

Both average grey value and standard deviation kinetics could be
accommodated with a typical sigmoidal shape (Fig. 1) with a
transition from “fresh mushroom” to “brown mushroom” and were
modelled using a logistic model (Pinheiro and Bates, 2000):

\[ \text{Grey} = \text{Grey}_{\text{final}} - \frac{\text{Drop}}{1 + e^{-\frac{\text{t}}{\text{t}_{\text{crit}}}}} \]  (2)

where Grey is the particular response under observation, Drop is
the change from the initial (white) state to the final (brown) state,
\text{t}_{\text{crit}} is the transition time needed to get the middle point of the
transition, and \text{t}_{\text{crit}} stands for the critical time defining the speed of the
transition.

2.5. Variability modelling

Two nested random effects (batch to batch and sample to sam-
ple inside a batch) were assigned to each parameter (Drop, \text{t}_{\text{crit}}
and \text{t}_{\text{crit}}) to construct a nonlinear mixed effect model.

2.6. Secondary modelling

The main secondary variables affecting the storage kinetics of
the mushroom were the temperature and the relative humidity.
The Vapour Pressure Deficit (VPD) was used instead of the RH in
order to avoid the interaction between T and RH (Aguirre et al.,

2
An initial model was built with random effect terms and no dependence of the parameters with T or VPD.

The secondary model was built by adding polynomial model terms with storage conditions (fixed effects) of temperature (T) and vapour pressure deficit (VPD) and product variability (random effects) to the primary model parameters and then tested for model improvements using a log-likelihood ratio test in a stepwise fashion. The final model components were as follows:

The model building process followed a series of steps:

1. An initial model was built with random effect terms and no dependence of the parameters with T or VPD.

2. A summary of the model was produced with t-statistics for each individual model coefficient and Wald tests for each model term.

3. Based on the Wald test statistics of significance for the fixed effect non-significant terms of the model were eliminated (Pinheiro and Bates, 2000).

4. Polynomial model terms with storage conditions (T and VPD) were added.

5. A summary of the new model was produced with Wald tests for each model term.

6. The logarithm likelihood ratio test and the Akaike Information Criteria were employed to compare the new model with the previous one.

7. Steps 2–6 were repeated until a satisfactory model augmentation was achieved.

Finally, in order to assess the suitability of the best model, the random effects and residuals were studied for seasonality effects.

3. Results and discussion

3.1. Results and discussion of the grey value

The whiteness kinetic of the mushroom decreased with time, and brown spotting kinetic showed an increase with time. Both kinetics were influenced by environmental conditions of T and RH (Fig. 1).
Although variability played an important role, the environmental conditions also exerted an effect on the colour kinetics, with some storage conditions slowing colour degradation kinetics. After the model building work outlined a final candidate model was selected. Table 1 shows the estimated different parameters for the grey value kinetics. The asymptotic estimate of the grey value decrease depended on the temperature (T). The time required to reach half of the Browning transition point (t90 parameter) was significantly affected by the temperature, vapour pressure deficit, quadratic effect of the temperature and the interaction between the temperature and the vapour pressure deficit. The speed at which the Browning transition process took place, expressed by t15 parameter, was only affected by temperature. The quadratic effects on the transition time (t90) pointed to possible optimal storage conditions that may decrease the kinetics of the Browning process and extend the time necessary to arrive to the middle point of the transition from white to brown.

Fig. 1 shows a selection of experimental data set with best linear unbiased predictions (BLUP) of the model. The BLUP may be used to compare the behaviour of the grey value for existing mushroom batches within the same environmental conditions. It was possible to see how the model accommodated the data and described appropriately their kinetics. The residual plot showed the residuals were randomly distributed. There were some outliers, which is a typical situation in modelling of continuous monitoring devices, where the magnitude of the data available (3664 experimental data) and possible instrumental deviations impairs the modelling process.

From Table 1 it was possible to generate a map of the dependence of the model parameters with storage conditions. Fig. 2 indicates how the transition time from “white” to “brown” changed with the temperature, the VPD and the RH. It can be seen in Fig. 2a that the best way to delay the Browning of the mushroom would be to employ a low storage temperature (5 °C–15 °C) and low water vapour pressure deficit (0.2). The decrease in whiteness was affected by the temperature, the VPD and the RH. It can be seen in Fig. 2a that the best way to delay the Browning of the mushroom would maintain its water content and the grey value would not start this transition decrease for at least 3.5 days. In the case of higher temperature, the water vapour pressure deficit also increased, therefore drying the mushroom. At these conditions the whiteness is maintained but the quality of the product is not acceptable. Although VPD is a conventional variable for refrigeration technology, package designers and food technologists usually employ the RH. Fig. 2b indicates that the best conditions to delay the onset of brown colour on the mushroom cap were low temperatures (4 °C–13 °C) and high RH (close to saturation). Under these conditions, the whiteness was maintained for a similar time. The Fig. 2b also shows that in storage conditions where the relative humidity was higher than 80% and temperature was between 0 °C and 18 °C the whiteness is maintained for three days.

In Fig. 2b at 25 °C and relative humidity below 40% the whiteness was maintained for 10 days. In this case, due to the high temperature the mushroom lost so much water that it was dried and the colour did not have time to change. Burton and Noble (1993), Pai (2000) and Pardo et al. (2001) proved that the decrease in whiteness was affected by the temperature and reported that lower temperatures decreased this whiteness loss, which was confirmed by the present finding. Lukkasse and Polderdijk (2003) reported that the temperature affected the shelf life of the mushrooms, being shorter when the temperature was increased. Furthermore, the present study showed that the relative humidity also affected the transition process from “white” to “brown”. Fig. 2 shows that the relative humidity was an important factor and the maintenance of a high relative humidity during storage was necessary to maintain the whiteness of the mushrooms. It is possible to allow higher storage temperatures if the relative humidity is higher than 90%. These results for the grey value decrease are in accordance with the previous results for the L* value obtained by Aguirre et al. (2008). The improvement in whiteness from storage at 25 °C to 3 °C is a 75% less Browning (as seen by the relative change in the Drop parameter).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drop [0–255]</td>
<td>À6.7 (4.8–7.5) T</td>
</tr>
<tr>
<td>Transition Time [h]</td>
<td>7.9 (1.4) + 5.5 T + 22 (10.5) VPD</td>
</tr>
<tr>
<td>Critical Time [h]</td>
<td>Â0.32 (0.15) T + 10.72 (0.6) T * VPD</td>
</tr>
<tr>
<td>Variability between samples</td>
<td>38.9 (4.6)</td>
</tr>
<tr>
<td>Critical time [h]</td>
<td>15.1 (10.7)</td>
</tr>
<tr>
<td>Error term</td>
<td>2.14 (2.16)</td>
</tr>
</tbody>
</table>

The standard error of each coefficient is presented in subscripted brackets. All effects were significant (p < 0.05).

![Fig. 2](image-url) (a) Contour plots of the dependence of the transition time of the grey value damage process on the water vapour pressure deficit (VPD) and/or (b) RH with the temperature of mushroom batches.
3.2. Scenario analysis and between batches variability assessment

Using the random effects sources of variability (and excluding the uncertainty from the estimated parameters, arising from the instrumental error of the acquisition system) stochastic simulations of different storage scenarios were performed in order to compare the average colour evolution of batches of mushrooms between (i) abused storage (25 °C and 90% RH), (ii) retail guideline storage (Pai, 2000 and Pardo et al., 2001) conditions (5 °C and 90% RH) and (iii) a proposed optimal arising from this study (11 °C and 95% RH). A Monte Carlo simulation (n = 15,000) was performed to obtain the 5% and 95% percentiles of the population of stored batches of mushrooms at each one of 50 equally spaced time points between the initial day and the 7th day of storage.

Storage of mushrooms in an abused situation produced a decrease of the grey value of an important part of the population of mushroom batches, with losses in the grey value becoming very important as storage sets on and for the first days (Fig. 3a). As mushrooms decayed and entered the later phases of senescence, the differences in the grey value population decreases.

Fig. 3b shows that taking into account the variability between batches of the system there was a marginal difference between the retail guidelines and a higher storage temperature optimal arising from this study. The effect between batches variability was very important in the fate of a mushroom during storage and the effect of decreasing temperature might not yield an effect of maintaining the whiteness of the mushrooms for all the energy that has been spent compared to storage at 11 °C. This storage of mushrooms at higher temperature occurs in the retail sector, at the point of receipt of the product and the following display of the product in the vegetable cabinets. Using this storage temperature mushrooms would keep, taking into account for variability, in a similar quality standard as mushrooms stored at very much lower temperatures for at least two days, which is the turnover time of a fresh product. However, after the consumer bought this product, the toll of the high temperatures employed during retail would decrease the time during which mushroom would keep at high quality in the consumers refrigeration unit. In order to pass to the consumer the maximum shelf life time of the product, the lower refrigeration temperatures should be employed, not only for post-harvest and distribution, but also for retail.

3.3. Results and discussion of the Standard deviation kinetics (SD)

The SD, a measurement of the image contrast, may be employed to follow the development of browning spots in the mushroom cap. The kinetics of the local SD of the grey value of a whole mushroom cap region of interest (ROI) was monitored for this purpose.

The secondary model was built by adding linear model terms with storage conditions (fixed effects) and product variability (random effects) to the primary model parameters and then testing for model improvements using a log-likelihood ratio test in a stepwise fashion. The final model components were as follows.

(1) Estimated fixed effects: (i) a linear effect with temperature was found for the Drop, (ii) a constant ttrans parameter and (iii) a quadratic dependence with temperature for the tcrit.

(2) Estimated Random effects: Independently distributed random effects associated to each of the mushrooms measured were assigned to the Drop, ttrans and tcrit to include the mushroom-to-mushroom variability in the shape of normal distributions and to Drop, ttrans to describe the batch-to-batch variability.

Table 2 shows that brown spotting was affected by the temperature but not by the relative humidity. The asymptotic of the grey value decrease depended on the temperature (T). In the case of the speed at which browning took place (the tcrit parameter), the parameters that affected were the temperature and the quadratic effect of the temperature. Due to the presence of the quadratic ef-

![Fig. 3](image-url)
fect of the temperature it was possible to calculate an optimal storage temperature (11 °C) to slow down the brown spotting which is indicated in Fig. 6. Fig. 4 showed a selected example data set compared to the best linear unbiased predictions (BLUP) of the model. It can be seen how the model accommodated the experimental data. The residual of SD were randomly distributed. The effect of the variability between mushrooms in the model parameters was assessed, affecting the final SD value ($62\%$), the transition time ($50\%$) and finally the critical time ($28\%$). The uncertainty of the estimated parameters was lower than the random effects, except for the final SD value.

3.4. Scenario analysis and between batches variability assessment

Stochastic simulations of different storage scenarios were performed in order to compare the average brown spotting process in different batches of mushrooms between (i) abused storage.
In the case of the grey value, the study showed that to maintain the grey value for as long as possible, four days, it was necessary to have low temperatures (4 °C–13 °C) and high RH (close to saturation). The study showed that to maintain the grey value for three days, such extreme conditions are not necessary. If the temperature was between 0 °C and 18 °C and the relative humidity higher than 80% the grey value was maintained for three days. These conditions can be more achievable because they were the conditions that the mushrooms are stored at the supermarket. However, if the retailer wished to pass the six days of shelf life to the consumer, lower storage temperatures at high relative humidity should be required during retail.

In the case of the standard deviation, the study proved that the appearance of the brown spotting was only affected by the temperature.

References


