

# Optimisation of humidity absorbers in active packaging of button mushroom by response surface methodology and genetic algorithms

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## RESEARCH PAPER

### Abstract

Button mushroom is one of the most perishable products. Respiration of product leads to an accumulation of moisture within the package and the speed of product spoilage increases. The use of active packaging with moisture absorbers results in absorption of the excess moisture within the package and a slowing down of the speed of product spoilage. In this work the effects of three moisture absorbers (silica gel, CaCl<sub>2</sub> and sorbitol) were studied. The amount of absorbers with respect to quality indicators like weight loss, cap size, stem length and colour parameters was optimized in a response surface methodology (RSM) design. The coefficients of the RSM models were optimised using the genetic algorithm (GA) method. The results showed that using 5 g silica gel and 5 g CaCl<sub>2</sub> during storage time of button mushrooms led to the lowest weight loss, changes of cap size, stem length and colour difference ( $\Delta E$ ). Predicted values by GA optimised model with experimental values had a higher correlation coefficient than predicted values by RSM model that showed the performance of GA optimisation. Usage of the moisture absorbers led to an increase in the shelf life of button mushrooms.

**Keywords:** active packaging, button mushroom, genetic algorithm, humidity absorber, optimisation, response surface methodology

## 1. Introduction

Button mushroom (*Agaricus bisporus*) is one of the most popular mushrooms traditionally cultivated in the world. Consumption and production of this edible mushroom have grown continuously in recent years. It is the most extensively cultivated edible mushroom, comprising 32% of worldwide production (Chang, 1999). Mushrooms are a good source of vitamin B2, niacin, folates and many mineral elements (Mattila *et al.*, 2001).

Mushrooms are highly perishable due to their thin and porous epidermal structure resulting in high respiration rates which induce deterioration immediately after harvest. More importantly, the high tyrosinase and phenolic content of mushrooms makes them prone to enzymatic browning (Brennan *et al.*, 2000) which is the major cause

of quality loss that accounts for a reduction in market value (Mohapatra *et al.*, 2008).

However, button mushrooms only have a short shelf life of 3-4 days; they lose their commercial value within a few days, due to browning, water loss, senescence and microbial attack. The short shelf-life of mushroom is an impediment to the distribution and marketing of the fresh product. Thus, prolonging post-harvest storage while preserving their quality would benefit the mushroom industry as well as consumers (Jiang *et al.*, 2011).

There are many methods to extend the shelf-life of mushrooms. They include modified atmosphere packaging (Roy *et al.*, 1995), controlled atmosphere storage (Lopez-Briones *et al.*, 1992), coating (Nussinovitch and Kampf, 1993), refrigeration (Gormley, 1975; Mau *et al.*, 1993), cultivating with CaCl<sub>2</sub> solution (Miklus and Beelman,

1996) and using absorbers (Roy *et al.*, 1995). Lowering the temperature of mushrooms reduces respiration and transpiration, delaying senescence, preventing wilting and shrivelling and thus extending shelf life (Burton, 1989).

To improve mushroom packaging further, the relative humidity inside the package can be controlled to minimise moisture loss from the produce. Loss of 3-6% of fresh weight is usually enough to cause a marked deterioration in quality for most kinds of products and for mushrooms the acceptable weight loss is about 2% (Sveine *et al.*, 1967).

Villaescusa and Gil (2003) used sorbitol and silica gel to modify the in-package relative humidity (RH). High humidity conditions prevailing in the packages lead to moisture condensation, microbial growth and decay of the product. Results showed that lower RH was observed in packages containing silica gel and this did not affect the quality of the mushrooms. In another study moisture absorber with high moisture-holding capacity and slower rate of moisture absorption was used to package fresh mushrooms (Mahajan *et al.*, 2008). These researchers mixed the fast absorbing moisture absorbers (CaCl<sub>2</sub>, KCl and sorbitol) with a slow absorbing desiccant (Bentonite) in different proportions. The best combination was found to be bentonite, sorbitol and CaCl<sub>2</sub> in proportions of 0.55, 0.25 and 0.2 g/g desiccant, respectively (Mahajan *et al.*, 2008).

Roy *et al.* (1995) showed that mushrooms packaged with 10 and 15 g sorbitol lead to constant surface moisture content and the best overall colour respectively. Lowering of the in-package RH did not affect the maturation rate of mushrooms, but reduced bacterial growth and improvement in colour was probably due to a reduction in bacterial activity.

The use of moisture absorbers such as sorbitol, sodium chloride, propylene glycol and polyvinyl alcohol have resulted in better mushroom colour (Roy *et al.*, 1995, 1996). Therefore, mushrooms need special attention to retain freshness (Kim *et al.*, 2006).

There are several indicators that determine the quality of mushrooms, such as visual appearance, cap development, stripe elongation, number of ripe spores, texture, respiration rate, mannitol content, weight loss and microbial deterioration, size, colour, maturity stage and development stage (Aguirre *et al.*, 2008; Bartley *et al.*, 1991; Gormley and O'Sullivan, 1975; Gowen *et al.*, 2008; Lopez-Briones *et al.*, 1992).

Response surface methodology (RSM) is a statistical method for determining and simultaneously solving multivariate equations. It usually uses an experimental design such as central composite rotatable design to fit a first- or second-order polynomial by a least significance technique. An

equation is used to describe how the test variables affect the response, and to determine the relationship between the test variables in the response. The contour plots can be used to study the response surfaces and locate the optimal parameters (Sobukola *et al.*, 2009).

Genetic algorithms (GAs) are powerful optimisation techniques based on the methods of evolution (Gen and Cheng, 1997). GAs solve optimisation problems by simulating the biological evolutionary process. Optimisation using this technique includes the generation of possible solutions, application of selection, crossover and mutation operations, and evaluation of each solution over an objective function (fitness function) until a certain stopping criterion is met.

The ability of GAs to solve complex problems suggests that they are valuable tools for food processing systems. Madaeni and Kurdian (2011) stated that GA is an efficient and systematic method employed in the design of fuzzy model for optimisation of the poorly understood problems. Mohebbi *et al.* (2011) have presented an application of a versatile approach for estimating the moisture content of dried banana using neural networks and GA. Zahedi *et al.* (2010) optimised supercritical extraction of nimbin from neem seeds using a GA technique. Hatami *et al.* (2010) performed mathematical modelling for the extraction of essential oil from clove buds using supercritical carbon dioxide.

The objective of this work was to optimise the amount of absorbers (silica gel, CaCl<sub>2</sub> and sorbitol) with respect to quality attributes like weight loss, cap size, stem length and colour parameters in active packaging of fresh button mushroom.

## 2. Materials and methods

### Materials

Button mushrooms, grown on a commercial farm in Tehran, Iran, were purchased. The mushrooms were carefully collected at the closed cap stage with a pilei diameter of 4-5 cm. After picking, mushrooms were transferred within 30 min to the laboratory, where they were stored at 3 °C and 80% RH for 24 h in order to reduce the respiration rate. Humidity absorbers (silica gel, CaCl<sub>2</sub> and sorbitol) were prepared from Sigma-Aldrich, Co. Ltd. (Munich, Germany)

### Packaging

Approximately 200 g of mushrooms were packaged in polyethylene trays (5.5×4.5×2.4 cm). Different amounts of humidity absorbers were weighed (0, 2.5 and 5 g based on RSM design) and packed in high moisture permeability

wraps (5×2 cm<sup>2</sup>) and then placed under or on top of the mushrooms (there was no direct contact with the mushrooms) and overwrapped with cellophane. Thirty tests were performed based on the RSM design. The conditions of each test are shown in Supplementary Table S1. After complete packaging, depending on the time designated in RSM, the packages were stored at 3 °C.

### Quality attributes

#### Weight loss, changes of cap size and stem length

The percentage of weight loss, changes of cap size and stem length of samples were obtained from Equations 1 to 3. Measurements were performed in triplicate.

$$\text{Weight loss percentage} = \frac{m_1 - m_2}{m_1} \times 100 \quad (1)$$

Where  $m_1$  is the initial weight and  $m_2$  the final weight.

$$\text{Changes of cap size percentage} = \frac{c_1 - c_2}{c_1} \times 100 \quad (2)$$

Where  $c_1$  is the initial average of cap diameters (average of the largest and smallest diameters) and  $c_2$  the final average of cap diameters.

$$\text{Changes of stem length percentage} = \frac{l_1 - l_2}{l_1} \times 100 \quad (3)$$

Where  $l_1$  is the initial stem length and  $l_2$  the final stem length.

#### Colour

To measure representatively and accurately the colour of the mushrooms, the following computer vision system was implemented. A brief description of each step is as follows:

Image acquisition: images were captured using an image acquisition system for colour digital camera similar to that developed by Papadakis *et al.* (2000), namely:

- Samples were illuminated using four fluorescent lamps (length of 60 cm) with a colour temperature of 6,500 K (Natural Daylight, 18W) and a colour rendering index (Ra) close to 95%. The four lamps were arranged as a square 35 cm above the sample and at an angle of 45° with the sample plane to give a uniform light intensity over the food sample.
- A colour digital camera (CDC) Power Shot SX40 (Canon, Japan) was located vertically at a distance of 15 cm from the sample. The angle between the camera lens axis and the lighting sources was around 45°. The setting of the camera is shown in Table 1.
- Images were captured with the above-mentioned CDC at its maximum resolution (4,000 × 3,000 pixels); the images were converted directly in the computer in TIFF format without compression.

- Image processing was done using ImageJ software (version 1.44; NIH, Bethesda, MD, USA) as follows:
  - The noise of captured picture was reduced by using the Noise Despeckle within the Process menu.
  - The colour space of pictures was converted from RGB to CIEL\*a\*b\* by using Converter Space Colour of the Plugin menus under 6,500 K illumination.
  - For each of the colour parameters (L\*a\*b\*) the software gives separate pictures and by using Measure Stack from the Stacks menu the minimum, maximum and mean of each colour parameter of the samples can be obtained from the Results window.

### Optimisation procedure using response surface methodology

We supposed that quality attributes (weight loss, changes of cap size, changes of stem length and colour parameters) of mushroom were functionally related to amount of humidity absorbers and attempts were made to fit multiple regression equations describing the responses. Four coded independent variables in the process were the amount of silica gel (X1), amount of sorbitol (X2), amount of CaCl<sub>2</sub> (X3) and storage time (X4), as shown in Table 2.

Three levels of each of the independent variable were chosen for the study; thus thirty combinations including six replicates of the centre point were performed in random order, based on a central composite experimental design. Range-finding experiments were performed at the outset of this work in order to ascertain what amount of humidity absorbers and storage time could be applied to the mushroom packaging so that the product would be

**Table 1. Setting of the camera.**

Flash	off
Zoom	on
ISO velocity	100
White balance	fluorescence H
Aperture AV	F/2.6
Macro	on
Shutter speed	1/10 s

**Table 2. Coded independent variables in the process.**

Name	Units	Coded values (min ... max)	Mean
Silica	g	-1.000=0.00 ... 1.000=5.00	2.5
Sorbitol	g	-1.000=0.00 ... 1.000=5.00	2.5
CaCl <sub>2</sub>	g	-1.000=0.00 ... 1.000=5.00	2.5
Storage time	day	-1.000=1.00 ... 1.000=9.00	5

acceptable to consumers on the basis of quality attributes. The dependent variables considered include weight loss (Y1), changes in cap size (Y2), changes in stem length (Y3), lightness (Y4), redness (Y5) and yellowness (Y6). The RSM's optimisation was performed with Design Expert 8.0 trial (Stat-Ease, Inc., Minneapolis, MN, USA).

### Optimisation procedure using genetic algorithms

GA optimisation was used for optimisation of the RSM model. By using GA optimisation, the coefficients of polynomials model were optimised and the accuracy of the predicted model was improved. Obtained models constants from RSM were optimised by Year stretch GA Solver 2.7.7 software ([www.yearstretch.com](http://www.yearstretch.com)). All the coefficients were considered as a chromosome and were coded by software. Maximum and minimum of the limiting points were set based on ranges from input cell estimates. Maximize  $R^2$  between predicted and actual values, considered as the target function of the optimisation process. The GA optimisation was performed in 100 generations.

In order to evaluate the GA models, the runs of experiment design were repeated for weight loss response and the experimental results obtained were compared with the predicted data.

## 3. Results and discussion

### Weight loss, changes in cap size and changes in stem length

RSM analysis results for studied responses (variation of weight loss, cap size, stem length and colour parameters) with respect to the amount of silica gel,  $\text{CaCl}_2$ , sorbitol and storage time are shown in Supplementary Table S1. Variation in weight loss, change in cap size and change in stem length of mushrooms under different amounts of silica gel,  $\text{CaCl}_2$  is shown in Figure 1.

Weight loss increased in line with the increase in silica gel,  $\text{CaCl}_2$  and storage time. These results are in agreement with those obtained by Mahajan *et al.* (2008) and Song *et al.* (2001). By increasing the amount of silica gel,  $\text{CaCl}_2$  the changes in cap size and stem length significantly decreased ( $P < 0.05$ ) while storage time caused them to increase (not shown).

Variation in sorbitol had no significant effect on weight loss, changes in cap size and stem length ( $P > 0.05$ ). Villaescusa and Gil (2003) showed that sorbitol has no effect on quality of the mushrooms.

The results of analysis of variance (ANOVA) are shown in Supplementary Table S2. Changes in cap size and stem length due to a change in storage time was higher than

other parameters variation. Quadratic models described the effect of studied factors and their interaction on weight loss ( $R^2 = 0.9647$ ), changes in cap size ( $R^2 = 0.9568$ ) and changes in stem length ( $R^2 = 0.9760$ ). The coefficients of the model are shown in Supplementary Table S3.

### Colour changes

Effect of the different moisture absorbers studied (silica gel,  $\text{CaCl}_2$ ) on colour parameters changes are shown in Figure 2. As can be seen,  $L^*$  value increased with the increase in absorbers (silica gel and  $\text{CaCl}_2$ ) ( $P < 0.05$ ) and decreased with the increase in storage time (not shown). Different amounts of sorbitol led to a not significant change in  $L^*$  value of mushrooms.

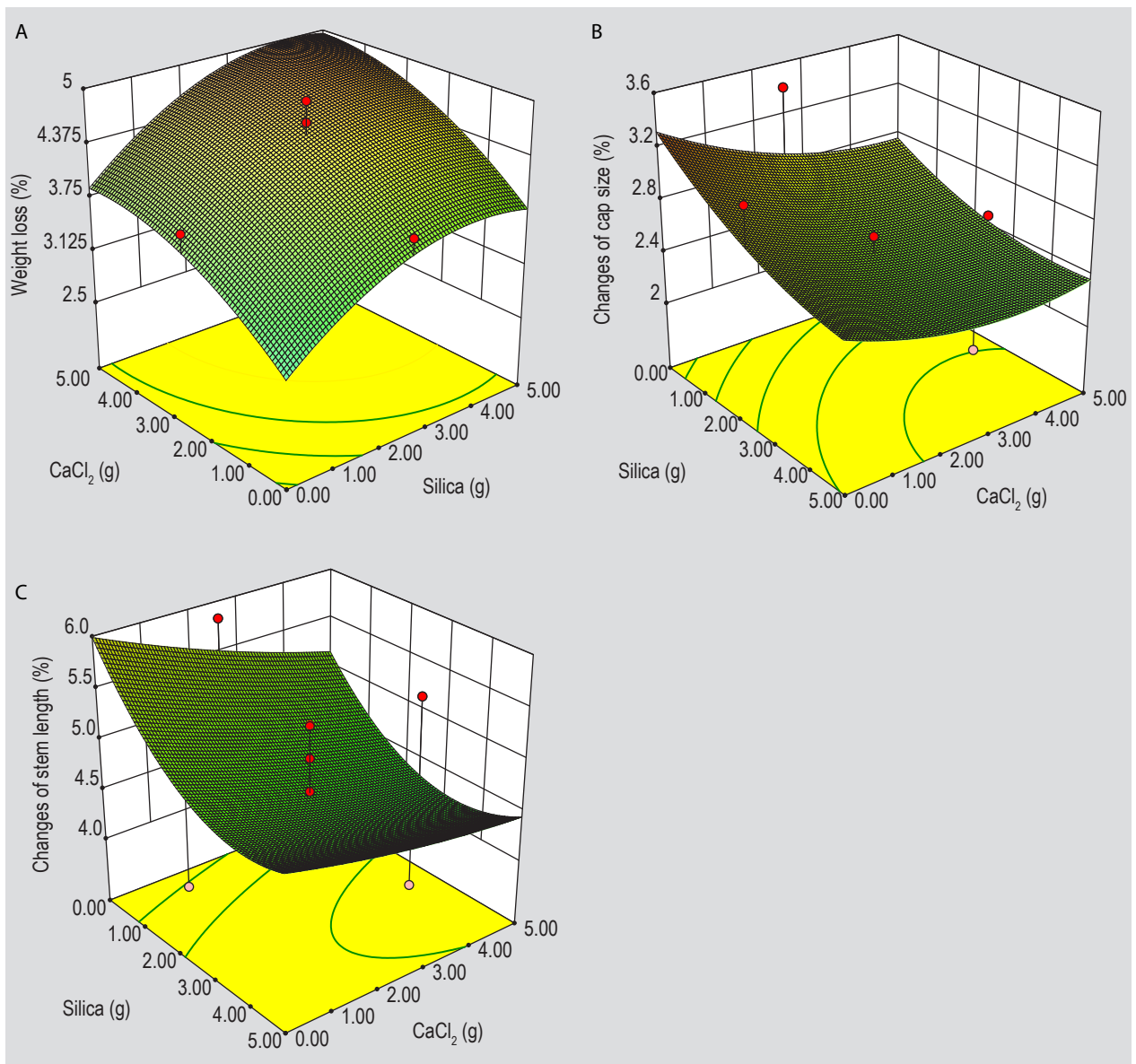
The  $a^*$  value of samples decreased significantly with an increase in absorber content (silica gel and  $\text{CaCl}_2$ ), increased with an increase in storage time ( $P < 0.05$ ) and no change was observed in the value with an increase in sorbitol. The  $b^*$  value changes due to studied treatments were similar to the  $a^*$  value changes. The colour difference ( $\Delta E$ ) decreased with an increase in silica gel and  $\text{CaCl}_2$  content (Figure 2). The results of ANOVA, which are given in Supplementary Table S4, showed that linear models were able to describe colour parameter changes due to independent factors. A high correlation coefficient showed the suitable correlation between this model with experimental lightness ( $R^2 = 0.9191$ ), redness ( $R^2 = 0.9516$ ) and yellowness ( $R^2 = 0.9844$ ) data. The coefficients of the model are shown in Supplementary Table S3.

The use of humidity absorbers led to reduced enzymatic browning in mushrooms due to decreased RH (Martinez and Whitaker, 1995).

Although humidity absorbers increased weight loss, they are used to reduce the rate of change in other quality attributes and prolong the mushroom shelf life.

### Optimising quantity of absorbers

A summary of the optimisation information such as the importance of quality attributes and range of the factors that are used in optimisation are shown in Table 3. As can be seen, weight loss is considered as the most important quality attribute. Minimum weight loss, changes in cap size, changes in stem length and  $\Delta E$  were considered. The optimisation was done in 30 solutions (not shown). The selected value of four factors and best results are shown in Figure 3. The best obtained desirability for each factor was: weight loss = 0.2654; changes cap size = 1; changes stem length = 0.9881; and  $\Delta E = 0.9467$ . Lowest desirability was obtained for weight loss and highest desirability was obtained for all factors and changes in cap size. Five g silica gel and 5 g  $\text{CaCl}_2$  were selected as the best quantity of



**Figure 1.** Variation in quality properties against different amount of silica gel (g) and amount of CaCl<sub>2</sub> (g). (A) Interaction effect on weight loss (%). (B) Interaction effect on changes in cap size (%). (C) Interaction effect on changes in stem length (%) of mushroom. Other variables are constant at mean values.

absorbers for reaching the best response levels (weight loss, changes cap size, changes stem length and  $\Delta E$ ).

### Genetic algorithm optimisation

In Table 4 the  $R^2$  after and before GA optimisation are compared. The correlation coefficient was improved after GA optimisation and showed better correlation between the GA optimised model and actual data. Optimised coefficients are shown in Supplementary Table S3.

### Evaluation of models

The results of the replicated weight loss tests (experimental weight loss data) are shown in Supplementary Table S5. The fitness for RSM model and GA based optimised RSM model with experimental weight loss data are shown in Figure 4. RSM model and GA optimised model had a good fit with the experimental results. The predicted values using the GA optimised model with experimental results had a higher correlation than predicted values using the RSM model that shows an improvement in the optimised model with GA. In other words, using a GA optimised model to predict the amount of humidity absorbers is more accurate and GA optimisation can be used in combination with RSM optimisation.

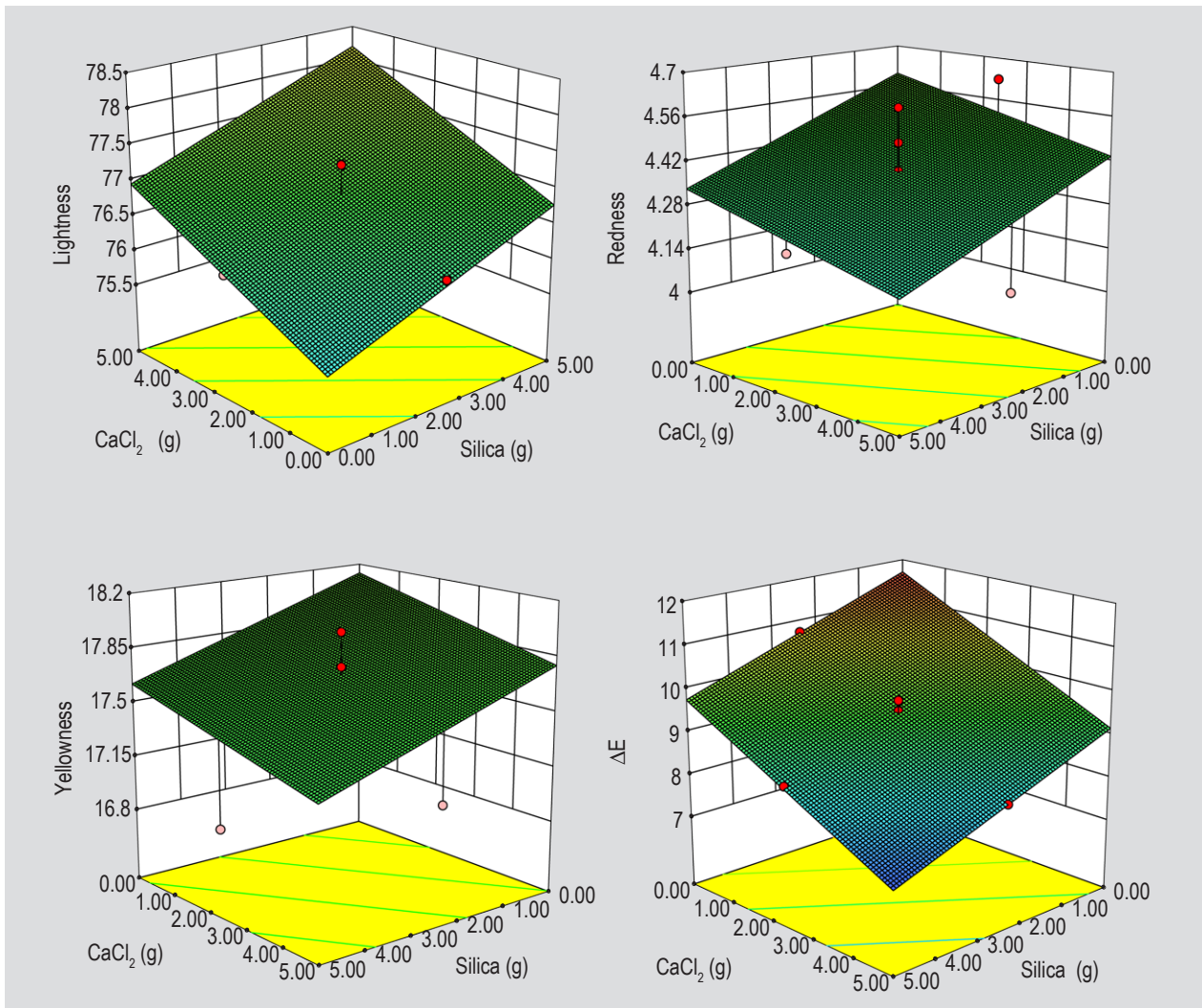


Figure 2. Variation in colour parameters against different amounts of silica gel (g) and of CaCl<sub>2</sub> (g). (A) Interaction effect on lightness. (B) Interaction effect on redness. (C) Interaction effect on yellowness. (D) Interaction effect on colour difference ( $\Delta E$ ) of mushroom. Other variables are constant at mean values.

Table 3. Summary of the optimisation information and range of the factor that was used for optimisation.

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Silica (g)	in range	0	5	1	1	3
Sorbitol (g)	in range	0	5	1	1	3
CaCl <sub>2</sub> (g)	in range	0	5	1	1	3
Weight loss (%)	minimise	2.88	5.59	1	1	4
Changes in cap size (%)	minimise	2.08	3.63	1	1	3
Changes in stem length (%)	minimise	4.27	7.69	1	1	3
Colour difference ( $\Delta E$ )	minimise	7.020036	11.78433	1	1	3

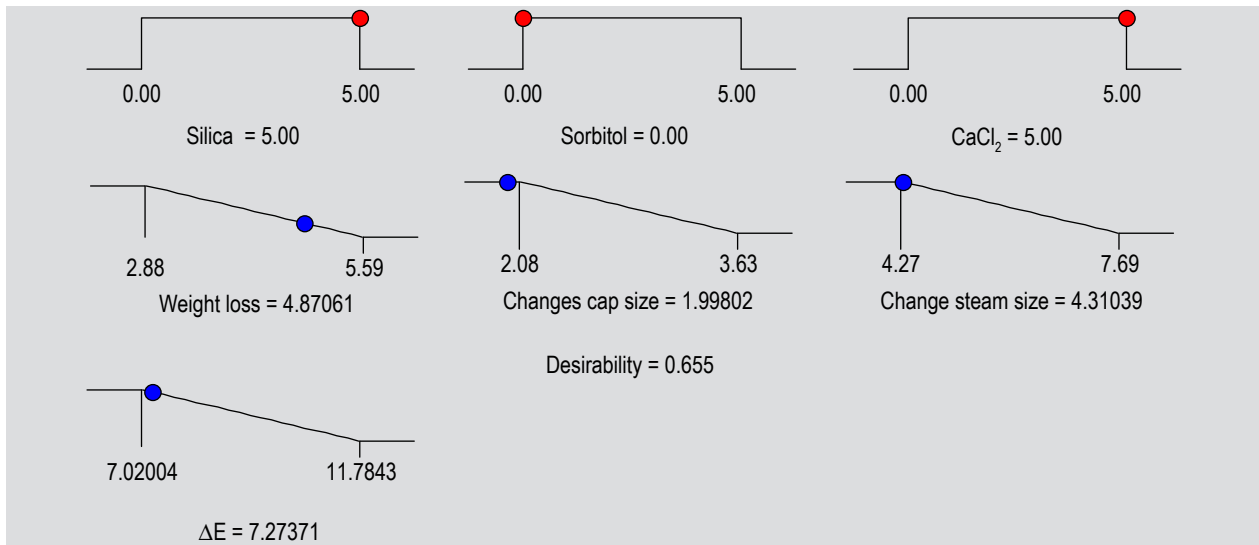


Figure 3. Selected values of four factors and the best results.

Table 4. Comparison of R<sup>2</sup> after and before genetic algorithm (GA) optimisation.

	Weight loss (%)	Changes in cap size (%)	Changes in stem length (%)	L*	a*	b*
Model	quadratic	quadratic	quadratic	linear	linear	linear
Before GA optimisation	0.9647	0.9568	0.976	0.9191	0.9516	0.9844
After GA optimisation	0.9928	0.9823	0.9898	0.9805	0.9942	0.999

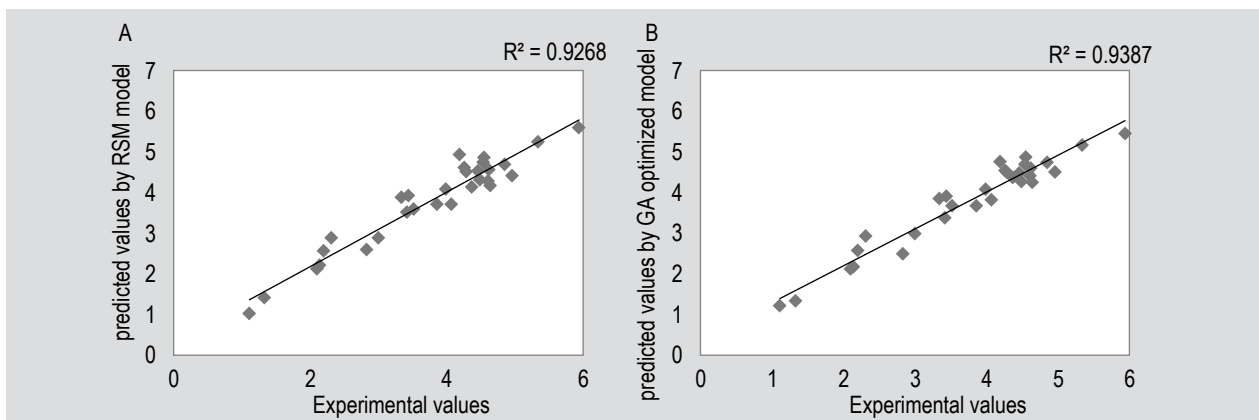


Figure 4. Experimental values vs. predicted weight loss values. (A) Experimental values vs. predicted values using the response surface methodology (RSM) model. (B) Experimental values vs. predicted values using the genetic algorithm (GA) optimized model.

## 4. Conclusions

Use of the moisture absorbers led to reduced humidity inside the package which prevents quality losses in storage periods, while an increase in moisture absorbers resulted in some losses such as weight loss. The use of silica gel and CaCl<sub>2</sub> had a significant effect on indicators that determine the quality of mushrooms such as weight loss, cap size, stem length and colour parameters, while sorbitol had no

considerable effect. The results of this study revealed that optimised RSMs models using GA showed a better fit with the experimental results as compared to RSM models. This study suggested using 5 g silica gel and 5 g CaCl<sub>2</sub>, which led to the best quality of button mushrooms. The digital image processing method allows measurements and analyses of the colour of food surfaces that are adequate for food engineering research.

## Supplementary material

Supplementary material can be found online at <http://dx.doi.org/10.3920/QAS2012.0116>.

Table S1. Response surface analysis conditions and results of quality parameters of mushrooms affected by the amount of silica gel, CaCl<sub>2</sub>, sorbitol and storage time.

Table S2. ANOVA for the response surface quadratic model for weight loss, changes in cap size and changes in stem length.

Table S3. Optimised coefficients of the model before and after genetic algorithm optimisation.

Table S4. ANOVA of the response surface linear model for L\*a\*b\*.

Table S5. Values of the actual data obtained from the replication of the test for weight loss and predicted by the models.

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