Evaluation of blanching effectiveness and optimal temperature and time conditions to minimize browning of commercial mushrooms

Avaliação da eficácia do branqueamento e condições ideais de temperatura e tempo para minimizar o escurecimento de cogumelos comerciais

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The bleaching operation has been utilized to increase the shelf life, reduce enzymatic activity, and lower the microbial load of foods. This study aimed to investigate the effects of blanching time and temperature on three commercial mushroom varieties' lightness and color variation and determine the optimal operation conditions. Three specimens were collected: *Agaricus bisporus* Paris Champignon, *Agaricus bisporus* Portobello, and *Lentinula edodes* Shitake. The composite central rotational design and the response surface methodology were employed to evaluate the combined effects of blanching temperature (ranging from 53.8°C to 96.2°C) and time (ranging from 10.3 seconds to 349.7 seconds) on the color parameters L* (lightness), a* (redness-greenness) and b* (yellowness-blueness) of mushrooms slices. Cutting, oxygen exposure, and temperature below 75°C significantly affected the color by reducing L* and b* values and increasing a* values, favoring the mushrooms' darkening (higher ΔL). The bleaching operation optimized to minimize color variation (ΔE) was at 80°C for 170 seconds for Champignon, 83°C for 209 seconds for Portobello, and 82°C for 186 seconds for Shitake. Blanching fresh mushrooms under these operational conditions can help prevent the development of dark color, thereby improving their quality for human consumption, extending their shelf life, and reducing waste caused by unsightly appearance.

Keywords: quality parameter, processing conditions, water blanching.

A operação de branqueamento tem sido utilizada para aumentar a vida útil, reduzir a atividade enzimática e diminuir a carga microbiana dos alimentos. Este estudo teve como objetivo investigar os efeitos do tempo e da temperatura de branqueamento na luminosidade e na variação de cor de três variedades comerciais de cogumelos e determinar as condições ótimas de operação. Três espécimes foram coletados: *Agaricus bisporus* Paris Champignon, *Agaricus bisporus* Portobello e *Lentinula edodes* Shitake. O delineamento composto central rotacional e a metodologia de superfície de resposta foram empregados para avaliar os efeitos combinados da temperatura de branqueamento (variando de 60°C a 96,2°C) e tempo (variando de 60 segundos a 349,7 segundos) nos parâmetros de cor L* (luminosidade), a* (vermelho-verde) e b* (amarelo-azul) de fatias de cogumelos. O corte, a exposição ao oxigênio e a temperatura abaixo de 75°C afetaram significativamente a cor, reduzindo os valores de L* e b* e aumentando os valores de a*, favorecendo o escurecimento dos cogumelos (maior ΔL). A operação de branqueamento otimizada para minimizar a variação de cor (ΔE) foi de 80°C por 170 segundos para Champignon, 83°C por 209 segundos para Portobello e 82°C por 186 segundos para Shitake. O branqueamento de cogumelos frescos nessas condições operacionais pode ajudar a prevenir o desenvolvimento de cores escuras, melhorando assim sua qualidade para consumo humano, prolongando sua vida útil e reduzindo o desperdício causado pela aparência desagradável.

Palavras-chave: parâmetro de qualidade, condições de processamento, branqueamento com água.
1. INTRODUCTION

Humans consume a vast array of mushrooms for health benefits, from 1 to 5 kg per person per year (fresh weight): thus, commercial mushroom cultivation demand and global markets have rapidly increased during the past decades. According to current estimates, out of more than 10,000 species reported, 3000 are recognized as edible, nearly 200 wild species are used for medicinal purposes, about 100 are being economically cultivated, about 60 mushroom varieties are grown commercially, and ten types are produced on an industrial scale in many countries [1-6].

In Brazil, mushroom production remains minimal compared to other countries. Per capita consumption in Brazil is only 288 grams per year. However, in recent years, the fresh niche market has emerged as a promising alternative for Brazilian mushroom producers. This market is particularly advantageous because mushrooms imported from China can not enter the Brazilian market in their fresh form due to their limited shelf life and specific conservation requirements. Additionally, fresh mushrooms do not require processing and face fewer marketing restrictions from Brazilian authorities compared to pickled products [7-9].

It is reported that the most cultivated mushrooms in Brazil and globally have been the *Agaricus bisporus* (Paris Champignon), *Lentinula edodes* (Shiitake), and species of the genus Pleurotus spp. such as *P. ostreatus*, the white *Agaricus bisporus* (Paris Champignon), the brown *Agaricus Bisporus* (Portobello), *Lentinula edodes* and *Pleurotus ostreatus* [1-6]. Annual production in Brazil is approximately 12,000 tons of fresh mushrooms. The state of São Paulo is the main consumer and producer, responsible for 90% of national production, followed by the state of Paraná, with emphasis on the cities of Curitiba, Castro, and Tijucas do Sul [7-9].

Mushrooms have become an essential part of our diet. They have been widely used for cooking and in different recipes due to their attractive velvety texture, umami taste and smooth aroma. Mushrooms are also sources of vitamins, minerals, protein, polysaccharides, fiber, unsaturated fats, phenolic compounds, flavonoids and tocopherol. These compounds are well known for their beneficial activities, such as anti-inflammatory, antioxidant, antifungal, antibacterial, and antitumor properties [2, 6, 10, 11].

As the consumption and production of fresh mushrooms have increased, so has the importance of maintaining their quality and appearance. The visual appeal of fresh mushrooms, characterized by a consistent texture and uniform and natural color (white, brown, orange, depending on the species) without changes (firmness loss, decrease in L* value, and increases in a* and b* values), is important for consumer acceptance [12]. Mushrooms are highly perishable due to their high moisture content and enzyme activity, which limits their shelf life of just 3-7 days even under cold storage conditions. Considering the high perishability, combined with the seasonal availability of a few varieties and their susceptibility to sensorial alterations such as browning or yellowing, underscores the need for effective preservation techniques. These methods are significant not only for extending the shelf life of mushrooms but also for maintaining their sensory perception and commercial value [6, 12-14].

However, water blanching is a simple and low-cost preservation procedure in industrial processing applied before storage to inactivate deleterious enzymes and reduce the microbial load. In blanching, the product is exposed to a heat source at a pre-designated temperature and time and subsequently to cooling. The efficiency of this thermal treatment is usually based on the inactivation of heat-resistant enzymes like peroxidase (POX) and polyphenol oxidase (PPO), which are also involved in the deterioration reactions and consequent undesirable changes in nutritional value, flavor, and color (including dark pigments). So, the blanching conditions must be controlled to keep mushrooms’ nutritional and organoleptic properties [14, 15].

This way, by utilizing the response surface method (RSM) in combination with multiple linear regressions, the optimization of mushroom blanching conditions becomes a viable approach to achieve the desired color and better use of temperature, time, and energy. Limited studies have been conducted on the impact of blanching on the color of Paris, Portobello, and Shiitake mushrooms, underscoring our research's originality. Therefore, this study aims to investigate the effects of blanching time and temperature on three commercial mushroom varieties’ lightness and color variation and determine the optimal operation conditions.
2. MATERIALS AND METHODS

2.1 Samples

Samples were supplied by Aguro mushroom farm (São José dos Pinhais, Curitiba, Paraná, Brazil) from August to October 2021. Approximately 2 kg of each mushroom species were collected and refrigerated to the Federal University of Paraná laboratory. Three specimens were collected at the commercial fruiting maturity defined by Aguro farm: *Agaricus bisporus* Paris Champignon (CP; Figure 1a); *Agaricus bisporus* Portobello (P; Figure 1d); *Lentinula edodes* Shiitake (S; Figure 1g). The Paris mushroom cap diameter was about 5.38 ± 0.33 cm, Portobello cap diameter was about 6.50 ± 1.41 cm and Shiitake cap was still slightly curled or just as the partial veil broke away.

2.2 Experimental design and statistical analyses

The factorial composite central rotational design (CCRD) was used, with two experimental factors: immersion time (60 and 300s) and temperature (60 and 90°C), control interval based on the literature [11, 16-18] four factorial points, four axial points, and two central points. The response variables were L*, ΔL*, a*, b*, and ΔE*. Table 1 shows the experimental matrix. The statistical effect of the experimental factors on ΔE* was determined by the response surface methodology, using a second-order polynomial model to describe the observed behavior (Eq. 1).

\[
Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1<j} \sum \beta_{ij} x_i x_j
\]  

Where: \(\beta_0\) is the width from the origin; \(\beta_i\) is the \(i^{th}\) linear regression factor; \(\beta_{ii}\) is the second-order regression coefficient of the \(i^{th}\) factor; \(\beta_{ij}\) is the interacting effect of the \(i^{th}\) factor plus the \(j^{th}\) one; and \(Y\) is the dependent variable.

<table>
<thead>
<tr>
<th>Run</th>
<th>Experimental conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X₁ (time, s)</td>
</tr>
<tr>
<td>2</td>
<td>X₂ (Temperature, °C)</td>
</tr>
<tr>
<td>1</td>
<td>-1 (60.0)</td>
</tr>
<tr>
<td>2</td>
<td>+1 (60.0)</td>
</tr>
<tr>
<td>3</td>
<td>-1 (300.0)</td>
</tr>
<tr>
<td>4</td>
<td>+1 (300.0)</td>
</tr>
<tr>
<td>5</td>
<td>-1.41 (10.3)</td>
</tr>
<tr>
<td>6</td>
<td>+1.41 (349.7)</td>
</tr>
<tr>
<td>7</td>
<td>0 (180.0)</td>
</tr>
<tr>
<td>8</td>
<td>-1.41 (53.8)</td>
</tr>
<tr>
<td>9</td>
<td>0 (180.0)</td>
</tr>
<tr>
<td>10</td>
<td>+1.41 (96.2)</td>
</tr>
</tbody>
</table>

Analysis of variance (ANOVA) was applied to determine the significant effects of immersion time and temperature on the responses. The determination coefficients (R²) and the adjusted determination coefficient (R_adj²) assessed the model quality. This study design was analyzed, and three-dimensional response surface plots were drawn using Statistic v. 10 (StatSoft, Inc., Tulsa, Ok, USA).
2.3 Blanching

All mushrooms were washed, sanitized, and dried at room temperature. The samples were sliced transversely on the cap with a 2.5 and 3.0 mm thickness. The fresh sample (control) was immediately color analyzed in the HunterLab MiniScan EZ colorimeter (Reston, Virginia, U.S.A.). The blanched samples were obtained according to each experimental run, where 100 g of cut mushroom were immersed in 1L deionized water in a thermostatic bath at different times and temperatures, as stated by the statistical design. After blanching, mushroom pieces were cooled in an ice bath until they reached 7°C. Then, the fractions were drained using a plastic sieve and blotted with absorbent material. After 300 min at ambient temperature, the non-blanched (control) and blanching samples were submitted to the color analysis to estimate their enzymatic browning after the blanching process.

The L* (lightness), a* (redness - greenness), and b* (yellowness – blueness) color parameters of the mushroom samples were determined using a HunterLab MiniScan EZ colorimeter (Reston, Virginia, U.S.A.). The L*, a*, and b* values were measured ten times by rotating the dish by 90° and taking readings in each position. The equation calculated the lightness variation: \( \Delta L^* = L^* - L_0^* \), where \( L^* \) was for blanching samples and \( L_0^* \) for control samples. The total color variation is evaluated by Equation 2:

\[
\Delta E = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}
\]

3. RESULTS AND DISCUSSION

Color is one of the most critical quality attributes of food matrices. The color values expressed in the L*, a*, b*, \( \Delta L^* \), and \( \Delta E^* \) can be observed in Table 1. Fresh mushrooms showed a tendency to bright yellow, as indicated by the CIE scale parameters: coordinate a* was 0.10; 1.28 and 1.36 for Shitake, Portobello, and Paris, respectively, which is next to the zero and represents an absence of green or red color but showing a tendency to red; the values of b* was positive from 10.98 (Shitake) to 12 (Paris and Portobello), showing a trend to yellow color; the L* parameter values were next to 100 (87.23 to 88.88) which represents a white color tendency, Figures 1a, 1d and 1g and Table 2 (Fresh).

![Figure 1. The visual appearance of mushrooms. Paris mushroom a) fresh, b) run 10, c) run 7; Portobello d) fresh, e) run 10, f) run 3 and Shitake g) fresh, h) run 10, i) run 3.](image-url)
Results expressed as mean obtained from assays (n=10). In Tukey’s analysis, different letters in the same column significantly differ (p<0.05).
The results were obtained from 10 experimental runs following the factorial composite central rotational design. Analysis of variance showed a significant difference (p < 0.05) between all color parameters (Table 2). As expected, slicing and exposure to oxygen led to a decrease in $L^*$ and $b^*$ and an increase in $a^*$ showing the browning of mushrooms, as can be seen comparing non-blanchled and fresh samples. The natural formation of dark brown pigments in mushrooms occurs due to endogenous polyphenolic compounds from the polyphenol oxidase family of enzymes, including tyrosinases and laccases, as was verified in previous works by fluorescence spectroscopy [19].

The luminosity ($L^*$) can be the primary parameter indicator associated with enzymatic browning. High $L^*$ values (>80) reveal a clearing sample, while lower values (close to 0) mean the mushroom slice does not differ from the brightness of the standard sample indicating a darkening or brownish and oxidized sliced mushroom [20]. The use of color space was related to ohmic and conventional blanching treatment for *Agaricus Bisporus*. Both technologies showed promise in inactivating PPO with less impact on color and texture [14].

The lowest values of $L^*$ for all mushrooms were evidenced for runs 2, 4, 5, 9, and 10 to Paris, run 6, 8, 9, and 10 to Portobello and run 4, 8, 9, and 10 for Shitake demonstrating that the lower temperature of scalding, lower than 75°C can favor the darkening of analyzed mushrooms (Table 2). The effect of $L^*$ (luminosity) in mushrooms was confirmed by Barrón-Garcia et al. (2022) [14], whose most significant observed darkening was verified with a reduction in the $L^*$ value. Comparing the $L^*$ results with the non-blanchled mushrooms sample shown in run 11, which was not subjected to blanching; it was just sliced, packed, and subjected to a spectrophotometer reading, the blanching was effective because $L^*$ values were significantly higher than run 11.

In the CIE $L^*$a*b* scale, the lower the redness ($a^*$) value, the minimum the enzymatic browning. The treatments were satisfactory in this regard, as they reduced the value of $a^*$ compared to the control treatment. Furthermore, the $b^*$ coordinate did not show significant variations between treatments, being in the positive range and tending toward yellow [21].

The lightness variation $\Delta L^*$ and the color difference parameter $\Delta E^*$ are also used to check for color changes caused by darkening. There was a tendency for $\Delta L^*$ and $\Delta E^*$ to increase over time, indicating the darkening of the samples. However, the control treatments showed the highest values for $\Delta E^*$ and $\Delta L$, showing that the blanching treatments were satisfactory, as they reduced the color difference. In addition to blanching and inactivating enzymes related to mushroom browning, heat treatment can minimize non-enzymatic browning reactions and reduce the microbial load on the food surface [22]. Color changes in mushrooms are mainly attributed to PPO-mediated browning reactions [16]. The lowest values for $\Delta E^*$ observed for all mushroom samples were at the central point run in a blanching for 180 seconds at 75°C. However, Paris is also practical at 90°C for 60 and 300 seconds.

The statistical parameters, such as coefficient ($R^2$) and p-value, were measured with the analysis of variance (ANOVA). The $R^2$ value was used to judge the adequacy of the models, and the results showed that the models developed for $\Delta E^*$ to Paris and Shitake were significant (Table 3). The multiple regression analysis of $\Delta E^*$ values showed that the model was effective (p < 0.05). The results of the correlation coefficient ($R^2$) values showed that the model could explain up to more than 76% of all variances in data with adjusted $R_{adj}^2 = 0.72$ for Paris, 0.67 for Portobello and 0.90 for Shitake [23].

<table>
<thead>
<tr>
<th>Variable</th>
<th>$Y_{\text{Paris}}$ Coefficient</th>
<th>p-value</th>
<th>$Y_{\text{Portobello}}$ Coefficient</th>
<th>p-value</th>
<th>$Y_{\text{Shitake}}$ Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_0$</td>
<td>406.45</td>
<td>&lt;0.001</td>
<td>381.18</td>
<td>0.005</td>
<td>364.213</td>
<td>0.0018</td>
</tr>
<tr>
<td>$X_1$</td>
<td>-0.215</td>
<td>0.669</td>
<td>-0.089</td>
<td>0.288</td>
<td>-0.091</td>
<td>0.5646</td>
</tr>
<tr>
<td>$X_1^2$</td>
<td>0.0002</td>
<td>0.276</td>
<td>0.001</td>
<td>0.002</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$X_2$</td>
<td>-9.241</td>
<td>&lt;0.001</td>
<td>-8.874</td>
<td>&lt;0.001</td>
<td>-8.507</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$X_2^2$</td>
<td>0.05</td>
<td>&lt;0.001</td>
<td>0.058</td>
<td>0.003</td>
<td>0.054</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$X_1X_2$</td>
<td>0.002</td>
<td>0.195</td>
<td>-0.004</td>
<td>0.104</td>
<td>-0.002</td>
<td>0.0434</td>
</tr>
</tbody>
</table>

$R^2$ 0.79845 0.76289 0.92768

Table 3. Regression coefficients of fitted models by the central composite design.
Concerning the effects of models, and $X_1^2$ showed a low significantly positive effect for Paris and Portobello and the slices Portobello was low on quadratic regression coefficient of temperature ($X_2^2$). The linear temperature ($X_1$) and the interactions of time ($X_1$) and temperature ($X_2$) had a significantly low positive effect on the total color variation $\Delta E^*$ for Portobello and Shiitake. The effects of parameters on the dependent variable are shown in 3D-response surface plots (Figure 2) as a function of time and temperature. The predicted model for Paris, Portobello, and Shiitake can be described by (Eq. 3, 4, and 5):

$$Y_{\text{Paris}} = 406.45 - 0.215 X_1 + 0.0002 X_1^2 - 9.241 X_2 + 0.056 X_2^2 + 0.002 X_1 X_2$$ (3)

$$Y_{\text{Portobello}} = 381.18 - 0.089X_1 + 0.001X_1^2 - 8.874X_2 + 0.058X_2^2 - 0.004 X_1 X_2$$ (4)

$$Y_{\text{Shiitake}} = 364.21 - 0.091 X_1 + 0.001 X_1^2 - 8.507 X_2 + 0.054 X_2^2 - 0.002 X_1 X_2$$ (5)

Response surface methodology and multiple regression analysis have been used to find the best conditions to perform blanching. In addition, the multi-response optimization procedure using the desirability function was conducted with the models to minimize the total color variation. The result for this optimization revealed that blanching at 80.31°C for 170.15 seconds for Paris, at 83.33°C for 209.51 seconds for Portobello, and at 82.20°C for 186.43 seconds for Shiitake were the best conditions for this combination of variables. Lespinard et al. (2009) [24], with the same type of treatment (conventional bleaching) in a similar range of 50 to 90°C for $A.\ bisporus$ mushroom, showed that the color change was also associated with the increase in temperature. The authors evidenced that as the bath temperature increased (60–80 °C), the enzyme remained active for less time, producing smaller quantities of colored products. The results also corroborated with Barron-García et al. (2022) [14] that use a bath temperature ranging from 67 to 90°C for $A.\ bisporus$ and the most significant darkening was also evidenced in the lower temperature, of 67°C.

Optimizing both time and temperature in the blanching process could yield numerous advantages, ranging from conserving energy in the industry to enhancing the quality of the final product. Another significant benefit of this optimization is the avoidance of prolonged cooking times. Extended cooking periods can have adverse effects on both the nutritional value and texture of edible mushrooms. Prolonged cooking may result in the leaching of water-soluble vitamins [25], leading to a loss of essential nutrients, phenolic compounds, and antioxidant compounds that are beneficial for our health [17]. Additionally, the suggested quick heat treatment can ensure that the visual and sensory aspects of the mushrooms meet consumers’ expectations [16] and can reduce the microbial load, thereby enhancing the safety of the mushrooms.
Figure 2. Response surface and contour diagrams for total color variation (ΔE) of (a) Paris, (b) Portobello and (c) Shiitake. According to the Tukey test, different letters correspond to a significant difference (p < 0.05).

4. CONCLUSION

The L* values showed that the bleaching treatments, in relation to the control, were satisfactory at temperatures above 76°C due to the tendency to increase clarification. Furthermore, the a* values showed that the treatments were adequate for color maintenance, and about the b* coordinate, there were no significant variations between treatments. Using response surface methodology coupled with multiple regression was possible to suggest mathematical models to evaluate the dependent variables. Multiple response optimization using color variation (ΔE) was adequate for the proposed aim targeted with the best process conditions to minimize the color parameters variations for Paris, Portobello and Shiitake. These results indicate the optimum blanching treatment to potentially save energy, reduce sensory quality losses and extend the shelf life of the mushrooms selected for the study. Furthermore, this study enlarges the knowledge of rapid and non-destructive analysis, such as colorimetry, which can be applied to evaluate the blanching effect on mushroom sensory characteristics.
5. ACKNOWLEDGMENTS

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