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Title: Fogging with antibrowning agents as an alternative to conventional immersion treatments to extend shelf life of mushrooms

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Fogging with antibrowning agents as an alternative to conventional immersion treatments to extend shelf life of mushrooms

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ABSTRACT

Mushroom (*Agaricus bisporus*) is a highly perishable product and can be easily damaged when immersion in antibrowning solutions is performed; therefore, fogging application represents a less aggressive technique. The objective of this work was to evaluate the effect of different antibrowning agents (ascorbic acid, sodium metabisulphite and L-cysteine) applied by two methodologies (immersion and fogging) on the quality of mushrooms during storage at 5˚C. Weight losses, microstructural organization, colour and texture changes were evaluated as quality indicators. Polyphenol oxidase activity, phenolic content and antioxidant activity were also evaluated. Fogging achieved browning inhibition without major changes in firmness, while immersion treatments inhibited browning but significant changes in firmness were found. Browning inhibition achieved by fogging showed similar results when compared to the conventional immersion treatments. Fogging has the advantage of being less aggressive than immersion while requiring a significantly smaller amount of antibrowning solution than traditional immersion.

Keywords: enzymatic browning, mushrooms, polyphenol oxidase, antioxidants, microstructure, fogging.

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Introduction

Mushrooms (*Agaricus bisporus*) are a highly perishable fresh product, increased respiration rates, susceptibility to enzymatic browning; microbial spoilage, softening and dehydration are the main causes for shelf-life reduction (Jaworska *et al*., 2010; Paudel *et al*., 2016; Zhang *et al.*, 2018). Browning due to polyphenol oxidase (PPO) has a significant negative impact on the visual acceptance of the product.

In addition, mushrooms can be easily damaged when immersion in antibrowning solutions is performed (Paudel *et al.,* 2016); this technique has the disadvantage of requiring large amounts of water. Spraying of different agents has been applied in several products (Fu *et al.*, 2016; Peretto *et al.*, 2016) showing a great reduction in water consumption. However, spraying technique wets the product surface and could alter its quality. Fogging technique could be an alternative because particle size of water droplets is small enough, to avoid wetting. This methodology was applied to control of postharvest diseases of fruits (Van de Velde *et al.*, 2016). However, fogging with antibrowning agents has been poorly studied.

Sulphites are well known reducing agents and were for a long time the most effective and frequently used antibrowning agents (Xue *et al.,* 2017); however, their use has been restricted because of their adverse effects on human health (Stohs and Miller 2014). Actually, ascorbic acid (AA) is the most widely used antibrowning agent; some reports attributed its action to the reducing effect on quinones or as PPO inhibitor (Olotu *et al*., 2015).

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Cysteine was also investigated as antibrowning agent; its activity could be attributed to various mechanisms such us its nucleophilic reactivity towards quinones to give colourless adducts, inhibitory effect on PPO or its ability to reduce o-quinones (Ding *et al.,* 2016).

The objectives of this work were to compare and evaluate the effect of different antibrowning agents in aqueous solutions (ascorbic acid, sodium metabisulphite and L-cysteine) applied by two different methodologies (immersion and fogging) on the quality of mushrooms during storage at 5˚C. Weight losses, microstructural organization, colour and texture changes were evaluated as quality indicators. The effects of the different antibrowning agents on PPO activity, phenolic content and antioxidant activity were also evaluated.

Materials and Methods

Mushrooms (*Agaricus bisporus*) were kindly provided by Abrantes SRL (Escobar, Buenos Aires, Argentina). Six kilograms from the second flush were harvested at commercial maturity stage (3–4 cm tall). Some of them were separated as CONTROL and the rest were immediately submitted to one of the following treatments.

Immersion treatments

Mushrooms were dipped for 10 min in one of the following solutions: a) 5% (w/v) ascorbic acid solution (AAS) b) 1% (w/v) sodium metabisulphite solution (MBS), c) 5% (w/v) L-cysteine solution (CYS). The ratio between sample : solution was set at 1:3 depending on

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volume. Immediately after treatments mushrooms were drained for 30 minutes in a colander externally covered with paper towelling to prevent absorption of additional solution (Brennan *et al.,*1999; Olotu *et al.,* 2015).

Fogging treatments

Fogging was carried up in a closed glass chamber connected to an ultrasonic aerosol generator unit, (Ultrasonic Nebulizer Twister 3019, San Up, Argentina), with a liquid reservoir of 8 mL producing fog droplets between 1.5–5.7 µm in diameter having a mass median aerodynamic diameter (MMAD) of 3.8 µm. Particles were carried away by the airflow and blown inside the glass chamber (capacity 8000 cm^3), containing the mushrooms placed in a plastic basket. Fogging treatments were: a) 5% (w/v) ascorbic acid solution (F-AA) b) 1% (w/v) sodium metabisulphite solution (F-MBS), c) 5% (w/v) L-cysteine solution (F-CYS). All fogging treatments lasted 10 min and were performed at room temperature (25 ˚C).

Treated and CONTROL mushrooms (80 g per tray) were packed in polystyrene trays (15 x 10 x 3 cm), covered with self-adhesive film. Seventy trays (two per condition) were stored at 5 ± 1 °C for 16 days. Samples were analyzed at initial time and every 4 days during storage. To determine phenolic content, antioxidant capacity and PPO activity, samples were cut in pieces of 1 x 1 x 1 cm and immediately frozen at – 20° C until analysis.

Weight losses

After removing trays from storage, they were weighted and weight losses were calculated.

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Firmness

Firmness was evaluated using EZ-LX texture analyzer (Shimadzu, China) equipped with a compression probe of 40 mm diameter. Firmness (expressed in Newtons, N) was measured as the maximum force needed to compress 5 mm of one-half of the mushroom. Test speed was 15 mm s^{-1}. At least 6 determinations from two replicate packages were evaluated for each treatment at each sampling time (Oms-Oliu *et al.*, 2010).

Scanning electron microscopy (SEM)

A thin portion of the cap was obtained with a razor blade. Then, the samples were prepared following the procedure proposed by Sorrivas de Lozano *et al.*, (2014) and observed under a scanning electron microscope (JEOL, 5800 LV, Japan) at 15 kV.

Phenolic content

Phenolic content was determined on a methanolic extract as Ding *et al.*, (2016) proposed, with some modifications. Briefly, frozen tissue (5 g) was homogenized with a manual processor, and then magnetically stirred with 30 mL of 80% (v/v) methanol for 60 min in ice bath, filtered and referred as methanolic extract. Phenolic content was determined by mixing 500 µL of the extract with 2 mL of distilled water and 125 µL of Folin Ciocalteu reagent; after 3 min 250 µL of 7.5 % (w/v) Na_2CO_3 was added. The reaction mixture was left in dark and after 60 min, the absorbance was determined at 760 nm. Three replicate analyses were performed, and results were expressed as mg of gallic acid g^{-1} of fresh weight.

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Antioxidant capacity (AOC)

The free radical-scavenging effects of the methanolic extracts on 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulphonate (ABTS•+) radicals were determined.

Dilutions of the methanolic extract were mixed with prefixed volumes of freshly prepared radical solutions and allowed to react for one hour. The absorbances were determined at 517 nm and 734 nm for the DPPH• and ABTS•+ assay respectively (Ding *et al.,* 2016).

The percentage of inhibition was calculated using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as standard. The final DPPH and ABTS values were calculated by using a calibration curve based on % of inhibition and results were expressed as mg of Trolox equivalents g^{-1} of fresh weight.

PPO Activity

Frozen tissue (10 g) and 50 mL of cold acetone were homogenized with a manual processor, magnetically stirred in ice bath for 30 min and filtered through paper. The dry acetonic powder (0.2 g) was dissolved in 50mM phosphate buffer (pH = 7.0) 1 mM EDTA and 0.5 % (w/v) polyvinylpyrrolidone under magnetic stirring in ice bath for 30 min. The product was centrifuged at 8,000 x g for 10 min at 4°C and the supernatant was referred as enzyme extract.

To determine PPO activity, 2 mL of enzyme extract was mixed with 1 mL of 50 mM catechol in 50 mM phosphate buffer (pH = 7.0). The reaction mixture was incubated at 25°C, and the absorbance was recorded every 10 s for 120 s at 420 nm. One unit of PPO activity was equivalent to a variation in absorbance of 0.001 at 420 nm per minute and it was expressed

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as U/kg based on protein content. Total protein content in the enzymatic extracts was determined by the Lowry's method using bovine albumin as a standard.

Colour

Colour was measured with a colourimeter (Minolta, Japan). The equipment was set up for a D65 illuminant and a 10° observer angle. Four samples from two replicate packages were evaluated for each treatment and sampling time. CIE L* (lightness), a* (red-green) and b*(yellow-blue) parameters were measured on the cap of the mushrooms. These values were used to calculate the browning index (BI)(Eq. (1))(Cliffe-Byrnes and O'Beirne 2008).

$$BI = 100(x - 0.31)/ 0.17 \text{ where } x = (a^* + 1.75 \cdot L^*)/(5.645 \cdot L^*) + (a^* - (3.012 \cdot b^*)) \quad \text{(Eq. 1)}$$

Statistical analysis

The results were statistically analyzed using analysis of variance (ANOVA) ($\alpha = 0.05$). The differences among means were tested for statistical significance using a multiple-range least significant difference (LSD) test with the Info Stat-Statistical Software 2015 (Córdoba, Argentina).

Results and Discussion

Weight losses

Weight loss due to dehydration is particularly important in mushrooms since they do not have barriers, such as peels, to prevent water losses (Olotu *et al.,* 2015).

CONTROL mushrooms had final weight losses of 3.63 ± 0.38 %; this result was not

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significantly different ($p>0.05$) from those found in fogging treatments F-MBS (3.83 ± 0.13), F-CYS (3.52 ± 0.01) and F-AA (3.50 ± 0.12) indicating that fogging might not affect significantly water exchange between mushrooms and the surrounding atmosphere (Supplementary Table 1).

Immersed samples had final weight losses that were significantly higher than those found in the other treatments, which might be a consequence of tissue softening and disruption of the natural structures in mushrooms. Therefore, cells dehydrated faster and weight loses were higher (Zhang *et al.,* 2018) than that in CONTROL mushrooms.

Fogging technique seems to be less invasive than immersion since the small droplets are deposited on the surface of the product, avoiding pressure or bump damage (Van de Velde *et al.*, 2016). Because of this, the tissue could be more efficient in retaining water; therefore, dehydration was not as high as in immersed samples.

Firmness

Mushrooms stored under refrigeration conditions could suffer some chilling injury below 0° C or dehydration that leads to hardening (Sapers *et al.*, 2001; Zhang *et al.*, 2018). Softening may also be a problem in mushrooms subjected to immersion treatments as a consequence of over-wetting, enzymes activity or microbiological spoilage (Cliffe-Byrnes and O'Beirne 2008; Singh *et al.,* 2010).

Fresh mushrooms had initial firmness of 26.68 ± 2.24 N. During storage, CONTROL showed a progressive decrease in firmness (final value = 9.80 ± 1.50 N) indicating that softening was the prevailing effect over hardening due to dehydration (Supplementary Table 1). Softening

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could be related to microbial spoilage (Zhang *et al.,* 2018) and the activity of lignin degrading enzymes (Jaworska *et al.,* 2010).

Firmness in MBS and CYS treatments had a significant reduction of about 60 % until day 12 after which a significant increase was observed, reaching an average final value of 14.62 ± 3.13 N. These results could be a consequence of a delayed response of secondary metabolisms involved in lignin and other structural components synthesis (Xue *et al.,* 2017).

AAS treatment in fog-treated and immersed mushrooms showed a significant decrease in firmness ($p<0.05$) after 4 days of storage and this tendency was maintained until the end of the experiment. Nevertheless, at day 16, these samples presented firmness values that were significantly ($p<0.05$) higher than in CONTROL (Supplementary Table 1). The final average value of 13.19 ± 1.58 N was significantly higher than the value found in CONTROL. Therefore firmness was improved suggesting that fogging with antibrowning solutions did not have undesirable effects on the texture of mushrooms stored under refrigeration conditions.

Scanning electron microscopy

Scanning electron photographs of *A. bisporus* at different magnifications are presented (Figures 1 and 2). Immediately after treatments were applied, fog samples had slight modifications on the surface but the cellular structure remained similar to CONTROL (Figure 1a, g). A dense area was observed in the surface of immersed samples, suggesting major changes.

After 8 days, CONTROL (Figure 1b) did not have significant changes on the surface, however, the intercellular spaces were smaller than those observed initially, and this might be a consequence of dehydration process. Fog-treated samples (Figure 1h, I, j) showed dense

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areas (black arrows), which could be attributed to lignin formation (Oms-Oliu *et al.*, 2010; *Zhang et al.,* 2018). A thick dense area (white arrow) was observed in immersed samples (Figure 1d, e, f); indicating major changes. This might be a consequence of water absorption, tissue disruption and lignification. Immersion has been proved to alter cell structures and to remove natural barriers, which enhances mass transfer during the process (Zivanovic *et al.*, 2006).

At the end of storage, disruption in cell organization on the surface of CONTROL (Figure 2a) was observed; this could be related to dehydration. In fog-treated samples (Figure 2 e, f, g), dense areas (black arrows) were observed on the surface, however, they were significantly thinner than those observed (white arrow) in immersed mushrooms (Figure 2b, c, d).

The SEM analysis indicated that the application of immersion treatments had a significant impact on cell structure and organization. The surface tissue was affected and this could change texture properties of the mushrooms. Fogging treatments seemed to be less harmful than immersion, producing less structural changes.

Antioxidant capacity (AOC)

The scavenging effects of the extracts on DPPH• and ABTS•+ radicals were similar, but some differences were detected among treatments and antibrowning agents. This could be related to the differential reactivity of DPPH• and ABTS•+ in determining antioxidant capacities due to organic acids and some phenolic compounds (Floegel *et al.*, 2011).

The initial total ABTS•+ and DPPH• radical-scavenging activities for CONTROL were 0.264 ± 0.049 and 0.380 ± 0.060 mg eq g^{-1} respectively (Supplementary Table 2). An increase in AOC

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was observed during storage, coincided with changes in phenolic compounds content, which are the major antioxidant compounds in mushrooms (Oms-Oliu *et al.*, 2010; Ding *et al.*, 2016). AAS and CYS treatments raised the AOC up to 5 times in comparison to CONTROL, while MBS doubled the AOC in comparison to CONTROL. These results are in coincidence with the differential antioxidant potential of these antibrowning compounds (Floegel *et al.*, 2011). AAS treated mushrooms showed a continuous decrease in the AOC, which could be related to AA degradation. However, at the end of storage, the AOC for AAS was still significantly higher than CONTROL (Supplementary Table 2).

Mushrooms treated with MBS did not have significant changes in AOC during 8 days of storage, then a significant increase was observed at day 12 (Supplementary Table 2); this was in coincidence with a slight increase in phenolic compounds. The AOC in mushrooms immersed in CYS had similar tendencies, however, the maximum value for AOC evaluated with ABTS•+ was detected at day 8; this could be related to secondary reactions that led to compounds with different reactivity (Ali *et al.*, 2015).

A progressive decrease in AOC was detected in all fog-treated mushrooms. Considering that no significant changes in phenolic compounds content were detected during storage, these results could indicate that the degradation of the antibrowning agent could be responsible for the decrease in the AOC. Even though fogging treatments did not significantly increased the AOC of mushrooms, these treatments had some regulatory effects on the metabolism of phenolic compounds, maintaining its content almost invariable during storage (Supplementary Table 2).

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Antibrowning agents applied by immersion were responsible for a significant increase in AOC of mushrooms, while fogging application did not have a significant effect in this parameter.

Phenolic content

Initial phenolic content in CONTROL was 0.181 ± 0.024 mg g^{-1}. A progressive increase during storage was observed (Supplementary Table 2), having a maximum value at day 16 (0.240 ± 0.023 mg g^{-1}). An increase of phenolic compounds content was reported in previous works (Oms-Oliu *et al.*, 2010; Xue *et al.,* 2017) associated with an increased activity of secondary metabolism enzymes.

Immersed samples had initial values that were significantly ($p<0.05$) higher than CONTROL (Supplementary Table 2). This might be a consequence of an overestimation, considering that when immersion treatments are applied the amounts of antibrowning agent retained on the surface would be considerable. Therefore, the results found in immersed samples are not representative of the phenolic content, especially in CYS and AAS treated samples.

Initially, all fogging treatments increased phenolic content immediately after application; these values were significantly higher ($p<0.05$) than in CONTROL but significantly lower than those achieved by immersion due to the mentioned interferences (Supplementary Table 2). After 4 days of storage, a reduction of 10-15 % in phenolic content was observed and, it would be a consequence of early reversible browning reactions (Zhang *et al.,* 2018). However, since day 8 a progressive increase was observed, indicating that the antibrowning effect of the different substances would not be enough to inhibit the production of phenolic

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compounds. In average, fog-treated mushrooms had final values that were not statistically different from CONTROL.

Fogging treatments were able to slow down phenolic synthesis during early stages of storage, and this might be interesting since major colour changes develop during early stages of storage (Sapers *et al.*, 2001; Zhang *et al.,* 2018).

PPO activity

Discolouration due to browning reactions in mushrooms is generally assumed as a consequence of PPO action on polyphenols (Zhang *et al.,* 2018).

Fresh mushrooms had initial PPO activity of 683.62 ± 10.68 UA. A significant ($p<0.05$) increase at day 4 was observed, after which it decreased and maintained without major changes until the end of storage (Figure 3). This decrease could be a consequence of direct inhibition of some oxidized products (Ali *et al.*, 2015).

Immersion in MBS achieved initial inhibition of almost 90%, followed by AAS (85% of inhibition) and CYS (60 % of inhibition). After 4 days of storage, these samples had a significant increase in PPO activity; however, its activity was still significantly lower ($p<0.05$) than CONTROL. AAS samples had the lowest values for PPO activity from day 4 and throughout the experiment (Figure 3). This could indicate that immersion in AAS was more effective in inhibiting PPO when compared to MBS and CYS.

Fogging with antibrowning agents achieved partial inhibition of PPO activity; this inhibition was of 40% for F-MBS and F-AA and 30 % for F-CYS. After 4 days of storage an increasing

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trend in PPO activity was observed for F-MBS and F-AA samples, reaching values of 432.85 ± 10.73 and 521.01 ± 16.47 UA, respectively by day eight. In average, the final value for PPO activity was 637.97 ± 20.30 UA, still significantly lower than CONTROL at day 4 (Figure 3). The inhibition in fog-treated samples was smaller than in immersed samples because the antibrowning agent did not penetrate deeply the tissue.

Colour changes

Discolouration is mainly related to the formation of melanoidins, these colour changes are mainly detected in the cap and reduce the visual acceptability of the product. Although lamella is rather sensitive to discolouration, the analysis of the cap is more suitable for industrial applications (Ding *et al.,* 2016; Sapers *et al.,* 2001).

CONTROL presented a progressive increase in BI throughout the experiment. After 12 days of storage, BI indicated colour differences that were visually confirmed with the development of brown colour (Figure 4). Samples immersed in MBS and CYS initially presented BI values lower than CONTROL; this was visually correlated with a whitening effect as previously reported (Brennan *et al.*, 1999). Major colour changes were detected after 4 days of storage (Figure 4), especially in CYS treated mushrooms, which presented a greenish colour that could be related to the formation of adducts between o-diquinones and cysteine (Ali *et al.*, 2015). The abrupt increase in BI after 4 days could be related to disruption of natural structures and cell compartmentalization during immersion treatments, which enhance browning development (Xue *et al.,* 2017).

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Samples immersed in AAS did not present initial whitening (BI=23.76 ± 1.11) and had BI values that showed a similar tendency as in CONTROL. Browning development was not as intense as in the other immersion treatments and this could be related not only to the antibrowning effect of ascorbic acid but also to its effect on secondary metabolism reactions (Ali *et al.*, 2015).

Fogging treatments were efficient to reduce BI until day 8; F-AA achieved the lowest values of BI (23.87 ± 0.90) when compared to F-MBS (27.36 ± 1.03) and F-CYS (26.42 ± 1.00). At the end of storage, F-AA and F-MBS had values that were significantly lower than CONTROL but not statistically different from MBS and AAS (Figure 4). These results indicate that fogging treatments (F-AA and F-MBS) could achieve similar results to those obtained with conventional immersion treatments (MBS and AAS). Application of cysteine by fogging was effective in maintaining low values of BI during 8 days of storage after which a significant increase was observed.

At the end of storage F-AA and F-MBS had BI values that were not significantly ($p>0.05$) different (Figure 4). These results are interesting since the same antibrowning effect was achieved with AA, which is a natural alternative to the traditional use of sulphites as antibrowning agent. Fogging technique showed similar results in browning inhibition when compared to the conventional immersion treatments.

General discussion

The application of antibrowning agents by fogging seems to be an interesting methodology for industry purposes when several aspects are considered.

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Immersion in MBS and AAS accomplished to maintain the colour of mushrooms, being AAS treatment the one showing the best results (+++) (Supplementary Table 3) as evidenced by BI values. MBS initially had the lowest values for BI, however, its antibrowning effect decreased significantly at day 4 and therefore its global effect (++) was not as good as AA. Immersion in CYS had similar results than MBS but as undesirable colours were detected its effect on maintaining colour was poor (-).

The presence or development of off-odours is a quality indicator of great importance when choosing a fresh product. MBS could impart some 'chemical odour', therefore, it could be inferred that MBS treatments could leave more residual off-odour (--) than F-MBS (-). L-cysteine has an 'onion-like' odour, even at low concentrations it is perceived by most consumers; therefore its application would have a negative impact on the quality of the product. On the other hand, AA is an odourless product; therefore, it would not impart of-odours to the product neither by immersion nor by fogging (Supplementary Table 3).

MBS and CYS treated samples showed some hardening at the end of storage, which could also have a negative impact on texture properties. In contrast, fog treated samples had a similar firmness profile evolution as in CONTROL samples, therefore it could be inferred that these treatments might not affect texture significantly. Dehydration is a major problem that could develop mushrooms during refrigerated storage; therefore those treatments that had a minor effect on firmness were also effective in preventing excessive dehydration.

Health-related incidents are one of the main restrictions that led to ban sulphites by the FDA on products intended to be consumed raw or to restrict its use to very low concentrations (Brennan *et al.*, 1999). Therefore, treatments with MBS could have a negative perception (---) by consumers. Since the amounts of preservative applied by

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fogging, would be significantly lower than immersion, the negative impact of F-MBS (-) is smaller than MBS. The application of AA or L-cysteine could be associated with beneficial effects on the health of consumers because both are antioxidant substances. Therefore, the application of AA by both methodologies would have a positive effect on consumers´ perception of health properties of the product (+++). L-cysteine could also have beneficial effects on consumer´s health; however, its antioxidant potential is lower than AA and consumers do not usually know about L-cysteine benefits (++).

From an economic perspective, immersion treatments are simple in terms of the technology needed to apply them, being MBS relatively accessible (++). Ascorbic acid and L-cysteine are expensive antibrowning agents in comparison to MBS; therefore, its application by immersion would imply spending a significant amount of money (---). All immersion treatments have the disadvantage of requiring large amounts of water in comparison to fogging; therefore, this should be considered in process optimization. Fogging treatments might need an initial high investment in the fog-generating device, but in the long term, the economic benefit will be greater given that the amount of water and antibrowning agent required are significantly reduced (Burfoot *et al.,* 1999).

If all the analyzed factors are considered together, fogging treatments would have a positive effect on edible mushrooms. Particularly, the application F-AA that has no significant effect on the texture of the mushrooms prevents browning development and enhances beneficial health properties.

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Conclusion

In the present work, two methodologies for antibrowning agents application on edible mushrooms were evaluated.

Fogging achieved browning inhibition without major changes in firmness, while immersion treatments were able to inhibit browning but significant changes in firmness were found. These results were correlated with scanning electron microscopy findings. Browning inhibition achieved by fogging technique was similar to conventional immersion treatments. However, fogging technique has the advantage of being less invasive than immersion while the amounts of antibrowning solutions are significantly reduced.

Fogging with ascorbic acid and sodium metabisulphite exhibited similar results for browning inhibition related parameters, but health beneficial effects of ascorbic acid make it an interesting alternative to sulphites in the industry of edible mushrooms.

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Legends to Figures

Fig. 1. SEM of CONTROL (a), immersed and fog (c-g) at t=0; CONTROL (b), MBS, CYS, AAS (d-e-f); F-MBS, F-CYS, F-AA (h-i-j) t=8.

Fig. 2. SEM of CONTROL (a), MBS (b), CYS (c), ASS (d), F-MBS (e), F-CYS (f) and F-AA (g) after 16 days at 5 ºC.

Fig. 3. Polyphenol oxidase in CONTROL, immersed (MBS, AAS, CYS) and fogged (F-AA, F-MBS, F-CYS) samples (superscripts, $p<0.05$).

Fig. 4. Browning Index in CONTROL, immersed (MBS, AAS, CYS), fogged (F-AA, F-MBS, F-CYS) mushrooms (superscripts, $p<0.05$).

Supplementary Items

Supplementary Figure: Evolution of visual appearance of CONTROL mushrooms and treated by immersion (MBS, AAS, CYS) or by fogging (F-AA, F-MBS, F-CYS) during refrigerated storage**.**

Supplementary Table 1: Weight loss and firmness changes in control mushrooms and treated groups during storage at 5 °C.

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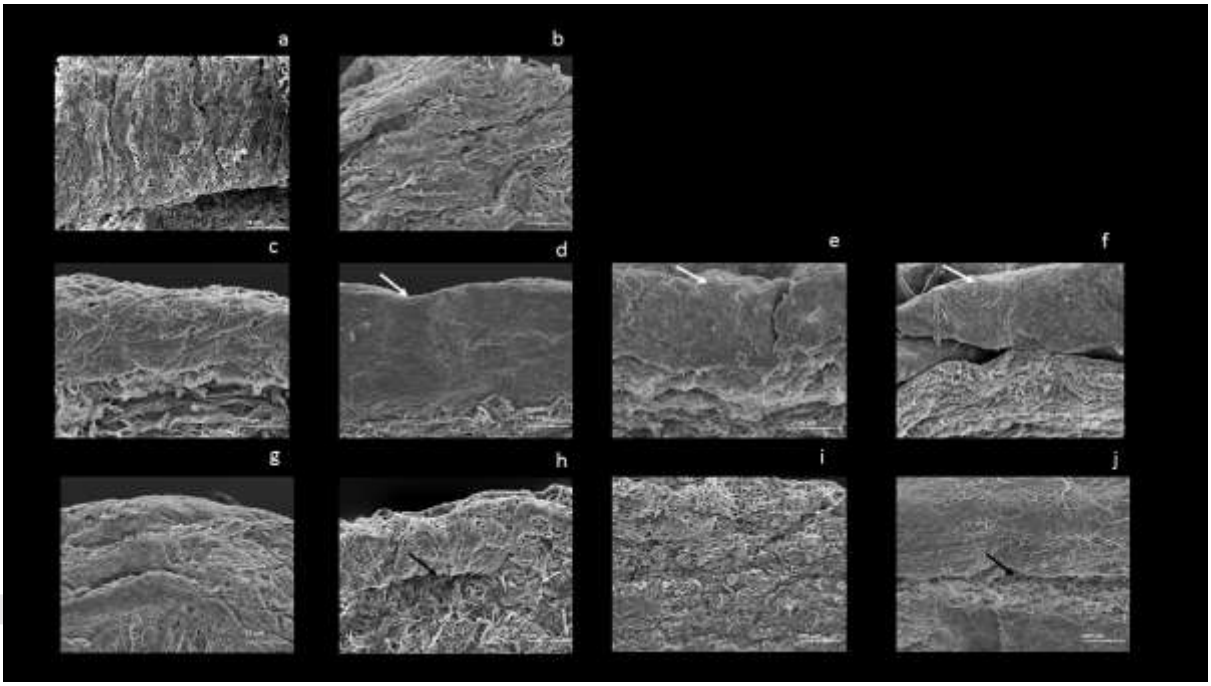
Supplementary Table 2: Antioxidant capacity evaluated by DPPH and ABTS assay and phenolic content changes in control mushrooms and treated groups during storage at 5 °C.

Supplementary Table 3: Evaluation of immersion and fogging treatments considering quality attributes, health issues and cost.



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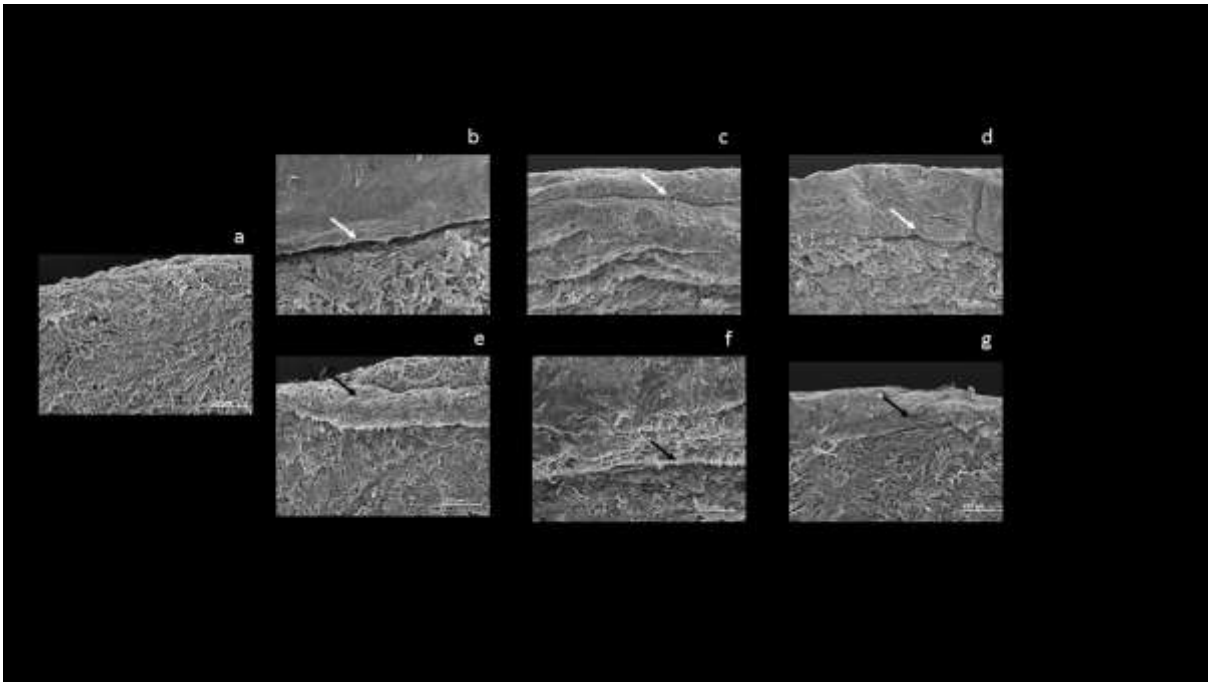

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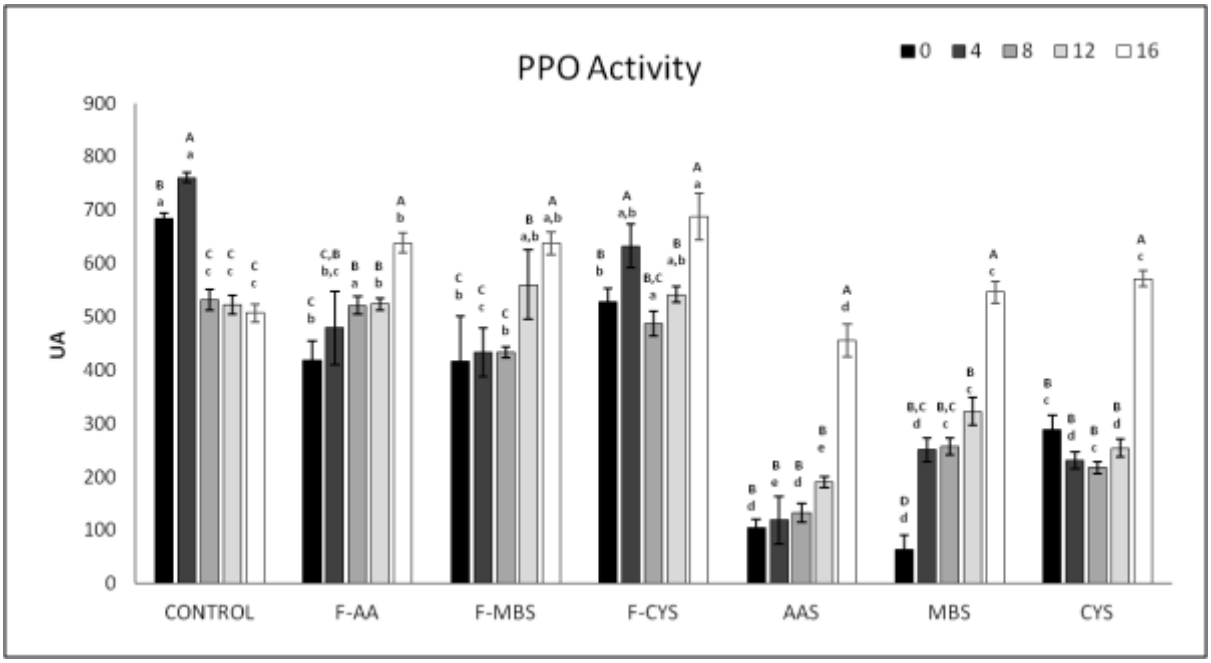

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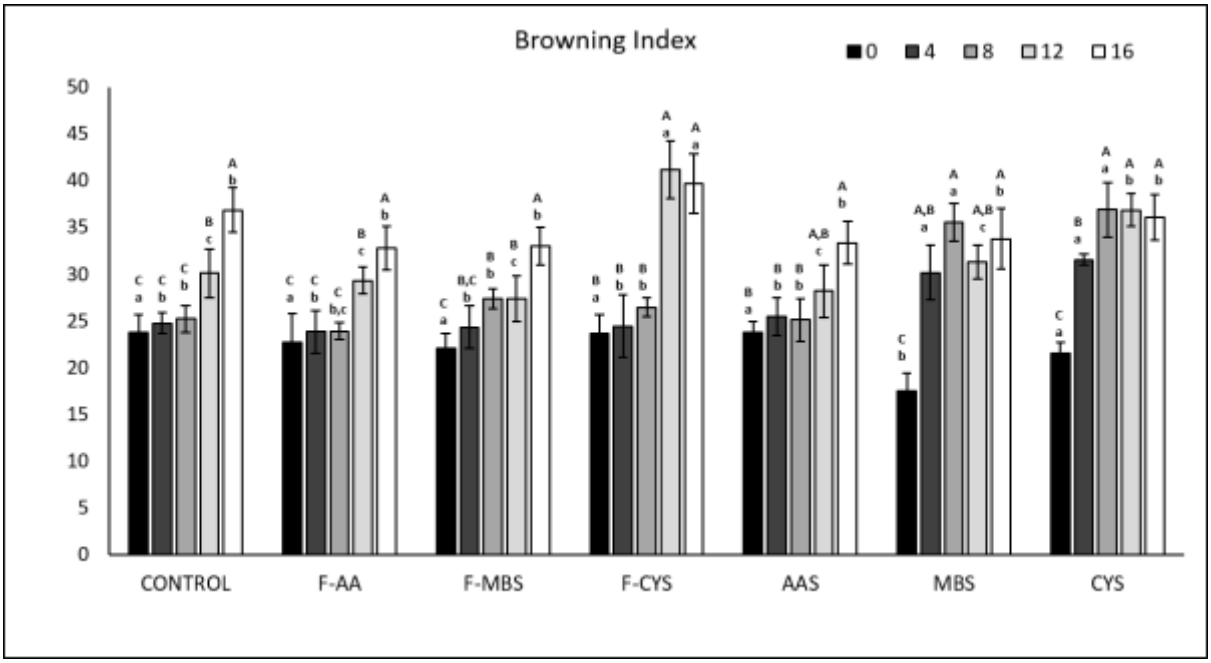

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