Comparison of Fumigation and Immersion of Essential Oils on Quality and Physiology of Fresh Shiitake Mushrooms (Lentinus edodes)

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Abstract: The effects of different essential oils with fumigation or immersion on sensory and physiological quality of fresh shiitake mushrooms were evaluated during the storage. Shiitake mushrooms were treated with essential oils (grapefruit essential oil, rosemary essential oil, cinnamon essential oil) by fumigation and immersion respectively. The surface color, browning degree, sensory evaluation, hardness, soluble protein content, total phenolic content and DPPH· scavenging ability of shiitake mushrooms were measured during the storage. The results showed that hardness, sensory qualities and soluble protein decreased gradually with the extension of storage period in all the treatments and rosemary essential oil had better effect on maintaining cap color, sensory quality, total phenolic content and DPPH· scavenging ability compared with other treatments. In general, immersion treatment showed better effect on shiitake mushrooms preservation than fumigation treatment.

Keywords: Essential Oil, Shiitake Mushrooms, Quality, Physiology, Preservation Effect

Introduction

Shiitake mushrooms (Lentinus edodes) cultivated and consumed widely in the world. shiitake mushrooms have special taste and affluent nutrient compounds, such as polyphenols, polysaccharide, protein and multiple vitamins (Jiang et al., 2013). Among the various bioactive substances, phenolic compounds were recognized as one of effective antioxidants and anti-tumour agents (Putteraju et al., 2006). Moreover, strong radical scavenging ability in shiitake mushrooms could be attributed to the phenolic compounds, polysaccharide and protein (Ares et al., 2006). However, fresh shiitake mushrooms are easily perishable for their high respiration and metabolism. Therefore, appropriate preservation methods for fresh shiitake mushrooms are very necessary after postharvest.

Essential oils are mainly obtained from plant tissues. Most of the essential oils are liquid with strong volatile aroma and safe at lower dosage (Jia et al., 2019). Essential oil as a natural, green preservation approach, it was applied widely. It was also utilized as antioxidants, antimicrobial and anticancer reagent. Rosemary essential oil was proved to inhibit the pathogenic bacteria such as Pseudomonas Flourescens, E. coli on fish (Gómez-Estaca et al. 2010). Essential oils are very complex compounds, mainly consisting of terpenoids, aromatic families, fatty families and compounds containing nitrogen and sulfur. The essential oils also contained phenols, aldehydes and terpenes, which contribute good antibacterial activity and promote the antioxidant effect (Tu and Hu, 2018). Essential oils were reported to be effective to inhibit some microbiology including fungi, bacteria and virus on fresh fruit and vegetables. It was also reported essential oils have potential to increase antioxidant ability of some fruit and vegetables (Nasiri et al., 2018; Cristóbal-Luna et al., 2018; Rahmanzadeh et al., 2019). Essential oils extracted from natural plant, have advantages of high safety low cost, thus they are often used as natural preservatives for food. Till now, essential oils could be treated by immersion or fumigation when used on the postharvest fruit and vegetables. However, the effect of immersion or fumigation of essential oils on preservation of shiitake mushrooms still need to be confirmed and compared.

The objective of this study was to compare the effect of fumigation and immersion treatments of three essential oils (grapefruit, rosemary and cinnamon) on hardness, cap...
color, sensory quality, browning degree, soluble protein, total phenols and DPPH-scavenging ability of shiitake mushrooms during the storage.

Materials and Methods

Materials and Treatments

Fresh shiitake mushrooms were harvested from Zibo in the Shandong province of China, then they were transported to the laboratory within 3 h. Shiitake mushrooms were selected for uniform size, color and maturity, without mechanical damage. In this experiment, grapefruit, rosemary and cinnamon essential oils were used to treat shiitake mushrooms. According to our previous study, essential oil concentration below 10 µL L⁻¹ did not show obvious effect on quality maintenance of shiitake mushrooms, while essential oil aroma would cover the aroma of shiitake mushrooms when the concentration was above 50 µL L⁻¹. Therefore, the concentrations of essential oil treatments were selected as 10, 30 and 50 µL L⁻¹ for both fumigation and immersion treatments. The shiitake mushrooms were treated as follows: (1) Fumigation treatment: A total of 2 kg shiitake mushrooms were placed in a 5 L sealed container with a filter paper inside the cover. Essential oils were spread onto filter paper to final concentrations of 10, 30 and 50 µL L⁻¹ solution. No essential oil applied shiitake mushrooms were used to treat shiitake mushrooms. According to our previous study, essential oil aroma would cover the aroma of shiitake mushrooms when the concentration was above 50 µL L⁻¹. Therefore, the concentrations of essential oil treatments were selected as 10, 30 and 50 µL L⁻¹ for both fumigation and immersion treatments. The shiitake mushrooms were treated as follows: (1) Fumigation treatment: A total of 2 kg shiitake mushrooms were placed in a 5 L sealed container with a filter paper inside the cover. Essential oils were spread onto filter paper to final concentrations of 10, 30 and 50 µL L⁻¹. Then the fumigation was kept at 15°C for 12 h. (2) Immersion treatment: Essential oil was emulsified to final concentrations of 10, 30 and 50 µL L⁻¹ with 0.05% Tween 20. No essential oil applied shiitake mushrooms were taken as control set (CK). Three replicates were evaluated per treatment. Then, the mushrooms were air-dried to a stable mass after treatment by packaging 70±5 g shiitake mushrooms in low density polyethylene film (LDPE, 16 cm × 46 cm in area and 120 µM in thickness). All the samples were stored at 15°C with 90% RH for 6 days.

Cap Color Determination

Cap colors of shiitake mushrooms were determined by assessing three equidistant points on the shiitake mushroom cap with SC-80 C automatic chromatometer and L* value was recorded (Jiang et al., 2011).

Hardness

Hardness was measured by hand-held hardness tester. Each mushroom was evenly measured at three points (Jiang et al., 2010a). The probe pressed down at a certain speed with a depth of about 6 mM. The result was recorded as kg cm⁻².

Sensory Evaluation

Sensory evaluation was carried out according to the referenced methods with some modifications (Antmann et al., 2008; Han et al., 2015; Mohapatra et al., 2011; Phat et al., 2016). The sensory evaluation parameters and scoring method were discussed and determined in a preliminary experiment. The overall visual quality, aroma, texture, cap color, gill color and gill integrity were chosen as the sensory evaluation parameters. Scoring was carried out by a panel of 10 trained assessors. The scoring scale was 1-9, higher scores represented good quality of shiitake mushrooms.

Browning Degree

Shredded shiitake mushrooms of 5 g were mixed with 50 mL cold distilled water. The mixture was homogenized for 30 s and centrifuged at 8000 r min⁻¹ for 15 min, then the supernatant was kept at 25°C for 5 min and absorbance at 410 nM was determined. The browning degree was calculated as 10 × A₄₁₀ (Jiang, 2013).

Soluble Protein Content

Shiitake mushroom sample with 4 g in each treatment was added to 20 mL of deionized water and extracted for 20 min. Then the mixture was centrifuged at 10000 r min⁻¹ and 4°C for 10 min. The standard curve was made by determination of adding different concentration of BSA (Bull Serum Albumin) with CBB (Coomassie brilliant blue) dye. The result was expressed as µg g⁻¹ fresh sample (Jiang et al., 2010b).

Total Phenolic Content

Folin-Ciocalteu Reagent (FCR) method was used to determine the total phenolic content (Cheung et al., 2003; Li et al., 2014; Wu et al., 2016). Shiitake mushroom sample of 1 g for each treatment was added with 20 mL 95% methanol and then extracted at 60°C for 30 min. After centrifugation at 10000 r min⁻¹ and 4°C for 10 min and the supernatant was taken. The extraction was carried out twice. The two supernatants were combined with 95% methanol to a final volume of 50 mL. The extract of 0.4 mL was added to 2 mL 10% Folin-Ciocalteu reagent and 1.8 mL 7.5 % sodium carbonate solution. The mixture was kept in the dark condition for 1 h and then absorbance at 765 nM was measured. Gallic acid was taken as the standard solution. The result was expressed as µg GAE g⁻¹ fresh sample (GAE represents Gallic Acid Equivalent).

DPPH Scavenging Ability

The 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) scavenging activity of shiitake mushroom samples was determined according to the referenced method with some modifications (Jiang et al., 2010a). Shiitake mushroom sample of 1 g in each treatment was added to 20 mL 80% methanol and extracted at 60°C for 30 min, then the mixture was centrifuged at 10000 r min⁻¹ and 4°C for 10 min. The extraction was carried out twice and supernatant was collected and diluted with 80% methanol to a final volume of 50 mL as sample extract. Then 400 µL sample extract was added with 3.5 mL 0.14
mmol L\(^{-1}\) DPPH\(^{-}\) solution. The mixture was placed in dark for 30 min and the absorbance at 517 nM was determined. The radical scavenging activity (\(\%\)) = 100 (1-A\(_S\)/A\(_C\)), where A\(_S\) is the absorbance of the methanol extract and A\(_C\) is the absorbance of the DPPH\(^{-}\) solution.

Results

Changes in Cap Color of Shiitake Mushrooms

Table 1 showed that \(\text{L}^*\) decreased during storage. No significant difference could be detected on day 3 in all the treatments. However, till the 6 days of storage, \(\text{L}^*\) in all the fumigation treatments decreased significantly, while there was no significant decrease of \(\text{L}^*\) in 10 \(\mu\)L L\(^{-1}\) rosemary immersion or cinnamon immersion treatments. Compared with essential oil treatments, \(\text{L}^*\) was the lowest in control at the end of the storage.

Changes in Hardness of Shiitake Mushrooms

As can be seen from Fig. 1, the overall trend of the hardness value of shiitake mushrooms was decreasing during storage. On the third day, the hardness of shiitake mushrooms decreased significantly under different treatments and concentrations of essential oils. At the end of storage period, the hardness of shiitake mushrooms also decreased significantly in all the treatments. After harvesting, respiration and senescence mainly led to the softening of shiitake mushrooms. In addition, decrease of hardness was also related to the degradation of lignin and cellulose in shiitake mushrooms (Gao et al., 2014).

Changes in Sensory Qualities of Shiitake Mushrooms

The results of sensory quality changes of shiitake mushrooms during the storage are shown in Fig. 2-1 and 2-2. All the sensory parameters decreased during the storage compared with 0 day. On day 3, the sensory quality of shiitake mushrooms under rosemary essential oil with fumigation and immersion treatments could still maintain high levels. Till the day 6, quality deterioration could be observed in shiitake mushrooms under all the treatments. However, immersion treatment presented better effect on maintaining sensory quality than fumigation treatment. At the end of the storage, all the sensory attributes performed better in 10 and 30 \(\mu\)L L\(^{-1}\) rosemary essential oil immersion treatment, as well as a good performance of sensory quality also could be detected under cinnamon essential oil immersion treatment.

Changes in Browning Degree of Shiitake Mushrooms

The browning degree of shiitake mushrooms under different treatments increased significantly during storage in all treatments and the browning degree of shiitake mushrooms in control group increased the fastest (Fig. 3). The browning degrees treated with grapefruit essential oil, rosemary essential oil and cinnamon essential oil were lower than that of the control group and other experimental groups during the 6-day storage period.

Changes in Soluble Protein of Shiitake Mushrooms

It was indicated that, soluble protein content showed a downward trend except for the minor increase in grapefruit fumigation and immersion treatments with 10 and 30 \(\mu\)L L\(^{-1}\) in the 0-3 days of storage period (Fig. 4). From 3 to 6 days, soluble protein content in all the treatments kept declining. The soluble protein content of shiitake mushrooms in both grapefruit essential oil fumigation and immersion treatments decreased slowly and kept a higher level compared with the control group. In postharvest period, shiitake mushrooms still have respiration and physiology metabolism, but shiitake mushrooms have no supply of exogenous nutrients and can only consume its own accumulated nutrients. Protein is one of the sources of nutrients for postharvest life activities of shiitake mushrooms, which induced the soluble protein content decreasing after postharvest (Jiang et al., 2010b; Burton et al., 1997).

Changes in Total Phenolic Content

Figure 5, the total phenolic content of shiitake mushrooms increased after 6 days of storage. Till the day 3, the total phenolic content of shiitake mushrooms had an overall increase trend except for some slight decrease in fumigation treatments with 50 \(\mu\)L L\(^{-1}\) grapefruit essential oil, 50 \(\mu\)L L\(^{-1}\) rosemary essential oil, 10 \(\mu\)L L\(^{-1}\) cinnamon essential oil and 30 \(\mu\)L L\(^{-1}\) rosemary immersion treatment. At the end of storage, in all grapefruit essential oil treatments with different concentrations, the total phenolic content in 30 \(\mu\)L L\(^{-1}\) fumigation treatment was higher than that of others; in all rosemary essential oil treatments with different concentrations, 10 \(\mu\)L L\(^{-1}\) fumigation and 10 \(\mu\)L L\(^{-1}\) immersion treatments were higher than others; in all cinnamon essential oil treatments with different concentrations, 30 \(\mu\)L L\(^{-1}\) immersion treatment was higher than others.

Changes in DPPH- Scavenging Ability

Figure 6, DPPH- scavenging abilities of shiitake mushrooms in all treatments increased during storage. During the 0-3 days of storage, DPPH- scavenging rates of shiitake mushrooms treated with fumigation of grapefruit and cinnamon essential oil increased slightly but rose rapidly in 3-6 days. DPPH- scavenging rate of shiitake mushrooms fumigated with rosemary essential oil increased rapidly and then decreased rapidly during storage. DPPH- scavenging rates of shiitake mushrooms immersed with three essential oils increased rapidly in 0-3 days and maintained high levels in the following storage period.
Table 1: Changes in cap color (L*) of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Different letters represent the significant differences between mean values of different treatments at p=0.05 according to the ANOVA and LSD test.

<table>
<thead>
<tr>
<th>Essential Oil</th>
<th>Treatment</th>
<th>0d</th>
<th>3d</th>
<th>6d</th>
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<tr>
<td></td>
<td></td>
<td>40.18±1.55^a</td>
<td>42.75±1.47^a</td>
<td>28.97±5.06^ab</td>
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<tr>
<td>grapefruit</td>
<td>10 µL L⁻¹</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>30 µL L⁻¹</td>
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<td>42.19±4.51^a</td>
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<td>50 µL L⁻¹</td>
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<td>40.11±7.38^a</td>
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<td>42.81±2.93^a</td>
<td>24.72±2.18^b</td>
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<tr>
<td>rosemary</td>
<td>10 µL L⁻¹</td>
<td>40.18±1.55^a</td>
<td>38.60±1.15^a</td>
<td>32.45±5.19^a</td>
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<tr>
<td></td>
<td>30 µL L⁻¹</td>
<td>40.18±1.55^a</td>
<td>40.02±2.57^ab</td>
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<td>50 µL L⁻¹</td>
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<td>39.17±3.79^ab</td>
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<tr>
<td>cinnamon</td>
<td>10 µL L⁻¹</td>
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<td>37.90±1.33^b</td>
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<td>39.41±2.92^b</td>
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<tr>
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<td>42.81±2.93^a</td>
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<td>grapefruit</td>
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<td>40.18±1.55^a</td>
<td>40.61±2.46^a</td>
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<td>42.81±2.93^a</td>
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<td>rosemary</td>
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<td>39.83±1.96^ab</td>
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<td>42.81±2.93^a</td>
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</tr>
<tr>
<td>cinnamon</td>
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<td>38.08±3.58^b</td>
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<td>24.72±2.18^b</td>
</tr>
</tbody>
</table>

Fig. 1: Changes in hardness of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments at p = 0.05 according to the ANOVA and LSD test.
Fig. 2-1: Changes in sensory qualities of shiitake mushrooms under different treatments of essential oils during the storage at 15°C (0 day and 3 days)

Fig. 2-2: Changes in sensory qualities of shiitake mushrooms under different treatments of essential oils during the storage at 15°C (6 days)
Fig. 3: Changes in browning degree of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments at p = 0.05 according to the ANOVA and LSD test.

Fig. 4: Changes in soluble protein content of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments at p = 0.05 according to the ANOVA and LSD test.
Fig. 5: Changes in total phenolic content of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments at p = 0.05 according to the ANOVA and LSD test.

Fig. 6: Changes in DPPH· scavenging abilities of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments at p = 0.05 according to the ANOVA and LSD test.
**Discussion**

The results indicated that browning procedure of shiitake mushrooms could be retarded by essential oils treated with both fumigation and immersion. And 10 μL L⁻¹ rosemary immersion and all the cinnamon immersion treatments had the better effect on the cap color of shiitake mushrooms. However, essential oils did not have obvious advantages to prevent hardness decreasing. Meanwhile, grapefruit essential oil fumigation and immersion treatments were beneficial to the preservation of soluble protein in shiitake mushrooms. In this study, Total Phenolic (TP) content of shiitake mushrooms treated with rosemary essential oil was the highest among the three essential oils. Phenolic compounds are related to the browning of many plants. Phenolic compounds could be oxidized affected by polyphenol oxidase and oxygen. Increase of total phenolic content could be explained as the release of phenolic compounds from the cell for the softening and degradation of shiitake mushrooms during the storage (Alasalvar et al., 2005; Amanatidou et al., 2000). DPPH- scavenging ability in control set did not show the obvious disadvantages compared with essential oil treatments. Phenolic compounds could contribute the DPPH-scavenging ability by scavenging and chelating processes or as free radical terminators. It was indicated that DPPH-scavenging rates increase was related with the process of stimulating phenolic compounds in shiitake mushrooms. Phenolic compounds release could stimulate the increase of antioxidant activity of shiitake mushrooms. Besides, antioxidant ability of shiitake mushrooms were also related with other compounds, such as polysaccharide, flavonoid. Senescence or damage of the tissue in plants would lead to the release of phenolic compounds, polysaccharide or flavonoid, which could result in the increase of antioxidant ability. In control set, the higher levels of DPPH- scavenging rate might be attributed to the release of antioxidant compounds during the senescence process.

**Conclusion**

It could be concluded that the hardness of shiitake mushrooms decreased in general under different conditions of essential oil and the sensory attributes and soluble protein content decreased gradually during the storage. Total phenol content and DPPH-scavenging ability increased. In general, immersion treatment of the essential oils performed the better effect on quality and physiology of shiitake mushrooms. Moreover, rosemary essential oil with immersion treatment contributed the best effect on preservation among three essential oils.

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**Author’s Contributions**

Yanjie Li: Conception, experiment execution and paper writing.
Yanxin Wang: Picture drawing, data curation.
Shudong Ding: Data curation.
Yujiao Zhao: Participated in part of experiment.
Haifang Xiao: Ameliorated the manuscript.
Yuedong Song: Ameliorated the manuscript.

**Ethics**

The article is original and the content was unpublished. The authors declared that they have approved the manuscript and no ethical issues involved.

**References**


Phat, C., Moon, B., & Lee, C. (2016). Evaluation of umami taste in mushroom extracts by chemical analysis, sensory evaluation and an electronic tongue system. Food Chem. 192, doi.org/1068-107710.1016/j.foodchem.2015.07.113


