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

Yanjie Li, Yanxin Wang, Shudong Ding, Yujiao Zhao, Haifang Xiao, and Yuedong Song

School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, China

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Corresponding Author:
Yanjie Li
School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, China
Email: yanjie0227@163.com

Abstract: The effects of different essential oils with fumigation or immersion on the 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 the storage period in all the treatments, and rosemary essential oil had a better effect on maintaining cap color, sensory quality, and total phenolic content and DPPH· scavenging ability compared with other treatments. In general, immersion treatment showed a better effect on shiitake mushrooms preservation than fumigation treatment.

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

Introduction

Shiitake mushrooms (*Lentinus edodes*) are cultivated and consumed widely in the world. shiitake mushrooms have a special taste and affluent nutrient compounds, such as polyphenols, polysaccharides, protein, and multiple vitamins (Jiang *et al*., 2013). Among the various bioactive substances, phenolic compounds were recognized as effective antioxidants and anti-tumor agents (Puttaraju *et al*., 2006). Moreover, the strong radical scavenging ability in shiitake mushrooms could be attributed to the phenolic compounds, polysaccharides, 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 a strong volatile aroma and are safe at a lower dosage (Jia *et al*., 2019). Essential oil is a natural, green preservation approach, it was applied widely. It was also utilized as an antioxidant, antimicrobial, and anticancer reagent. Rosemary essential oil was proved to inhibit the pathogenic bacteria such as Pseudomonas fluorescens, and *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 viruses 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 plants have the advantages of high safety and 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 postharvest fruit and vegetables. However, the effect of immersion or fumigation of essential oils on the preservation of shiitake mushrooms still needs 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 an obvious effect on the 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}. 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} solution with 0.05% Tween 20. No essential oil applied to shiitake mushrooms was taken as a 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 *the* L^* value was recorded (Jiang *et al*., 2011).

Hardness

Hardness was measured by a 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 the good quality of shiitake mushrooms.

Browning Degree

Shredded shiitake mushrooms of 5 g were mixed with 50 mL of 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 $10 \times A_{410}$ (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 the determination of adding different concentrations of BSA (Bull Serum Albumin) with CBB (Coomassie brilliant blue) dye. The result was expressed as a 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 the 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 the 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 L^* decreased during storage. No significant difference could be detected on day 3 in all the treatments. However, till the 6 days of storage, L^* in all the fumigation treatments decreased significantly, while there was no significant decrease of L^* in 10 μL L^{-1} rosemary immersion or cinnamon immersion treatments. Compared with essential oil treatments, 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 the 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, a decrease in 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 days. On day 3, the sensory quality of shiitake mushrooms under rosemary essential oil with fumigation and immersion treatments could still maintain high levels. Till day 6, the quality deterioration could be observed in shiitake mushrooms under all the treatments. However, immersion treatment presented a 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 μL 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 the 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 μL 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 the postharvest period, shiitake mushrooms still have respiration and physiology metabolism, but shiitake mushrooms have no supply of exogenous nutrients and can only consume their own accumulated nutrients. Protein is one of the sources of nutrients for postharvest life activities of shiitake mushrooms, which induced the soluble protein content to decrease after postharvest (Jiang *et al*., 2010b; Burton *et al*., 1997).

Changes in Total Phenolic Content

In 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 increasing trend except for some slight decrease in fumigation treatments with 50 μL L^{-1} grapefruit essential oil, 50 μL L^{-1} rosemary essential oil, 10 μL L^{-1} cinnamon essential oil, and 30 μL 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 μL L^{-1} fumigation treatment was higher than that of others; in all rosemary, essential oil treatments with different concentrations, 10 μL L^{-1} fumigation and 10 μL L^{-1} immersion treatments were higher than others; in all cinnamon, essential oil treatments with different concentrations, 30 μL 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

		0d	3d	6d
grapefruit	10 μL L^{-1}	40.18±1.55^{a}	42.75±1.47^{a}	28.97±6.06^{ab}
(fumigation)	30 μL L^{-1}	40.18±1.55^{a}	42.19±4.51^{a}	28.36±2.83^{ab}
	50 μL L^{-1}	40.18±1.55^{a}	40.11±7.38^{a}	30.26±3.69^{a}
	CK	40.18±1.55^{a}	42.81±2.93^{a}	24.72±2.18^{b}
rosemary	10 μL L^{-1}	40.18±1.55^{a}	38.60±1.15^{b}	32.45±5.19^{a}
(fumigation)	30 μL L^{-1}	40.18±1.55^{a}	40.02±2.57^{ab}	35.09±3.22^{a}
	50 μL L^{-1}	40.18±1.55^{a}	39.17±3.79^{ab}	31.34±4.82^{a}
	CK	40.18±1.55^{a}	42.81±2.93^{a}	24.72±2.18^{b}
cinnamon	10 μL L^{-1}	40.18±1.55^{a}	37.90±1.33^{b}	33.05±1.02^{a}
(fumigation)	30 μL L^{-1}	40.18±1.55^{a}	39.41±2.92^{b}	32.87±3.56^{a}
	50 μL L^{-1}	40.18±1.55^{a}	39.97±2.57^{ab}	33.45±5.23^{a}
	CK	40.18±1.55^{a}	42.81±2.93^{a}	24.72±2.18^{b}
grapefruit	10 μL L^{-1}	40.18±1.55^{a}	40.61±2.46^{a}	32.00±2.58^{a}
(immersion)	30 μL L^{-1}	40.18±1.55^{a}	40.64±1.48^{a}	33.37±5.62^{a}
	50 μL L^{-1}	40.18±1.55^{a}	41.55±3.44^{a}	31.75±4.02^{a}
	CK	40.18±1.55^{a}	42.81±2.93^{a}	24.72±2.18^{b}
rosemary	10 μL L^{-1}	40.18±1.55^{a}	39.83±1.96^{ab}	37.03±6.73^{a}
(immersion)	30 μL L^{-1}	40.18±1.55^{a}	39.05±3.65^{b}	30.92±5.50^{ab}
	50 μL L^{-1}	40.18±1.55^{a}	40.02±1.47^{ab}	31.25±7.49^{ab}
	CK	40.18±1.55^{a}	42.81±2.93^{a}	24.72±2.18^{b}
cinnamon	10 μL L^{-1}	40.18±1.55^{a}	38.08±3.58^{b}	35.12±5.12^{a}
(immersion)	30 μL L-1	40.18±1.55^{a}	38.91±3.10^{ab}	36.70±6.75^{a}
	50 μL L^{-1}	40.18±1.55^{a}	40.52±3.39^{ab}	37.08±5.78^{a}
	CK	40.18±1.55^{a}	42.81±2.93^{a}	24.72±2.18^{b}

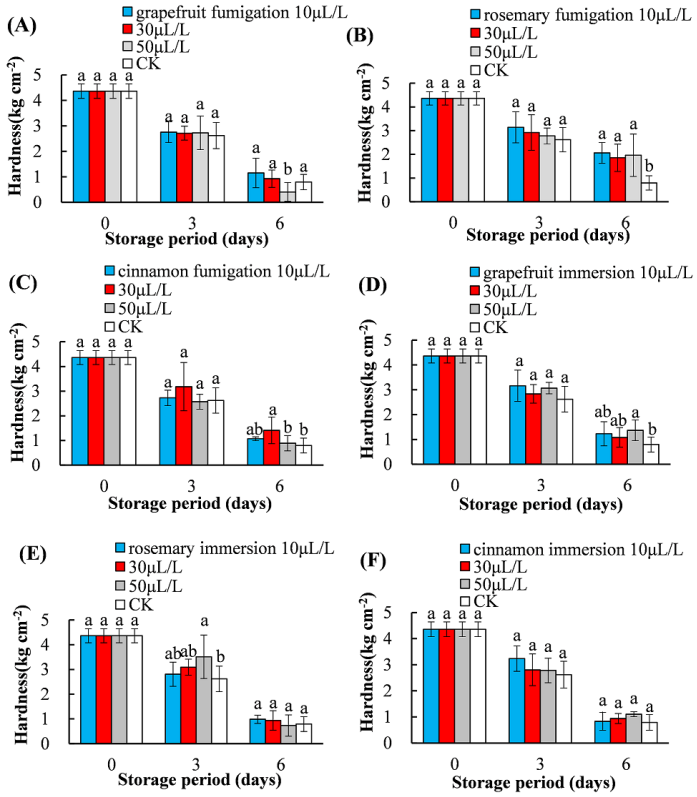

Fig. 1: Changes in hardness of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments at p = 0.05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

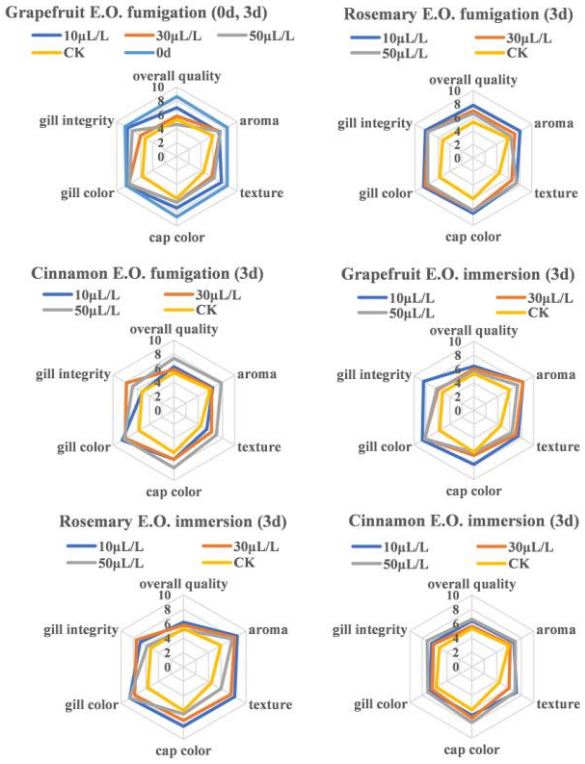

Fig. 2-1: Changes in sensory qualities of shiitake mushrooms under different treatments of essential oils during the storage at 15°C (0 days and 3 days)

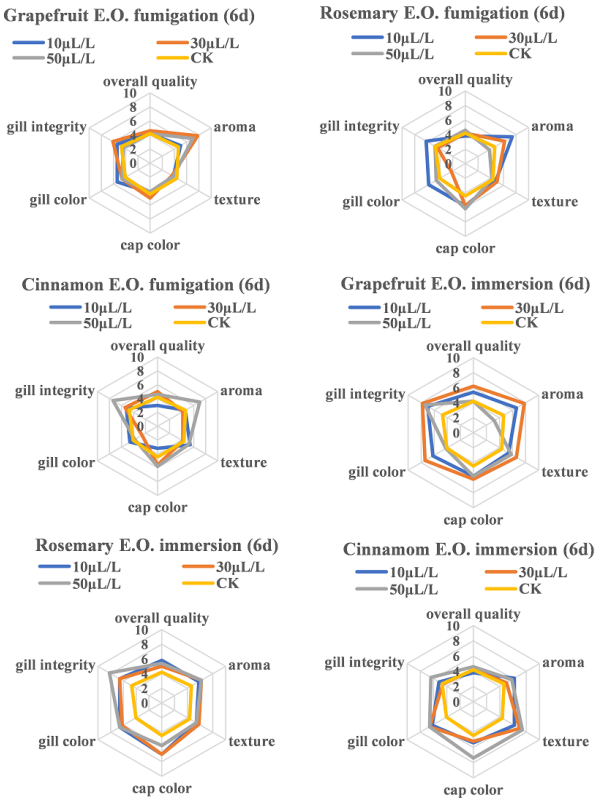

Fig. 2-2: Changes in sensory qualities of shiitake mushrooms under different treatments of essential oils during the storage at 15°C (6 days)

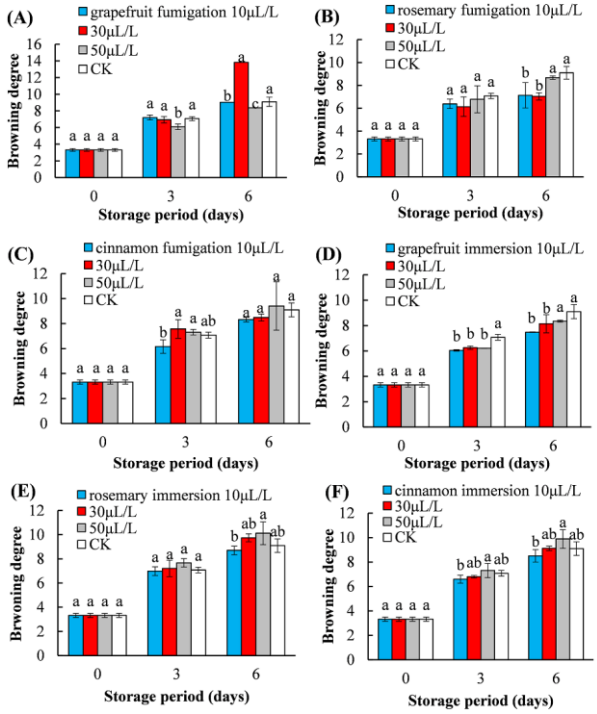

Fig. 3: Changes in browning degree of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments at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

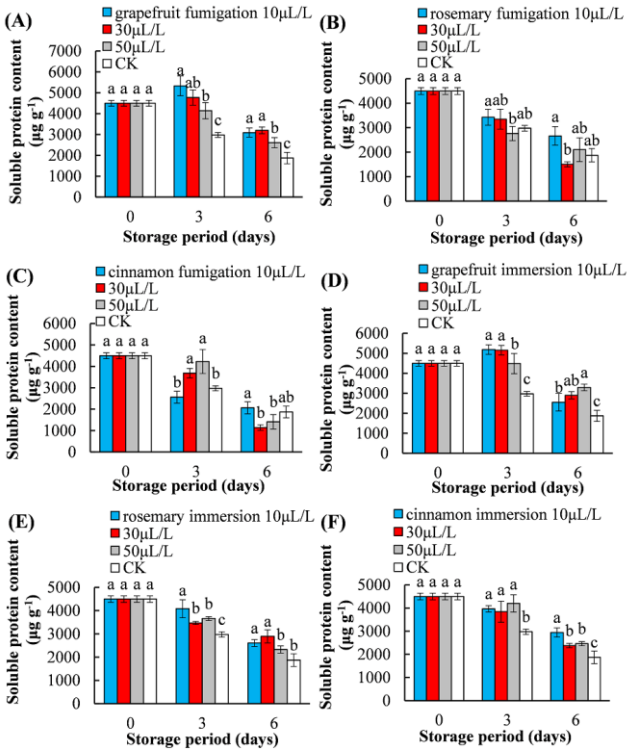

Fig. 4: Changes in soluble protein content of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments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

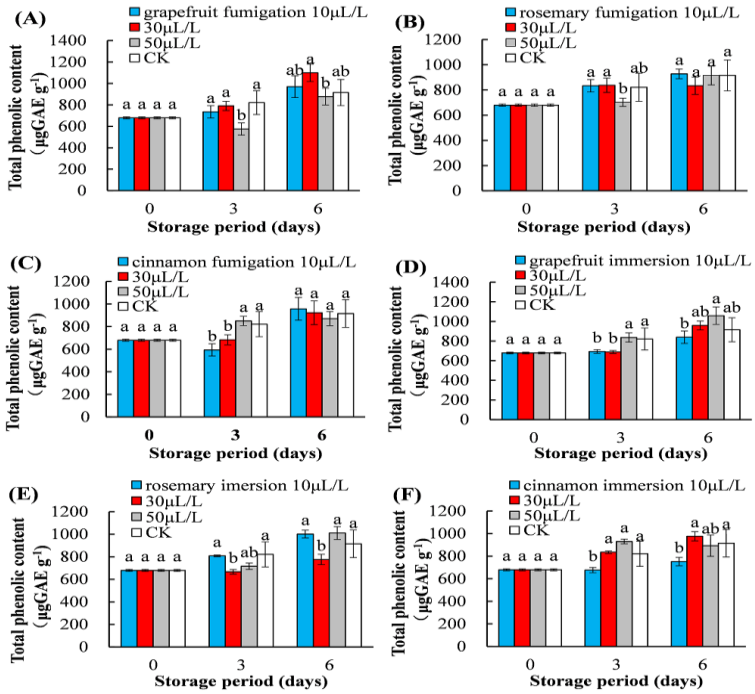

Fig. 5: Changes in total phenolic content of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments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

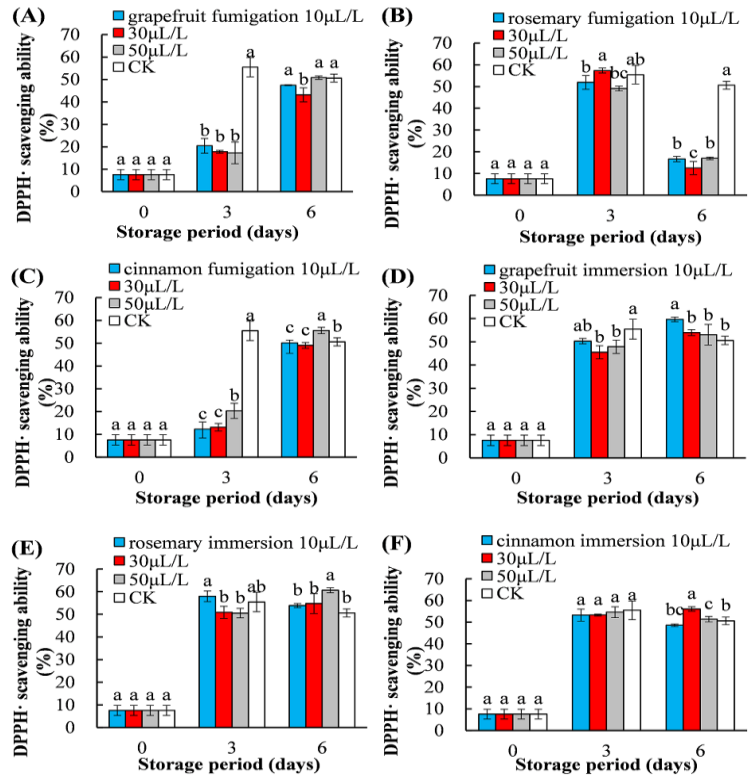

Fig. 6: Changes in DPPH· scavenging abilities of shiitake mushrooms under different treatments of essential oils during the storage at 15°C. Vertical bars represent the standard deviation of the mean (n = 3). Different letters represent the significant differences between mean values of different treatments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

Discussion

The results indicated that the 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 a 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, the 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 and affected by polyphenol oxidase and oxygen. An increase in 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 the control set did not show the obvious disadvantages compared with essential oil treatments. Phenolic compounds could contribute to the DPPH· scavenging ability by scavenging and chelating processes or as free radical terminators. It was indicated that DPPH· scavenging rates increase was related to the process of stimulating phenolic compounds in shiitake mushrooms. Phenolic compounds released could stimulate the increase of antioxidant activity of shiitake mushrooms. Besides, the antioxidant ability of shiitake mushrooms was also related to other compounds, such as polysaccharides, and flavonoids. Senescence or damage of the tissue in plants would lead to the release of phenolic compounds, polysaccharides, or flavonoids, which could increase antioxidant ability. In the 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 a better effect on the quality and physiology of shiitake mushrooms. Moreover, rosemary essential oil with immersion treatment contributed the best effect on preservation among the 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 an 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 are involved.

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