



The effect of edible coating with combined *Thymus vulgaris* extract and glycerol monostearate on oyster mushroom's shelf life

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Shelf-life of mushrooms is very low, because of several characteristics, such as their thin epidermal structure and high respiration rates. They tend to lose their quality after harvest. Hence, mushrooms need supportive care to keep freshness. Several protective methods have been recommended. In the current study, the effect of lipid-edible coating with different doses of glycerol monostearate and thyme extract, for the extending of edible mushroom's shelf life was evaluated. After, preparation of aqueous thyme extract (TE) by the Clevenger method, the mushroom treatments were prepared with different concentrations of the glycerol monostearate (GMS) and thyme extract. The chemical composition of the extract was performed using GC-MS method. The texture tightness, colour, and weight loss were respectively, measured using the texture analyzer, HunterLab, and digital balance. The Sensory and antimicrobial evaluations were also performed during the 15 days. Analysis of the extract has detected the 23 chemical compositions with the different structures and functional groups. The high texture tightness and the low weight loss determined for the mixture of GMS and 150 mg/kg TE, and the colour indices ($a^*b^*L^*$) have less significant change by adding the GMS with doses of 100 and 150 mg/kg of TE. Furthermore, the high antimicrobial activities resolution for GMS+TE150 mg/kg. In conclusion, the GMS+EO150 mg/kg coating could be used significantly for preserving the quality of oyster mushrooms throughout long-term storage.

1. Introduction

Today the production and consumption of edible mushrooms are increasing very fast throughout the world, mostly due to greater awareness of their nutritive and medicinal properties and their unique taste and texture (Jafri et al, 2013). Mushrooms are used not only as food but also as functional food and medicines due to high content of proteins and minerals, low cholesterol and starch, and also different bioactive compound (Sedaghat and Zahedi, 2012).

One of the most problems and challenges in the maintaining, post-harvest distribution, and retail of mushrooms is the short shelf life and highly perishable. This quick deterioration is mainly caused by high loss of water, metabolic activity, respiration rate, and dehydration (Fattahifar et al, 2018; Ares et al, 2006). Mushrooms, due to an inadequate cuticle layer on the cap surface are susceptible to shrivel and decay because the cuticle protects it from physical damage, water evaporation and microbial attack (Kim et al., 2006).



Transpiration losses of the edible mushrooms are the most major problem during the storage process because of their high moisture content (Gupta et al., 2016). About 90 % of mushroom's texture contains water, and their shelf life is between 3 - 5 days (Mohapatra et al., 2010). Though, they start deteriorating instantly within a day after harvest (Gupta et al., 2015). The polyphenol oxidase enzyme is the main cause of the colour changes of the mushroom (Mohapatra et al., 2010; Bonilla et al., 2012).

Due to the high unpreserved nature of mushrooms, several protective methods such as 1-MCP (Suna et al., 2020), pistachio green hull extract (Fattahifar, et al., 2018), modified atmosphere packaging (Jafri et al., 2013), edible coating (Sedaghat and Zahedi, 2012), washing with anti-microbial and anti-browning compound (Cliffe-Byrnes et al., 2008); drying (Villaescusa et al., 2003), packaging (Cliffe-Byrnes & O'Beirne, 2007), application of the polymeric films (Hardenburg, 1990), and moisture absorbers such as sorbitol, sodium chloride, propylene glycol and polyvinyl alcohol (Villaescusa & Gil, 2002), have been considered (Gupta et al., 2016). Recent years, the use of films and edible covers for the preservation and increase of durability of the food products and the struggle to replace the biodegradable materials in keeping food materials have been considered (Bonilla et al., 2012).

Medicinal plants play an excellent role not only as a traditional medicine but also in several sciences such as pharmaceuticals for the development of novel drugs and nutraceuticals (Xue et al., 2015; Jamshidi-Kia et al., 2018). The genus Thyme (*Thymus vulgaris* L), belongs to the Lamiaceae family and comprises more than 400 species plants with the medicinal and nonmedical uses, worldwide (Ozudogru et al., 2011). It has been used for many centuries in traditional medicine due to their amazing biological activities such as antiseptic, carminative, antiviral, antioxidant (Stahl-Biskup, 2002), anti-inflammatory, hepatoprotective, antimicrobial, anti-HIV-1, antiulcer, gastroprotective, hypoglycemic and antihyperlipidemic activities as well as particular cytotoxicity against a variety of tumour cell lines (Martins et al., 2015; Leal et al., 2017). This aromatic plant is geographically native to hot regions of Pakistan, Afghanistan, and south and southeast of Iran (Cristina et al., 2010). Due to the presence of secondary metabolites in the thymus plant, and their several biological activities according to previous

studies, it is likely that the extract of this plant may have a protective effect on the survival of some quickly biodegradable foods. Therefore, the main objective of the present research was to assay the inhibitory effect of edible coating with combined *Thymus vulgaris* extract and glycerol monostearate on extending shelf life and of oyster mushroom.

2. Materials and methods

2.1. Materials

Mushrooms

The white *Pleurotus* mushrooms were harvested in the first flush, and they were white in colour, with a cap diameter of 3–4 cm. They were transported by refrigerator vehicle at 2–4 °C during one hour to the laboratory.

Chemicals

All solvents and chemicals such as glycerol monostearate (GSM), polysorbate 80, sodium sulfate, deionized water, peptone water, plate dextrose agar and plate count agar were analytical grade and purchased from Merck Company (Merck, Germany).

2.2. Sample preparation

The preparation of aqueous thyme extract was performed according to the Samadloooy et al., (2007), by the Clevenger technique. Then, the extract was dried with sodium sulfate and kept at 4° C (Samadloooy et al., 2007).

2.3. Preparation of mushroom treatments

The mushroom treatments were prepared properly with different concentrations of the GSM and TE, according to Ayobi et al., (2013) method (Table 1).

2.4. Gas chromatography-mass spectroscopy (GC-MS) analysis of the thyme extract

The thyme extract (TE) was subjected to the 7890B (Agilent Technologies, USA) Gas chromatography-Mass spectroscopy (GC-MS). Electron ionization (EI) mass spectra (scan range, m/z 50-500) was obtained using electronic with an energy of 70 eV and filament emission of 0.5 Ma. The GC separations were conducted using an HP-5MS UI column (60 m × 0.025 mm i.d., film thickness 0.5 µm). Helium was used as



Table 1. Preparation of mushroom samples with different concentrations of thyme extract (TE), and glycerol monostearate (GMS).

Treatment	*TE (mg/kg)	**GMS (%)	***PS 80 (%)
1	-	0.5	0.5
2	100	0.5	0.5
3	150	0.5	0.5

*TE: thymus extract; **GMS: glycerol monoestearate; *** PS 80: polysorbate 80.

the carrier gas (flow: 0.8 ml min⁻¹). The GC oven was temperature programmed at 5 °C min⁻¹ from 60 °C after 3 min since the sample injection and held at 250 °C for 4 min. The injection port of the GC, transfer line, and ion source of 5977 MSD were respectively maintained at 240 °C, 250 °C, and 220 °C, respectively. The separated compounds were identified of standards and technology (NIST MS database, 2014) library. The relative per cent amount of each component was measured by comparing its average peak area to the total areas (Adams, 2007).

2.5. The texture tightness

A CNS Farnell texture analyzer (CNS Farnell, USA), was used to measure the texture tightness of the mushroom samples with different covering conditions. The permeability test was used to measure the sample texture. The diameter of the probe and permeability depth in the mushroom cap were respectively, 3 and 5 mm. Moreover, the permeability speed was 10 mm s⁻¹. The extreme required force (N), to create the hole in depth of 5 mm was recorded as the tightness (Sedaghat and Zahedi, 2012).

2.6. Colour measurement

The HunterLab device (HunterLab Scan XE, Reston, VA) was used to evaluate the colour of samples. For this purpose, three parameters of “a*”, “b*” and “L*” were measured in defining times according to Jiang et al., (2010).

2.7. Weight loss measurement

The sample weights were measured according to Poverenov et al., (2018), in days 0, 3, 6, 9, 12, and 15, using an analytical Sartorius digital balance (Sartorius, UK). The samples were kept at 4 °C. The weight loss is reported as:

$$\text{weight loss (\%)} = \frac{W_a - W_b}{W_a} \times 100$$

Where, Wa: the initial weight (g); Wb: the final weight (g).

2.8. The microbial assay

A volume of 225 ml of sterile peptone water was added to 25 g of the mushroom sample. Total bacteria counts were determined by surface inoculation of plate count agar (PCA) (Merck, Germany), as well as the yeast and mould counts by surface inoculation of potato dextrose agar (PDA) (Merck, Germany). Then, the PCA plates were incubated at 32 °C for 48 h and PDA plates at 28 °C for a week. All samples at days 0, 3, 6, 9, 12 and 15 were separately prepared. (Poverenov et al., 2018).

2.9. Sensory evaluation

The hedonic test was carried out with trained students, including the 6 females and 4 males at the laboratory of Food Science and Technology at Yasooj University, Iran. The examination was performed in triplicate on several days. Five samples were served in a randomized order at each session, and panellists were given a break and neutralized with water between each session (Geier et al., 2016). They evaluated the appearance, texture and colour of different concentrations of GMS, using a nine-point scale, where, “1” indicate to very weak, “5” indicate to moderate, and “9” indicate to very well (Phat et al., 2018).

2.10. Statistical analysis

Measurements in all the experimental analyses were expressed as Mean ± SD (n = 3). The statistical software package IBM SPSS V.21 (SPSS Inc., Chicago, IL, USA) was used for the analysis. The results of



the study were confirmed for statistical significance with Duncan's multiple range tests. Differences were considered statistically significant at the $p < 0.05$.

3. Results and discussions

3.1. GC-MS analysis of the thyme extract

Analysis of the extract using the GC-MS has detected the 23 chemical compounds with the noteworthy structures and diverse functional groups (C1-C23) (Table 2). The results illustrate that carvacrol (43.1 %), paracymin (19%) and thymol (14.8 %), were respectively the most abundant compounds occurred in the TE.

3.2. Texture tightness

The computer vision results of mushrooms coated at different concentrations are shown in Figure 1. The results illustrate that the texture tightness for all samples was decreased significantly during 15 days storage ($p < 0.05$). The results indicated that with adding the GMS the weight loss happened from 36.1 ± 0.42 g to 15.7 ± 0.63 g, during the 15 days; while, adding the 100 mg/kg thymus plant extract to GMS, the percentage of degradation in mushroom texture was decreased from 36.1 ± 0.49 g to 19.6 ± 0.27 g. Adding the 150 mg/kg of the thymus plant extract had the most impact on preserving the texture mushrooms. In the study of Salehi et al., (2019), the sample which coated with GMS exhibited the losing weight from

36.2 ± 0.61 g to 22.1 ± 0.33 g. On the other hand, the control sample was decayed earlier than other samples during the storage time. According to Lin et al., (2019), the weight loss in mushroom samples may be due to the pectolytic and microbial enzyme activities that make the cell wall degradation, and consequently the moisture loss, with the passage of time.

The GMS covers the surface of mushroom cells with appropriate treatment regarding its lipid structure, so that it prevents microorganism growth and moisture loss in addition to delay spoilage and the activity of destructive enzymes effectively (Pantoja-Romero et al., 2016). In a similar study, Li et al., (2011), were used the zinc oxide nanoparticles to cover the apple pieces, and they found, use of anti-microbial compounds in coverage retained the texture, fresh and significantly increased the shelf life of apple samples.

3.3. Huntr Lab colour measurement

The effect of mushroom colour on decision-making has an evident feature in consumer manners, such as how the color impact on price perceptions, consumers' state of mind and purchase intents (Kim et al., 2018). Accordingly, a study of the colour indices of "a*", "b*" and "L*" was assessed entirely by the Huntrlab colour measurement device.

The colour index of "a*" shows Red vs. Green; where a positive number indicates the red and a negative number indicates the green colour. The overview of

Table 2. Chemical compositions (C1-C23) obtained from the GC-MS analysis of the thyme extract.

No.	Compound	Abundance (%)	RI	No.	Compound	Abundance (%)	RI
C1	Alpha-thujene	0.15	927	C 13	Terpin-4-ol	0.2	1186
C2	Alpha-pinene	3.14	932	C 14	Dihydrocarvone	0.3	1199
C3	Camphene	0.2	958	C 15	Carvacrol methyl ether	3.5	1228
C4	Beta-pinene	0.4	972	C 16	Thymol	14.8	1231
C5	Myrcene	2.3	994	C 17	Carvacrol	43.1	1302
C6	Alpha-phellandrene	0.25	999	C 18	Carvacrol acetate	3.1	1373
C7	Delta-3-carene	0.25	1011	C 19	Beta-caryophyllene	2.2	1418
C8	Alpha-terpiene	0.9	1113	C 20	Alpha-humulene	0.2	1449
C9	P-cymene	19	1119	C 21	Allo-aromadendrene	0.2	1460
C10	1,8-cineol	0.2	1031	C 22	Bicyclogermacrene	0.2	1500
C11	Gama-terpienne	3.1	1050	C 23	Caryophyllene oxide	0.3	1569
C12	Linalool	0.5	1082				

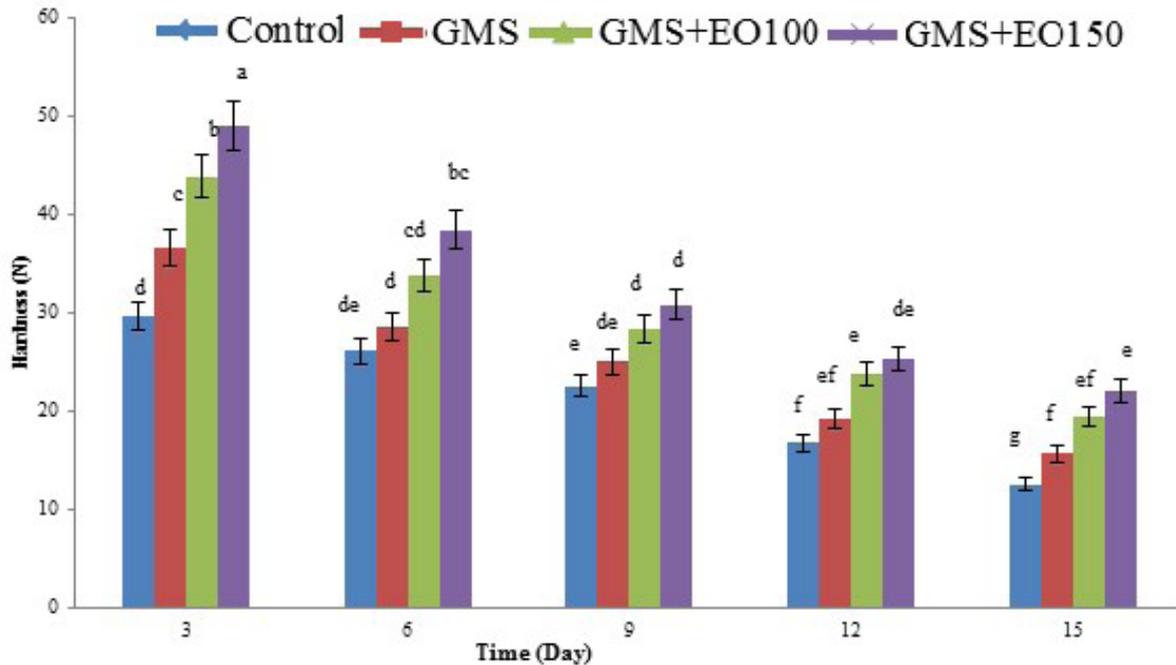


Figure 1. Effect of coating with glycerol monostearate (GMS) and thyme extract (TE) on the texture tightness of the mushroom samples * Non-identical letters in columns indicate the significant difference with the control group ($p < 0.05$).

“a*” colour index pointed out, there was increased during storage (Figure 2a), due to the physiological and microbial procedures (Cullere et al., 2018).

The use of GMS caused a slight change in the amount of “a*” colour index in comparison with the control groups. It was significantly increased from 2.71 ± 0.03 to 4.89 ± 0.08 , during the 15 days ($p < 0.05$). Correspondingly, there was a slight increasing amount of “a*” colour index by adding 100 mg/kg of the TE to the GMS from 2.80 ± 0.01 to 4.24 ± 0.04 . That led to an increase in durability and stability of the “a*” colour index in mushroom samples as the minimum changes of “a*” colour index was correlated to the mushroom samples, which were coated by GMS. A concentration

of 150 mg/kg, the thymus plant extract led to an insignificant increase from 2.82 ± 0.01 to 4.15 ± 0.03 , during the 15-days storage time. Noticeably, the control group had potentially accelerated changes in the “a*” colour index during the maintenance period.

The “b*” colour index shows yellow vs. blue colour, where a positive number indicates yellow and a negative number indicates the blue colours.

As Figure 2b illustrates, the “b*” colour index has approximately similar results to index “a*”, with increase durability and stability of colour of the mushroom samples during the 15-day storage time.

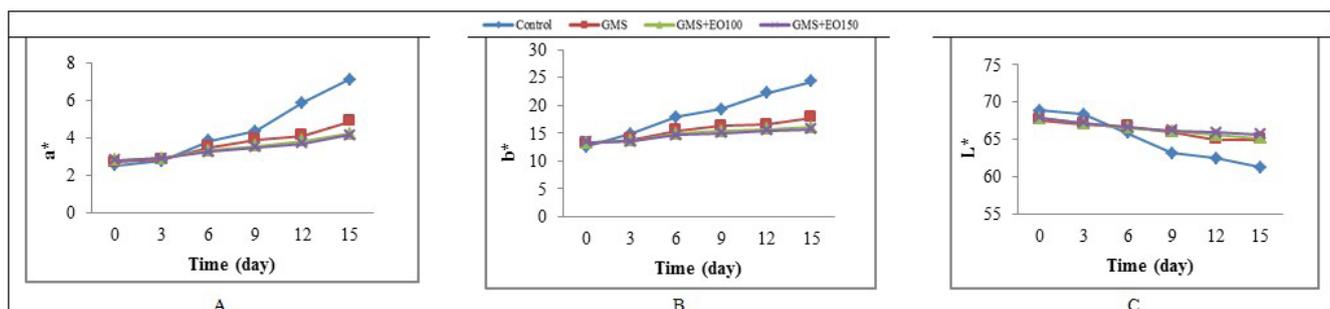


Figure 2. Effect of coating with glycerol monostearate (GMS) and thyme extract (TE) on “a*” (A), “b*” (B), and “L*” (C) indices of the mushroom samples.



The GMS decreased significantly the “b*” colour index ($p < 0.05$). Adding concentrations of 100 and 150 mg/kg of thymus plant extract to the glycerol monostearate (GMS+TE100 and GMS+TE150 mg/kg), led to an insignificant growing up in the “b*” colour index.

The “L*” colour index shows light vs. dark colour where a low number (0-50), indicates dark, and a high number (51-100), indicates brightness. The colour vividness of mushroom samples has a vital role to be purchased with an inclination of consumers (Sapers et al., 1994). Evidently, in the (Figure 2C), the amounts of “L*” colour index were decreased significantly in treated and non-treated samples during the 15 days ($p < 0.05$). Furthermore, in the control groups (non-treated), the fewest changes in the brightness and transparency of mushroom samples are related to adding the 150 mg/kg of the thyme extract to GMS. The TE could constantly maintain the “L*” colour index and had been effective in preserving brightness and appearance of mushroom samples.

The TE could strengthen the positive function of the GMS, because of the phenolic compounds content of the plant, which has too many anti-microbial (El-Din et al., 2009), and anti-oxidant activities (Ziani et al., 2019). According to Taghizadeh et al., (2009), appraising the mushroom’s shelf life through the imaging technique, it was shown the negative influences on all colour parameters during the storage period.

3.4. Changes in weight loss

The results of the firmness of coated and uncoated mushrooms demonstrated a clear favourable effect of GMS coating. Figure 3, represents the texture patterns of the coated and non-coated mushrooms during the 15 days of storage. The weight loss value increased over the total storage time in all samples. On day 12th, the GMS-coated mushrooms had a firmer texture than the uncoated ones. It is well proven that edible coatings physically developed significantly the

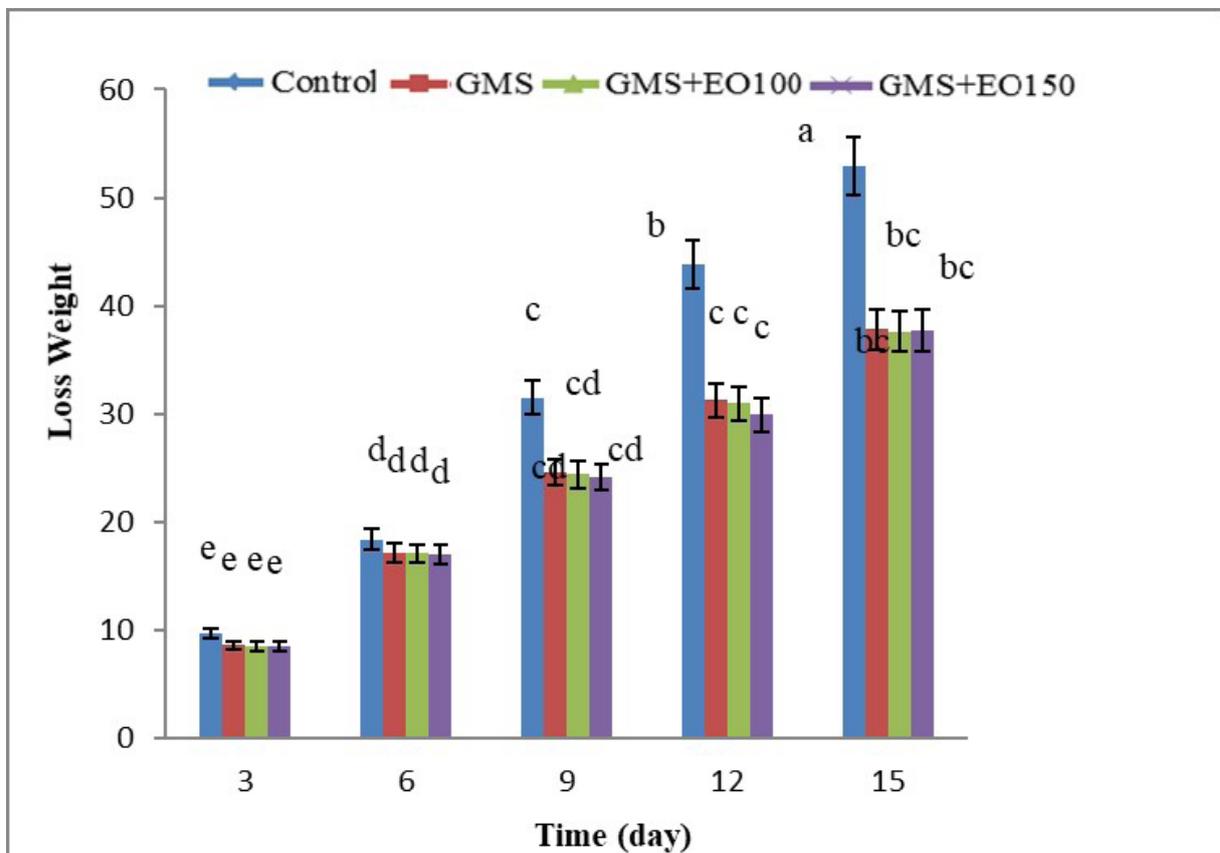


Figure 3. Effect of coating with glycerol monostearate (GMS) and thyme extract (TE), on weight loss of mushroom samples. * Non-identical letters in columns indicate the significant difference with the control group ($p < 0.05$).



structure of mushrooms and the texture's corruption was slower than the control sample (Poverenov et al., 2018). On the other hand, no effect of adding the TE to the GMS was detected on a delay of weight loss during the storage period. There is no significant difference in weight loss between GMS-coated mushroom and without thyme extract samples.

The most important cause of the inhibitory weight loss effect by GMS during the storage period maybe attributed to its compound lipid structure that leads to obstruction of mushroom surface holes, reduction of oxygen penetration on the surface and prevention of cell breathing (Talele et al., 2018). This decrease in weight loss is accordingly important, because of its effects on physiological, microbial activities, texture tightness and colour of mushroom samples.

Xu et al., (2015) show that the inevitable biological process after harvesting is breathing that accelerates the mushroom's shrinkage. During the storage period, the carbohydrate content in the product gradually

decreases and causes deleterious changes in the product (Christensen et al., 2019). In keeping with the Jiang et al., (2011), the exposure of oxygen to the surface of the mushroom cells is one of the main reasons for weight losing. The packaging process is an effective factor in avoiding oxygen penetration and delay the mechanism of breathing (Perdones et al., 2012).

3.5. The sensory evaluation

The colour and form of edible mushrooms are very important for consumers. Therefore, this study appraised the sensory evaluation based on the colour and appearance. The results are shown in Figure 4. The quality of edible mushroom was decreased significantly in all of the test and control groups during 15 days of storage period ($p < 0.05$). Presence of GMS has been significantly reduced the appearance and colour of mushroom samples, during the storage period ($p < 0.05$), as well as the declining effectiveness of the TE on colour specification. The control and

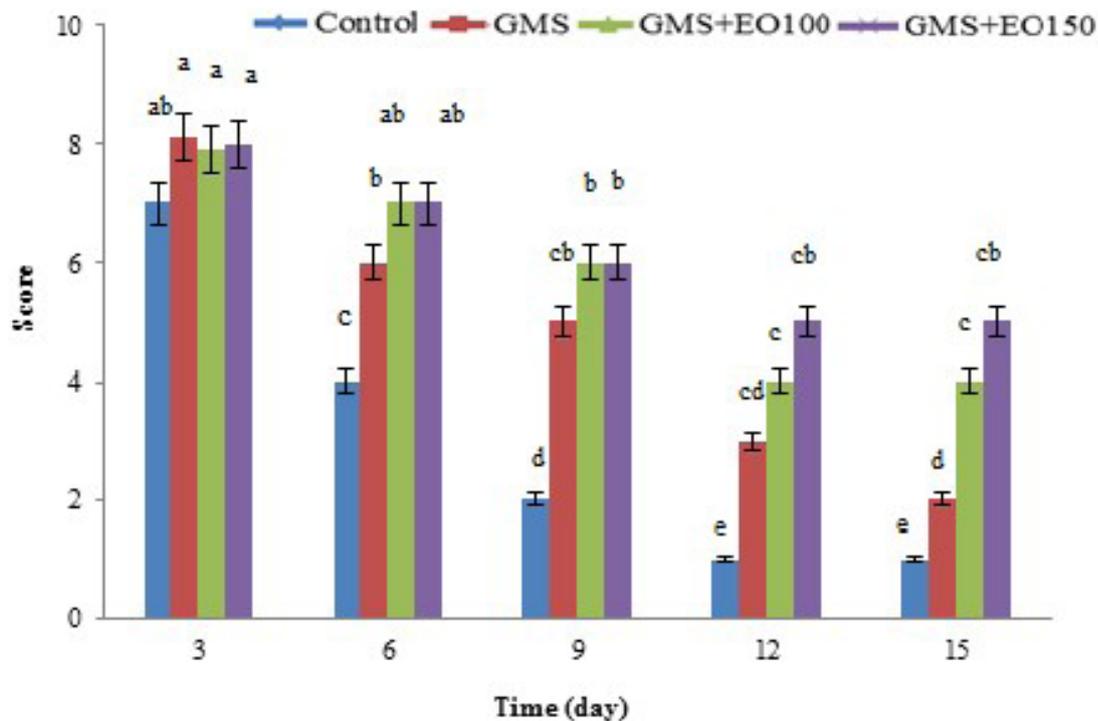


Figure 4. Sensory evaluations of the colour and appearance of mushroom samples covered with glycerol monostearate (GMS) and different concentrations (TE100, and TE150 mg/kg) of the thyme extract during storage time. * Non-identical letters in columns indicate the significant difference with the control group ($p < 0.05$).



the GMS+TE150 mg/kg groups had respectively, the minimum and maximum scores for the appearance and colour factors. Overall, the colour stability in the samples in the presence of the extracts can be attributed to the decrease in some factors such as microorganism growth, physiological activities, weight loss and cell breathing.

3.6. Antimicrobial assay

The medicinal thyme extracts are used in numerous fields of the industries and sciences such as food industries (Elgayyar et al., 2001), pharmaceuticals, and traditional and modern medicine (Raskin et al., 2002). They are expected as novel resources of the antimicrobial agents (Bankole et al., 2007). Traditionally, the extracts and essential oils of the TE are used as medicinal plants in several areas of the world, including Iran for many purposes, particularly for microbial disorders. The thymus essential oil has an excellent effect against *E. coli* O157: H7 (Maksimović et al., 2008).

Because the overall growth of microorganisms is the main cause of deterioration in sensitive products such as edible mushrooms; hence, in the current study, this parameter was measured during the 15 days in different conditions of coverage. Concerning the data shown in Figure 5A, the overall growth of microorganisms was significantly occurred with

increasing the storage period ($p < 0.05$). The results show that the use of GMS can significantly prevent the growth of microorganisms in comparison with the control group; especially, from the sixth day ($p < 0.05$). Also, adding the thyme extract to GMS as a preservative compound could play an inhibitory role in the growth of microorganisms. In a dose-dependent manner, it could significantly prevent the growth of microorganisms ($p < 0.05$).

In the current study, the effect of covering in different conditions using GMS and extract on the growth of mould and yeast was also evaluated. The results are shown in Figure 5B. The results indicate similar to studying the overall growth of microorganisms, by increasing the storage time, the amount of mould and yeast is increased significantly from the first day to the fifteenth day ($p < 0.05$). The most increase was related to the control group and the least increase of the mould and yeast amount was related to the sample that was covered by GMS+TE150 mg/kg. The results of this research indicated that use of the GMS to cover the edible mushroom could have a significant influence on decrease the amount of mould and yeast in mushroom ($p < 0.05$). On the other hand, use of the TE could intensify the effect of preventing mould and fungi growth of GMS compound. With increasing concentrations of GMS compound to 150 mg/kg, this influence was increased. It is proven that by covering the surface pores with GMS and therefore decrease the

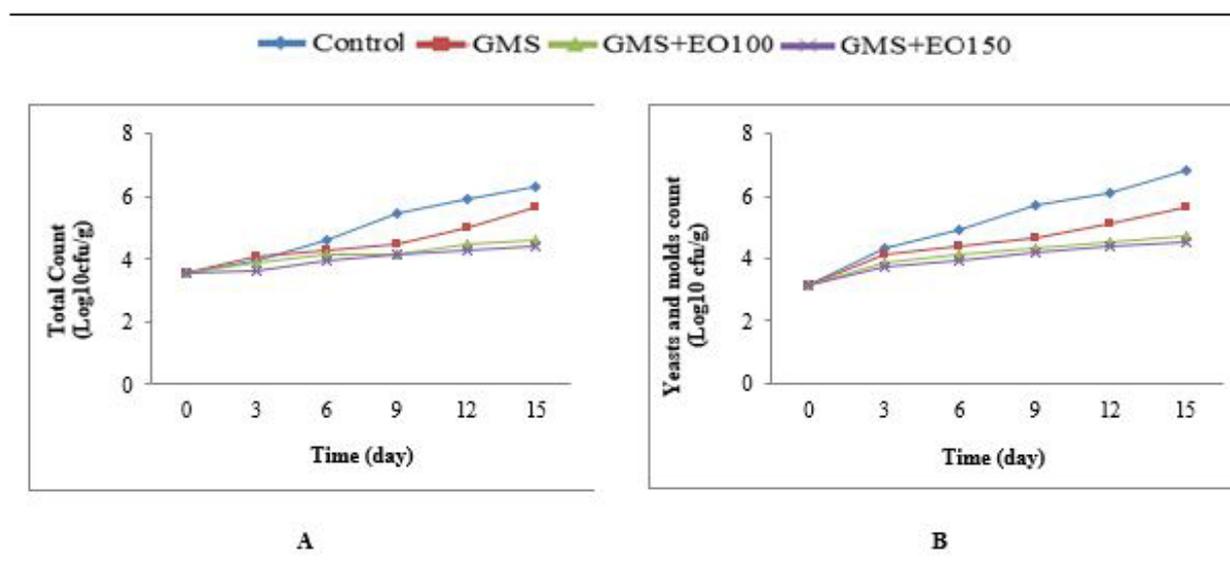


Figure 5. Effect of coating of mushroom with glycerol monostearate and thyme extract on total count (log10cfu/g) (A), and overall growth of moulds and yeast (B), during the storage period.



oxygen and available moisture prevents the excessive growth and reproduction of microorganisms. It was more effective than the control sample, which had no coating. On the other hand, this effect was dose-dependently increased with increasing concentrations of thymus extract.

In a comparable study, Jiang et al., (2011), showed that covering the mushrooms by chitosan can have a significant influence on decreasing the microorganism growth. These results were consistent with current studies. Similarly, in the study of Jiang et al., (2013), the coating of mushrooms with alginate and silver nanoparticles, had also significantly decreased the growth of microorganisms during the storage period.

4. Conclusion

In conclusion, the experiment conducted here indicated that the application of lipid-edible coating with different doses of glycerol monostearate (GMS) and thyme extract (TE) maintains the post-harvest mushroom's quality. The high texture tightness and the low weight loss determined for the mixture of GMS and 150 mg/kg TE, and the colour indices ($a^*b^*L^*$) have less significant change by adding the GMS with doses of 100 and 150 mg/kg of TE. Furthermore, the high antimicrobial activities resolute for GMS+TE150 mg/kg. In conclusion, the GMS+EO150 mg/kg coating could be applied to preserving the quality of oyster mushrooms throughout long-term storage.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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