

Effect of Mixing Rotational Speed on Characteristics of Edible Film Containing Tangerine (*Citrus reticulata*) Essential Oil to Extend the Shelf-Life of Mushroom (*Agaricus bisporus*)

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Abstract: Mushrooms are products which lose their unique organoleptic properties after harvesting and, it has a short shelf life. The effect of edible coating of mushroom on shelf life was evaluated by using carrageenan (1.5%) and different concentrations of tangerine (*Citrus reticulata*) essential oil (0.2, 0.4 and 0.6%). Edible coating solutions were prepared using different rotational speed of mixing (500, 1000 and 1500 rpm). Physicochemical properties of tangerine essential oil and rheological characteristics of edible coating solutions were measured. The results indicated that all edible coating solutions exhibited non-Newtonian pseudoplastic behavior. The highest value of water vapor permeability was for carrageenan (1.5%) incorporated with tangerine essential oil (0.2%) at 500 rpm sample. Essential oil and mixing speed affected the edible film morphology. Edible coating of mushroom affected the weight loss, firmness, moisture content and microbiological properties. The obtained results indicated the possibility of prolonging the period of preserving mushrooms by using the coating film prepared from carrageenan mixed with tangerine essential oil.

Keywords: Edible film, mushroom, tangerine essential oil, antioxidant, antimicrobial

INTRODUCTION

Citrus fruits of the Rutaceae family are extremely wealthy in essential oils. Citrus fruits production is the highest in the world and including species having a place with the "Citrus" genus as lemon (*Citrus limon* L. Burm. F), orange (*Citrus sinensis* L. Osbeck), tangerine (*Citrus reticulata* Blanco) and grapefruit (*Citrus paradise* Macfie). The essential oils of citrus fruit peels comprised a mixture of many components, e.g. terpenes, and oxygenated compounds. The major component of citrus essential oils is terpenes that reach approximately 47.5% of the total essential oil. The D-limonene represented a large portion and it has the specific aromatic smell of citrus (Fisher and Phillips, 2006; Bozkurt *et al.*, 2017).

Citrus essential oil (which produced from the peel as a by-product) is an economic and natural alternative as antioxidants. The peels of citrus fruits which are largely discarded as wastes cause environmental problems. The extracted citrus oils from discarded peels can be used as natural food preservatives. Other applications of essential oils are used in microencapsulation incorporating biodegradable polymers, nano-emulsion coatings, spray applications, and antibacterial action mechanisms of the active compounds present in the essential oils (Mahato *et al.*, 2019).

The volatile and non-volatile components of citrus essential oil were 85 to 99% and 1 to 15%, respectively (Fisher and Phillips, 2008). The active compounds of the essential oils are highly volatile and affected by oxygen, heat and light (Muriel-Galet *et al.*, 2015). The volatile components are monoterpene (such as limonene) and sesquiterpene hydrocarbons and their oxygenated derivatives, including aldehydes, ketones, acids, alcohols, and esters (Mahato *et al.*, 2019).

Coatings are considered as part of the final product by dipping or spraying the surface of food products with a thin layer of coating material. It used to protect food products (Cagri *et al.*, 2004; Cha and Chinnan, 2004). The edible films can be modified vegetable tissue metabolism where affecting respiration; they can be used as a carrier of antimicrobials, antioxidants, vitamins, minerals as well as other preservatives and they can be enriched product formulation. One of the most important roles that the ability of edible coating films to control the transport of moisture, oxygen, aroma, oil, and flavor compounds in food systems, depending on the nature of the edible film-forming materials. The properties of physical and mechanical edible films are of very important due to their influence on product behavior and consumer acceptance (Wiset *et al.*, 2014).

Carrageenan (polysaccharide compounds) can be used for making edible films. It can be obtained from seaweed which is one source of cheap raw materials for making edible films (Saiful *et al.*, 2013).

Saiful *et al.* (2013) prepared edible film from different concentrations of carrageenan (1, 1.25, 1.5 and 1.75%) and different concentrations of palm oil as plasticizer (10, 20 and 30%). They found that the optimized edible film structure was obtained when using carrageenan with concentrations of 1.25 and 1.5%.

Coating of *Manilkara zapota* fruits with 1.5% carrageenan could be prolonged *Manilkara zapota* shelf-life until 15 days. The use of carrageenan coating was one promising method to improve the stability of *Manilkara zapota* fruits during storage (Nguyen *et al.*, 2018).

Antimicrobial agents can be incorporated in the edible coating to prevent, or even inhibit the spoilage microorganisms and decrease the risk of pathogenic

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microorganisms on the surface of food, being considered an active packaging. The spoilage of mushroom mechanisms included moisture loss, enzymatic browning and bacterial growth. The rapid respiration rate of mushroom may be shorten its shelf-life compared to most ready-to-use vegetables as it has no barrier to protect it from water-loss or from microbial attack (Appendini and Hotchkiss, 2002).

The aim of this work was to study the effect of rotational speed (rpm) of laboratory mixer on the properties of edible coating film made from carrageenan and tangerine (*Citrus reticulata*) essential oil as an antibacterial coating to extend shelf-life of mushroom.

MATERIALS AND METHODS

Materials

Mushrooms (*Agaricus bisporus*) samples were purchased from a commercial market in Cairo, Egypt, and transported to the laboratory in one hour and selected for uniformity and size. Any bruised or diseased mushroom was removed. Carrageenan and glycerol was purchased from Achmetec Company (India).

Methods

Extraction of tangerine (*Citrus reticulata*) essential oil

The essential oil of tangerine (*Citrus reticulata*) was extracted by cold press according to Arnould-Taylor *et al.* (1981).

Physicochemical properties of tangerine (*Citrus reticulata*) essential oil

Physicochemical properties of tangerine essential oil including specific gravity (20°C), refractive index (at 20°C), solubility in alcohol (90%), acid value and aldehyde content as decanal were determined according to the methods described by Guenther (1948).

DPPH radical scavenging activity

The antioxidant activity of tangerine essential oil was determined using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Where, the odd electron in the DPPH free radical had a strong absorption maximum at 517 nm. The color turns from purple to yellow as the molar absorptive of the DPPH radical reduced when the odd electron becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H as mentioned by Choi (2010). The DPPH assay was measured according to the method reported by Brand-Williams *et al.* (1995). The tangerine essential oil was added by 25, 50, 100, 150, 200 and 250 µl, compared with ascorbic acid and butylated hydroxyl toluene (BHT), as references (control antioxidants). The radical-scavenging activity was expressed as the percentage quenching of the DPPH radical, and calculated as follows:-

$$\text{Inhibition of DPPH (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

Where, A is absorbance

Chemical composition of tangerine (*Citrus reticulata*) essential oil

The essential oil of tangerine was analyzed for their components using gas chromatography-mass spectrometry analysis (GC-MS) according to the method outlined by Fan *et al.* (2018).

Preparation of edible film

Laboratory mixer (Heidoiph RZR 2020, Germany) was used for mixing the edible film solutions containing tangerine essential oils using different rotational speeds. Coating solutions were prepared by dissolving 1.5% carrageenan in distilled water with the addition of 0.2, 0.4 and 0.6% tangerine essential oils, and 1% glycerol as a plasticizer and three different rotational speeds (500, 1000 and 1500 rpm) were used as shown in Table (1).

Table (1): Experimental samples

Sample code	Rotational speed, rpm	Treatments
C	----	Control (Without edible coating film)
T1	500	1.5% Carrageenan + 0.2% tangerine essential oil
T2	1000	1.5% Carrageenan + 0.2% tangerine essential oil
T3	1500	1.5% Carrageenan + 0.2% tangerine essential oil
T4	500	1.5% Carrageenan + 0.4% tangerine essential oil
T5	1000	1.5% Carrageenan + 0.4% tangerine essential oil
T6	1500	1.5% Carrageenan + 0.4% tangerine essential oil
T7	500	1.5% Carrageenan + 0.6% tangerine essential oil
T8	1000	1.5% Carrageenan + 0.6% tangerine essential oil
T9	1500	1.5% Carrageenan + 0.6% tangerine essential oil

Rheological properties

The rheological properties of the prepared blends were studied to investigate the flow behavior of blends which is an important factor for food coating materials. Rheological parameters (shear stress and shear rate) of carrageenan essential oil blends were measured using Brookfield Engineering labs DV-III Rheometer (USA),

SC₄₋₂₁ spindle was selected for the measurement according to Brookfield Manual (1998).

Thickness of different edible films

The thickness of the films was determined using a digital micrometer (MITUTOYO, Model MDC- 25S, resolution 0.001 mm, USA)

Determination of water vapor permeability (WVP)

The water vapor transmission rate (WVTR) [$\text{g}/(\text{s}\cdot\text{m}^2)$] and water vapor permeability (WVP) through films was determined gravimetrically using the ASTM Method (1996). A circular test cup was used to determine the WVP of the film. The film was first cut into circular shape that was larger than the inner diameter of the cup, the cup was filled with 50% distilled water and the film was sealed at the top using Paraffin oil, then the cups were placed in a desiccators containing calcium chloride with relative humidity RH (0%) and RH for water (100%). The weights of the cups were recorded every hour during 10 hours and two specimens of each film were tested. Linear regression was used to estimate the slope of this line in g/h. The water vapor transmission rate (WVTR) and water vapor permeability (WVP) were determined using the following equations:

$$WVTR = \frac{\Delta m}{\Delta t A}$$

$$WVP = WVTR \frac{L}{\Delta RH}$$

Where, $\frac{\Delta m}{\Delta t}$ is the moisture gain weight per time (g/s),

A is the surface area of the film m^2 , L is the film thickness (mm) and ΔRH is the difference in relative humidity.

Microstructure analysis

The microstructure of the film was done using scanning electron microscopy (SEM) model (JEOL JSM 5200, Tokyo, JAPAN) according to Shamseldean and Platzer (1989).

Coating application

Sample preparation and coating treatment

Mushrooms were selected, based on the pileus size of 30 to 40 mm, no mechanical damage and fungal infection, and refrigerated in darkness at 4°C. Mushrooms were dipped in the coating solution for 30 sec and the samples were air-dried for 15 min at room temperature. The coated mushrooms were wrapped in polyethylene stretch film and kept at 4°C for a period of 21 days.

Determination of weight loss for mushroom during storage

Weight-loss for each package was determined according to Henriques *et al.* (2013)

$$\text{Weight loss (\%)} = \frac{W_o - W_f}{W_o} \times 100$$

Where, W_o is the original weight on the first day and W_f is the final weight on the end of storage period.

Mechanical properties (firmness)

The firmness of coated mushroom samples was conducted by Brookfield Texture Analyzer CT3 using force (N) 10-50 KN (USA) according to AACC (2010).

Moisture contents of different samples of mushrooms

Moisture contents of the fresh and coated mushrooms were determined according to the methods of AOAC (2005).

Microbiological analysis

Assessment of antimicrobial activity of tangerine essential oil

Antimicrobial activity of tangerine essential oil was determined by the disc diffusion method according to Rios and Recio (2005).

Total bacterial count and yeast and mold

Fresh and coated mushrooms were analyzed for total aerobic plate counts and yeast and molds according to procedures of FDA/BAM (2001).

Statistical analysis

Means of the results are statistically analyzed using one-way analysis of variance (ANOVA). Where significant differences were observed at 5% standard deviations (SD). Statistical software (Assistat Version 7.7, Brazil) was used for all statistical analyses according to Silva and Azevedo (2009).

RESULTS AND DISCUSSIONS

Physicochemical properties of tangerine essential oil

Physicochemical properties of tangerine essential oil are shown in Table (2). The results showed that specific gravity, refractive index, acid value and aldehyde content as decanal were 0.853, 1.4764, 0.89 (mg KOH/g oil) and 2.13 (%), respectively. Also, it could be noticed that the essential oil of tangerine was soluble in alcohol (90%). Javed *et al.* (2014) reported that the specific gravity and refractive index of tangerine essential oil were 0.86 and 1.465, respectively. However, Bhuyan *et al.* (2015) found that the acid value and the aldehyde content of tangerine essential oil were 0.59 and 2.1%, respectively. The slight differences in the results of the refractive index and the acid number of the tangerine essential oil in this study and the previous studies may be due to several factors such as geographical location, season and environmental factors (Bourgou *et al.*, 2012).

Table (2): Physicochemical properties of tangerine essential oil

Properties	Values
Specific gravity	0.853 ± 0.09
Refractive index	1.4764 ± 0.10
Solubility in alcohol (90%)	Soluble
Acid value (mg KOH/g oil)	0.89 ± 0.05
Aldehyde content as decanal (%)	2.13 ± 0.15

Each value is mean ± SD

Antioxidant activity of tangerine essential oil

The antioxidant has an important role in reducing the activity of free radicals, by being able to give electron or hydrogen, and then a large percentage of the DPPH radicals will be converted to stable molecules. The antioxidant efficiency is measured by its ability to inhibit the high percentage of free radicals. Therefore, the high values of the DPPH radical scavenging activity indicated the high strong of the material as an antioxidant (Lu and Foo, 2001).

Antioxidant activity values of tangerine essential oil, ascorbic acid and BHT are shown in Table (3). The results indicated that tangerine essential oil has low antioxidant activity when compared with ascorbic acid

and BHT for all concentrations studied. The antioxidant activity increased by increasing the concentration of tangerine essential oil. The highest antioxidant activity of tangerine essential oil was 89% at 250 µl/ml. This activity represents approximately 90% from antioxidant activity of ascorbic acid at 250 ppm and 94% from antioxidant activity of BHT at 200 ppm. These results are in accordance with work of Javed *et al.* (2014) who reported that tangerine have strong scavenging activity (91.1%). Correspondingly, Yang *et al.* (2010) found that citrus peel oil contained limonene which is a major component and having antioxidant activity similar to that of strong antioxidant.

Table (3): DPPH radical scavenging activity (%) of tangerine essential oil, ascorbic acid and BHT

Sample	Concentration					
	25	50	100	150	200	250
	Radical scavenging activity (%)					
Tangerine essential oils (µl/ml)	35.2 ^{cE} ± 0.23	51.7 ^{bd} ± 0.75	68.0 ^{bcD} ± 0.16	71.0 ^{bc} ± 0.23	82.0 ^{bb} ± 0.87	89.0 ^{ba} ± 0.95
Ascorbic acid (ppm)	50.2 ^{aF} ± 0.15	63.0 ^{aE} ± 0.33	77.5 ^{aD} ± 0.67	84.1 ^{aC} ± 0.25	95.2 ^{aB} ± 0.11	98.9 ^{aA} ± 0.12
BHT (ppm)	47.0 ^{bE} ± 0.13	61.1 ^{abD} ± 0.27	74.2 ^{abC} ± 0.83	85.5 ^{aB} ± 0.75	94.3 ^{aA} ± 0.19	ND

-The mean values with different small letter indicate significant differences between columns ($P \geq 0.05$)

-The mean values with different capital letter indicate significant differences within rows ($P \geq 0.05$)

- ND: not determined

Chemical profile of tangerine essential oil

The principal components of tangerine essential oil are presented in Table (4). Nine components were identified representing 100% of the total oil. The results indicated that D-limonene was the main component (80.55%). The other ingredients were in the following order heptadecanoic acid, 10-methyl-, methyl ester (4.95%), vinyl-2-methoxy-phenol (3.16%), methoxy-phenol (2.90%), cytidine (2.78%), myrcene (1.95%) and vinyl (1.86%).

Table (4): Essential oil profile of tangerine

Components	RA (%)
Myrcene	1.95
Limonene	0.54
Vinyl	1.86
Methoxy-phenol	2.90
Beta.-Myrcene	1.31
D-limonene	80.55
Vinyl-2-Methoxy-Phenol	3.16
Cytidine	2.78
Heptadecanoic acid, 10-methyl-, methyl ester	4.95
Total	100

RA= peak area relative to the total peak area

Previous studies revealed that there is a great variation between chemical components of citrus peel

essential oils and this may be due to several factors such as geographical location, season and environmental factors, in addition to the part of the plant used and the method of extraction (Bourgou *et al.*, 2012). Boudries *et al.* (2017) found that *Citrus reticulata* essential oil containing 12 components, representing more than 99%, and limonene was the main component (77.81%). While, Boughendjioua and Boughendjioua (2017) reported that *Citrus reticulata* essential oil contained 24 compounds, representing (95.41%) of the essential oil and Limonene (67.04%), γ -terpinene (15.50%) and α -pinene (2.75%) were the major components.

Rheological properties of edible coating blends

Three different rotational speed of mixing were used for the preparation of edible coating blends using carrageenan incorporated with tangerine essential oil. The flow behavior curves (shear rate versus shear stress) of carrageenan (1.5%) incorporated with different concentrations of tangerine essential oil (0.2, 0.4 and 0.6%) was plotted as shown in Figure (1). The results showed that all edible coating solutions exhibited non-Newtonian pseudoplastic behavior. Constitutive equations are also important for providing the material parameters required by process control, samples studied obey the following power law relationship:

$$\tau = k\gamma^n$$

Where, τ is the shear stress (Pa), k is the consistency index, γ is the shear rate, (1/sec), and n is the flow behavior index.

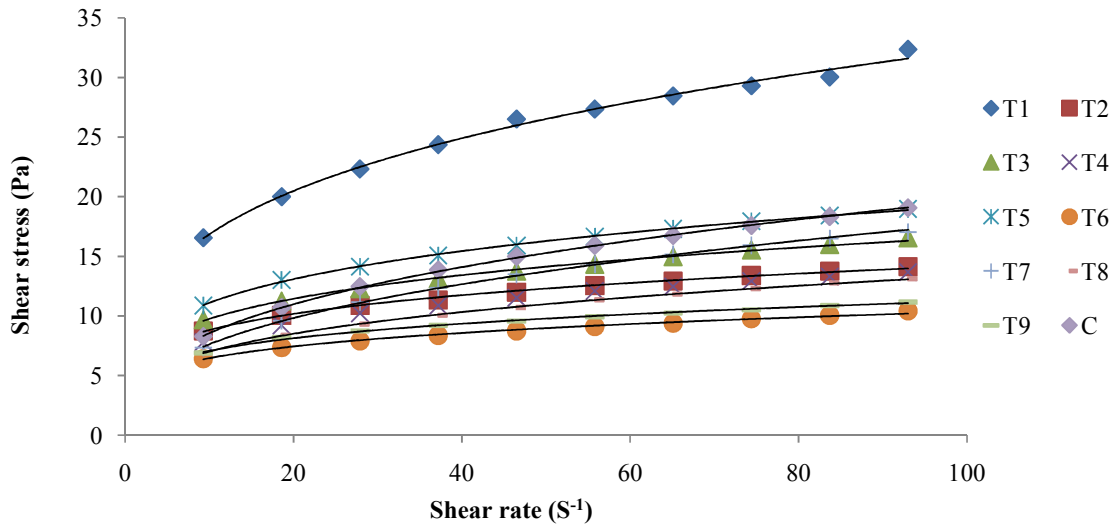


Figure (1): Shear rate – shear stress curves of different edible coating solutions

C: Control (Without edible coating film) - T1, T2 and T3: 1.5% Carrageenan + 0.2% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T4, T5 and T6: 1.5% Carrageenan + 0.4% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T7, T8 and T9: 1.5% Carrageenan + 0.6% tangerine essential oil at 500, 1000 and 1500 rpm, respectively

The thickness of edible film

Thickness values of the edible film made from carrageenan (1.5%) and tangerine essential oil (0.2, 0.4 and 0.6%) are shown in Table (5). The results observed that different mixing rotational speeds and the addition of essential oil affected the thickness of edible films and didn't give a good trend this may be due to intermolecular rearrangement of the matrix components by the incorporation of the essential oil as reported by Pelissari *et al.* (2009).

The thickness of films decreased with increasing concentration of tangerine essential oil. The highest value of thickness (0.193 mm) was observed for sample T1 [1.5% carrageenan + 0.2% tangerine essential oil] and the lowest value (0.1 mm) was that of sample T5 [1.5% Carrageenan + 0.4% tangerine essential oil].

Water vapor permeability of resultant edible films

Water vapor permeability (WVP) is an important property for films which are used as edible food coatings because most natural biopolymers are very sustain to water absorption (Pereda *et al.*, 2012). Incorporation of tangerine essential oil at a concentration of 0.2, 0.4 and 0.6% into carrageenan (1.5%) edible film affected the water vapor permeability as shown in Table (5).

The results indicated that WVP decreased with increasing the concentration of tangerine essential oil and increasing mixing rotational speed of mixing, this may be due to the hydrophobic nature of the films where tangerine essential oil had entered the matrix of the films which in turn prevented the water vapor to penetrate through the films (Siah *et al.*, 2018). The highest value of WVP was observed for sample T1 [1.5% carrageenan + 0.2% tangerine essential oil] and the lowest value of WVP was observed for sample T5 [1.5% carrageenan + 0.4% tangerine essential oil].

Edible film morphology

The scanning of electron microscope of edible film made from carrageenan (1.5%) and tangerine essential oil (0.4%) at different mixing rotational speed (500, 1000 and 1500 rpm) is shown in Figure (2). The results indicated that control sample (a) (1.5% carrageenan) seemed to be flat without pores or cracks. The addition of essential oil to carrageenan changed the microstructure of the edible film and it had more heterogeneous structure as increasing mixing rotational speed from 500 (b) to 1000 (c) rpm, while using 1500 rpm (d) decreased the heterogeneous structure of resultant edible film.

Table (5): Thickness and water vapor permeability (WVP) for carrageenan film incorporated with tangerine essential oil treatments

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9
Thickness (mm)	0.193	0.174	0.192	0.148	0.100	0.154	0.166	0.158	0.156
WVTR (g/s.m²)	9.910	8.160	8.399	8.280	8.041	8.201	8.120	7.603	7.365
WVP (g.mm/m².mmHg.day)	0.020	0.014	0.016	0.012	0.008	0.012	0.013	0.012	0.011

T1, T2 and T3: 1.5% Carrageenan + 0.2% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T4, T5 and T6: 1.5% Carrageenan + 0.4% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T7, T8 and T9: 1.5% Carrageenan + 0.6% tangerine essential oil at 500, 1000 and 1500 rpm, respectively

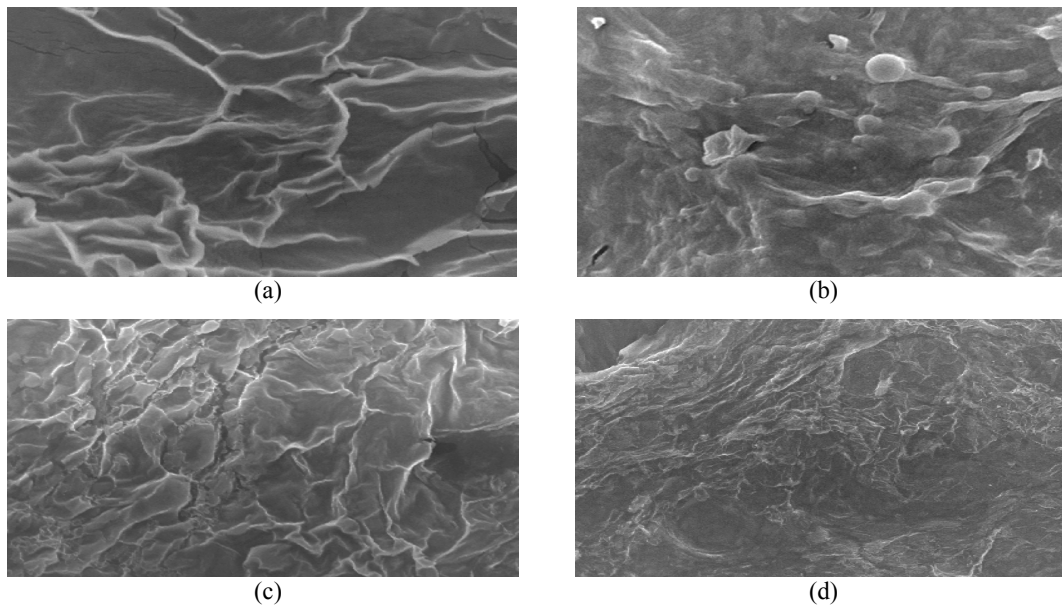


Figure (2): SEM image of edible film solutions using different mixing rotational speed (magnification of 500 x): (a) control, (b) 500 rpm, (c) 1000 rpm and (d) 1500 rpm.

Edible coating of mushroom samples

Effect of edible coating on weight-loss of mushroom during storage

Mushrooms are highly perishable products that tended to lose their unique organoleptic properties immediately after harvesting (Jiang *et al.*, 2010). Their short shelf-life is mainly explained by the water loss, high respiration rates and microbial colonization by bacteria or fungi (Mahajan *et al.*, 2008).

Water loss or transpiration is an important physiological process that affected the main quality characteristics of fresh mushrooms, such as weight, appearance and texture (Singh *et al.*, 2010). Figure (3) showed the effect of edible coating using carrageenan (1.5%) with different concentrations of tangerine essential oil (0.2, 0.4 and 0.6%) on weight loss of

mushrooms during storage (18 days) at 4°C. The results showed that the weight loss of uncoated mushroom (Control, C) was 12.85%, while edible coating reduced weight loss which varied from 0.77 to 4.42% after 18 days storage. These results could be attributed to the fact that mushrooms were protected by a thin and porous epidermal structure, which does not prevent a quick superficial dehydration, (Ares *et al.*, 2007). It was clearly observed that mushrooms coated samples (T5, T2 and T9) had the lowest weight-loss; this may be due to that edible coating provided a good barrier to moisture and minimized the loss of moisture during storage. It can also act as a gas barrier and slow down the respiration, senescence and enzymatic oxidation (Mohebbi *et al.*, 2012; Ghasemnezhad *et al.*, 2013).

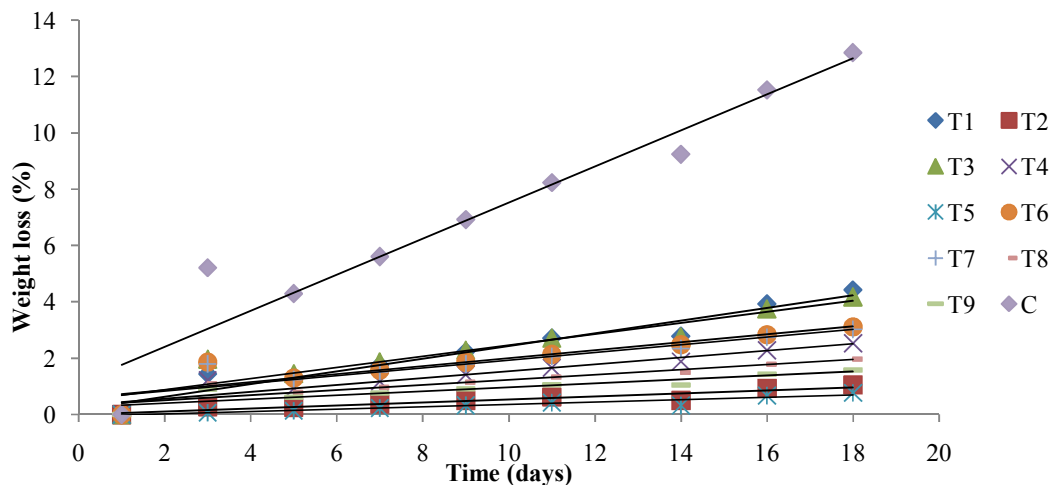


Figure (3): Effect of edible coating on mushroom weight loss during storage

C: Control (Without edible coating film) - T1, T2 and T3: 1.5% Carrageenan + 0.2% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T4, T5 and T6: 1.5% Carrageenan + 0.4% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T7, T8 and T9: 1.5% Carrageenan + 0.6% tangerine essential oil at 500, 1000 and 1500 rpm, respectively

Effect of edible coating on the firmness of mushroom

Coating plays an important role in the structure maintenance of membrane cell and cell walls in mushroom (Dhall *et al.*, 2013). One of the main changes associated with mushrooms deterioration are changes in their texture. Mushroom softening or loss of firmness during storage has been ascribed to changes in the membrane. These texture changes are also related to protein and polysaccharide degradation, hyphae shrinkage, central vacuole disruption and expansion of intercellular space at the pilei surface (Zivanovic *et al.*, 2000). Figure (4) showed the effect of the edible coating of carrageenan (1.5%) with different concentrations of tangerine essential oil (0.2, 0.4 and 0.6%) on the

firmness of mushroom samples. The results indicated that the uncoated mushroom sample began to collapse after five days and become gradually softer than the coated sample after nine days. However, the coating maintained its firmness for up to 18 days; this may be due to that edible coating directly affects fruit firmness by delaying the ripening process and decreasing the activity of cell wall degrading enzymes (Dang, 2008). It was observed that coated samples of mushrooms had higher firmness and better appearance concerning the control sample. The higher firmness (23.01 N) was observed for coated sample T5 and the lowest firmness (13.94 N) was observed for control sample (C) after 18 days.

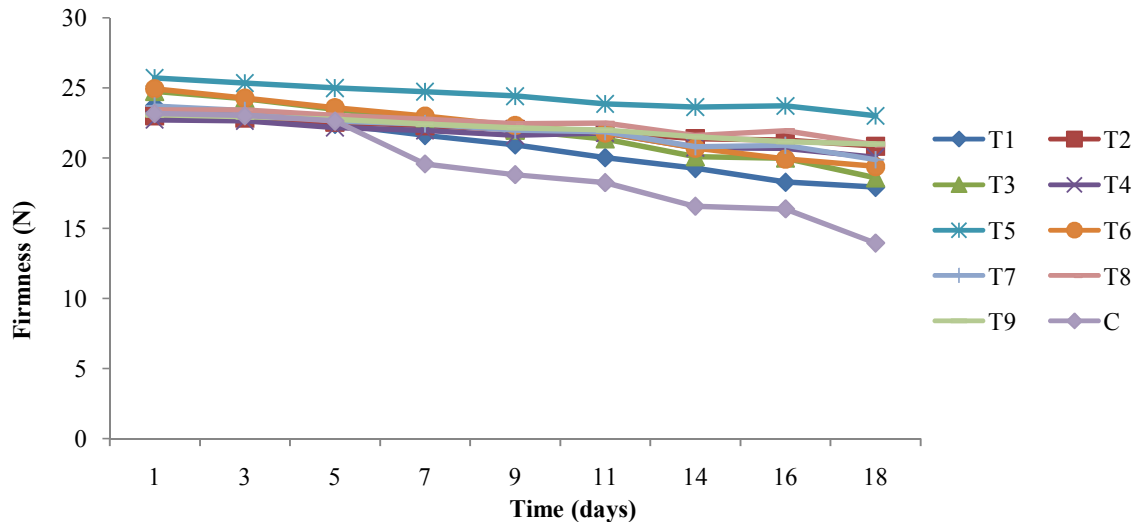


Figure (4): Effect of edible coatings on mushroom firmness

C: Control (Without edible coating film) - T1, T2 and T3: 1.5% Carrageenan + 0.2% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T4, T5 and T6: 1.5% Carrageenan + 0.4% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T7, T8 and T9: 1.5% Carrageenan + 0.6% tangerine essential oil at 500, 1000 and 1500 rpm, respectively

Moisture contents of mushrooms during storage

The moisture contents of fresh and coated mushrooms during storage at 4°C for 21 days are shown in Table (6). The results indicated that the moisture content of the fresh mushroom was 90.02% and it ranged between 89-90% for coated mushroom samples at zero time. Moisture contents reached to approximately 84% after 21 days. Results also showed that the moisture contents of all treatments reduced after 7 days by about 1.2-2.4%, and it reduced after 14 days by about 4.1-5.4%, meanwhile on day 21 it reduced by about 4.3-7.4%. It could be noticed that no significant differences were observed for reducing moisture contents with increasing mixing rotational speeds, but it was found significant differences with increasing the levels of tangerine essential oil concentrations on the day 21 it could be observed that no significant differences were found between both mushroom coated with carrageenan (1.5%) incorporated with 0.2, and 0.4% tangerine essential oil using rotational speed of mixing 1000 rpm. Also, the results indicated that the lowest moisture content was 83.3% after 21 days for coated mushrooms with carrageenan (1.5%) incorporated with 0.2% tangerine essential oil using

rotational speed of mixing 500 rpm. The fresh mushrooms did not continue for more than five days, and then it showed signs of deterioration where appeared brown spots and sticky areas, and these signs made the mushrooms unacceptable. The results indicated that the coating of mushrooms with a layer of carrageenan with essential oils of tangerine preserves the moisture content of mushrooms for 21 days at 4°C with the beginning of the first signs that made the mushrooms unacceptable after day 18. These results are in agreement with Brennan *et al.* (2000) who reported that the most important problem for mushrooms is a postharvest browning severe that reduced the shelf life. Also Singh *et al.* (2016) reported that mushrooms are extremely sensitive, fast deteriorated within one day after harvest due to high respiratory rate and sensitive skin structure. Thus, the shelf life of the freshly-cut mushrooms is no more than 1-3 days depending on the surrounding environment. Also, they found that the moisture content reduced from 90 to 85.39% and 85.5% after three days of the mushrooms preserved at ambient or refrigerated conditions, respectively after washing with plain water and polypropylene used as packaging material.

Table (6): Moisture contents of fresh and coated mushrooms during storage for 21 days at 4°C

Storage period (days)	Fresh mushrooms	Moisture contents (%)								
		Coated mushrooms treatments								
		T1	T2	T3	T4	T5	T6	T7	T8	T9
Zero time	90.02 ^a ±0.03	90.00 ^a ±0.01	90.00 ^a ±0.03	90.00 ^a ±0.02	89.04 ^b ±0.01	89.06 ^b ±0.05	89.03 ^b ±0.03	89.01 ^b ±0.06	89.03 ^b ±0.01	89.01 ^b ±0.05
7	ND	88.37 ^a ±0.04	88.96 ^a ±0.06	87.95 ^b ±0.07	87.94 ^b ±0.05	87.93 ^b ±0.07	87.94 ^b ±0.06	86.92 ^c ±0.03	86.91 ^c ±0.02	86.92 ^c ±0.05
14	ND	84.51 ^a ±0.04	85.31 ^a ±0.06	85.40 ^a ±0.03	85.38 ^a ±0.05	85.35 ^a ±0.07	84.35 ^b ±0.05	84.28 ^b ±0.08	84.22 ^b ±0.04	84.27 ^b ±0.03
21	ND	83.30 ^c ±0.08	85.20 ^a ±0.07	84.30 ^b ±0.03	84.10 ^b ±0.04	85.20 ^a ±0.02	84.80 ^b ±0.09	84.20 ^b ±0.04	84.14 ^b ±0.02	84.30 ^b ±0.03

- ND: not determined - Values are mean of three replicates ± SD - T1, T2 and T3: 1.5% Carrageenan + 0.2% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T4, T5 and T6: 1.5% Carrageenan + 0.4% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T7, T8 and T9: 1.5% Carrageenan + 0.6% tangerine essential oil at 500, 1000 and 1500 rpm, respectively

A lot of studies have been done to extend the mushroom preservation period. The use of essential oils become superior as an alternative to chemical preservatives and their use in foods meets consumers' demands for natural products (Alikhani-Koupae *et al.*, 2014; Ding and Lee, 2019).

Microbial status for mushroom samples during storage

For approximately five days after harvesting the texture of mushrooms became tougher. This toughening was followed by a softening phase which might be related to the breakdown in the mushroom tissue caused by bacteria (Zivanovic *et al.*, 2000).

Assessment of antimicrobial activity of edible coating containing carrageenan and tangerine essential oil

The obtained results in Table (7) showed that antimicrobial activity of the studied samples essential

oils against *Salmonella typhimurium* and *E. coli* increased as the increase of essential oils concentration from 0.2 to 0.6% as well as increasing mixing speeds from 500 to 1500 rpm.

Aspergillus niger and *Candida albicans* not detected (ND) at 0.2% concentration of the essential oils at different mixing speeds. But, the essential oil concentrations intensified up to 0.4 and 0.6% with increasing mixing speeds up to 1500 rpm caused an increase of inhibition zone against *Aspergillus niger* and *Candida albicans*.

These results are in agreement with Belletti *et al.* (2004) which reported that tangerine essential oil exhibited the highest antibacterial activity and it was effective against all the target bacterial species, especially against *E. coli*, *S. typhi* and *Streptococcus* sp.

Table (7): Antimicrobial activity of the edible coating containing carrageenan and tangerine essential oils (inhibition zone, mm)

Treatments	<i>Salmonella typhimurium</i>	<i>E. coli</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>
	Inhibition zone (mm)			
T1	6	6	ND	ND
T2	9	8	ND	ND
T3	8	7	ND	ND
T4	9	8	6	7
T5	13	11	8	9
T6	11	9	7	8
T7	11	12	8	9
T8	14	13	10	11
T9	13	13	9	16

ND: not detected - T1, T2 and T3: 1.5% Carrageenan + 0.2% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T4, T5 and T6: 1.5% Carrageenan + 0.4% tangerine essential oil at 500, 1000 and 1500 rpm, respectively - T7, T8 and T9: 1.5% Carrageenan + 0.6% tangerine essential oil at 500, 1000 and 1500 rpm, respectively

Total bacterial and yeast and mold counts of mushrooms

Data in Table (8), showed the microbial evaluation of mushroom coated with tangerine essential oil at different concentrations (0.2, 0.4 and 0.6%) and different mixing speeds (500, 1000 and 1500 rpm) compared with control. The results indicated that the highest values of total count of bacteria, yeast and mold of control sample achieved at all the different periods (zero time, after one and two weeks of storage time). The results revealed that the increase of tangerine essential oil concentration from 0.2 to 0.6% as well as raising mixing speeds from 500 to 1500 rpm caused a

decrease of total count of bacteria at zero time, after one and after two weeks of storage.

Similarly, total yeast and mold count markedly declined with increasing mixing speeds from 500 to 1500 rpm and raising the concentration of tangerine essential oil (that used for coating of mushroom) from 0.2 to 0.6% at zero time, after one and after two weeks of storage.

These results are in agreement with Wang *et al.* (2008), they reported that citrus oil rich in flavonoids which can inhibit the growth of microorganisms.

Table (8): Effect of tangerine essential oil on total bacterial, yeast and mold count during storage time

Treatments	Zero time		After one week		After two weeks	
	Total bacterial	Yeast and mold	Total bacterial	Yeast and mold	Total bacterial	Yeast and mold
Control	22×10 ²	12×10 ²	8×10 ³	2×10 ²	9×10 ⁵	8×10 ⁴
T1	8×10 ²	6×10 ²	12×10 ²	7×10 ²	1×10 ³	6×10 ³
T2	9×10 ²	4×10 ²	14×10 ²	10×10 ²	4×10 ³	4×10 ³
T3	9×10 ²	5×10 ²	14×10 ²	9×10 ²	3×10 ³	5×10 ³
T4	7×10 ²	8×10	8×10 ²	4×10 ²	7×10 ²	8×10 ³
T5	7×10 ²	9×10	8×10 ²	3×10 ²	5×10 ²	8×10 ²
T6	6×10 ²	7×10	7×10 ²	4×10 ²	6×10 ²	7×10 ³
T7	1×10 ²	2×10	2×10 ²	9×10	9×10 ²	2×10 ²
T8	2×10 ²	3×10	3×10 ²	8×10	7×10 ²	1×10 ²
T9	1×10 ²	2×10	2×10 ²	9×10	8×10 ²	1×10

CONCLUSION

The effect of edible coating on physicochemical and microbiological properties of mushroom were studied using carrageenan incorporated with tangerine (*Citrus reticulata*) essential oils. Physicochemical properties of tangerine essential oils were specific gravity, refractive index, acid value and aldehyde were 0.853, 1.4764, 0.89 mg KOH/g oil and 2.13%, respectively. Antioxidant activity increased by increasing essential oil concentration. Nine components of tangerine essential oils were identified representing 100% of the total oil and the main component was D-limonene (80.55%). Rheological properties indicated that all samples exhibited non-Newtonian pseudoplastic behavior. Also, edible coating affects thickness, water vapor permeability of edible film, firmness, weight loss, moisture content, microbiological tests of coated mushroom samples.

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تأثير سرعة الدوران أثناء الخلط علي خصائص الغشاء القابل للأكل المحتوي علي الزيت العطري لليوسفي لإطالة فترة صلاحية فطر عيش الغراب (المشروم)

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يفقد فطر المشروم خواصه الحسية الفريدة بعد الحصاد مباشرة، لذلك فإن له فترة صلاحية قصيرة. لذا اهتم هذا البحث بدراسة تأثير تغطية عيش الغراب (المشروم) بالغشاء القابل للأكل المكون من الكاراجينان والزيت العطري لقسور اليوسفي بتركيزات مختلفة (٠,٢، ٠,٤، ٠,٦، ١,٠) على فترة صلاحية عيش الغراب. تم تحضير محاليل الأعشبية القابلة للأكل باستخدام ثلاث سرعات مختلفة للخلط (٥٠٠، ١٠٠٠، ١٥٠٠ لفة في الدقيقة). تم تقدير الخواص الطبيعية والكيميائية للزيت العطري لقسور اليوسفي وكذلك الخصائص الريولوجية للأعشبية القابلة للأكل. أوضحت النتائج أن جميع العينات أظهرت سلوك غير نيوتوني (من النوع كاذب اللدانة). كانت العينة المحتوية علي ١,٥% كاراجينان + ٠,٢% زيت عطري لقسور اليوسفي بسرعة خلط ٥٠٠ لفة في الدقيقة هي الأعلى قيمة لنفاذية بخار الماء. أكدت النتائج أن الزيت العطري وسرعة الخلط أثرا علي الشكل الفراغي للغشاء القابل للأكل. وجد أن تغطية عيش الغراب بهذه الأعشبية أثر علي فقدان الوزن والصلابة ومحتوى الرطوبة وكذلك الخصائص الميكروبيولوجية. ونستنتج من هذه الدراسة إمكانية إطالة فترة حفظ عيش الغراب باستخدام التغطية بأعشبية قابلة للأكل مكونة من الكاراجينان المخلوط بالزيت العطري لقسور اليوسفي.