IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 11 Ver. I (November. 2018), PP 55-60
www.iosrjournals.org

Effect of TheLavanderEssential Oil Fortified Edible Film on Some Properties of Kashar Cheese During Storage Time

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Abstract: *This study focused on the use of antimicrobial edible coatings and films to reduce populations of foodborne pathogens in foods.The edible films were made by various lavander essential oil(Lavandula Stoechas L. Subsp. Stoechas) concentrations of 1 %, 2 %,egg white protein powder (EWPP) 5% (w/v) and sorbitol 3% (w/v) as plasticizer. Kashar cheese samples were artificially contaminated with Escherichia coli O157:H7 and Staphylococcus aureus at a level of 10^6 cfu^{-g}. After coating some of the Kashar cheese samples with film, all samples were stored at 4 °C for 30 days. Antimicrobial activities and selected physical-chemical parameters were assessed on the 1st, 7th, 15th and 30th days of storage.. The characteristics of the EWPP edible films have good water barrier properties, which improved with the addition of 2% (v/v) lavander essential oil to the film. All lavender essential oil containing films prevented the increase of Escherichia coli O157:H7 and Staphylococcus aureus counts in Kashar cheese for 4 weeks at 4 °C. During storage, Escherichia coli O157:H7 and Staphylococcus aureus levels increased in the control samples, while they decreased in the film-coated samples.*

Keywords: *Edible film, Kashar cheese, lavander essential oil*

Date of Submission: 17-11-2018 Date of acceptance: 03-12-2018

I. Introduction

Kashar cheese is the most produced cheese variety, after Beyaz cheese in Turkey. Kashar cheese is produced under different names in some Balkan and European countries. Kashkaval in Bulgaria, Kassari in Greece, Kachkawalj in Yugoslavia cheeses are similar to Kashar cheese .Kashar cheese is a semi-hard cheese type, whose production is not governed by one standard technique (Cetinkaya and Soyutemiz, 2004).

Edible films are defined as a thin layer, which can be consumed, coated on a food or placed as a barrier to nmoisture, oxygen and solute movement between the food and the surrounding environment. Edible films are produced with biologically hydrophilic materials including,starch, protein ,alginates cellulose, and carrageenan (Taqi et al., 2011). Edible films obtained exclusively from biopolymers (carbohydrate and protein based) have weak mechanical properties, are brittle and can crack during the drying stage (McHugh and Krochta 1994). These problems can be overcome by adding plasticizers to the film composition such as glycerol, propylene glycol, sorbitol or polyethylene glycol (Coupland et al., 2000; Dutta et al., 2009). Sorbitol (S) is an important plasticizer due to its lower moisture absorption and 100% dissolution capabilities (Ressouany et al. 1998). With the addition of different essential oils to protein based films, composite films with good water vapor barrier properties can be produced (Gennadios et al., 1998; McHugh et al., 1994). EWP based film coatings produced by the addition of different fruit and vegetable types are effective in food preservation (Sothornvit, 2005). Essential oils are frequently used to control the growth of pathogenic bacteria and degradation in foods due to their antimicrobial activities. (Burt, 2004; Padgett et al., 1998; Quattara et al., 2001; Zivanovic et al., 2005). In previous studies on EWP, it has been reported that edible films produced by using EW ovalbumin and lysozyme together provided a packaging material with high antimicrobial properties (Padgett et al., 1995). Different edible film formulations have been developed by using EWP with different biopolymers (Handa et al., 1999 ;Gennadios et al., 1998).

Lavandula (*Lavandula stoechas* L. subsp. Stoechas) is an aromatic plant of Lamiaceae family. (Goren et al., 2002). There are two subspecies in Turkey. These are: *L. stoechas subsp. stoechas* and *subsp. cariensis*(Davis, 1982). *Lavandula stoechas* L. *subsp. stoechas*flower has been reported to contain 0.77-1.2% volatile oil and essential ingredients in essential oil, phentermine, camphor, pinocarvyl acetate, eucalyptol and mirtenol (Gilani et al., 2000).Tanker (1990) determined that the main constituents of volatile oil were 23% camphor and 4% eucalyptol. In Turkey *subsp. stoechas* from the obtained essential oil composition, 23.29% camphor, 10.87% fenchone, 4.07% eucalyptol, 1.5% linalool and linalyl acetate weredetected. In the volatile oil composition obtained in the Aegean Region, 30% camphor, 18% fenchone, 5.3% eucalyptol, 2% linalool and

linalyl acetate were detected (Sarer et al., 1993). It has been determined that essential oil are sedative, anticonvulsant, antimicrobial, antifungal, antioxidant, antispasmodic, antithrombotic and cancer protective effects (Zeybek, 1999). In some studies antimicrobial activity was found to be associated with monoterpenoid compounds and linalool, 1,8-Cineole, Camphor, Terpinene-4-ol (Abchin et al., 2013; Rasouli and Rezaie, 2000).The antimicrobial activity in the volatile oil obtained from the flower was associated with α-pinene, 1,8-cineole, fenchone, camphor and myrtenyl acetate (Akgun et al., 2000; Zuzarte et al., 2013).

In this study focused to investigate the effect of coating kashar cheese with edible films obtained by the fortification of S+EWPP based film with lavander essential oils at different concentrations [1% (v/v); 2% (v/v)] on the extension of shelf-life. Also, it was aimed to determine the effects of the film coatings on the physicochemical properties of kashar cheese and specifically the effects of using two antimicrobial essential oils incorporated into the film against *Escherichiae coli* O157:H7 *(E.coli*O157:H7*)* and *Staphylococcus aureus (S. aureus).*

II. Material and Methods

Egg White Protein Powder (EWPP) and D-Sorbitol

For the preparation of coating material, Alfasol® egg white protein powder (EWPP) was purchased from Kimbiotek Chemical Agents Inc. (İstanbul-Turkey) and D-sorbitol (S1876) was obtained from Sigma-Aldrich.

Essential oilsand **volatile compounds by GC/MS**

The lavander (*Lavandula stoechas* L. *subsp. stoechas*)flower essential oils (LEOs) were purchased from flora Bornova-Izmir (Turkey). Essential oil was obtained by hydrodestillation for 3 h using a Clevenger-type apparatus (Bounatirou et al. 2007). The oils used were those of lavander, and the active components were obtained from Sigma-Aldrich (Steinheim, Germany).GC/MS analyses were carried out using Agilent 6890 GC system with Agilent 5973 MS system.

Column:DB-5MS 30m x 0.25mm x 0.25 um (5% Diphenyl / 95% Dimethylpolysiloxan) Agilent.

Oven temperature :initially held at 50 °C /3 min, programmed to 160 °C at 1.5 °C/min, then to 315 °C at 3 °C/min and the final temperature was held for 30 min.

The carrier gas : helium with a flow rate of 1.0 ml/min.

Split mode : 1:20

Enjection volume :1.0 µl.

The mass spectrometer was operated in the electron impact mode (70 eV). The ion source temperature was held at 230 °C. The transfer-line was maintained at 280 °C. The scanned mass range was from 30 to 500 u.

Fresh Kashar Cheese

Kasar cheese was produced by following the traditional production steps. The production steps of the kashar cheese was given in Table 2.

Coating and Films Preparationand preparation of Kashar cheese samples

Edible films were prepared according to Pintado et al. (2010) and Mchugh and Krochta (1994), with some modifications. (Table 1). Preparation of kashar cheese samples; *E. coli* O157:H7 (ATCC 43895) and *S. aureus* (ATCC 6538) strains used for the artificial contamination (10^6 cfu/g) of kashar cheese samples were obtained from Hemakim Corporation (Turkey)(Table 3).

Physical - Chemical Analysis

The cheese composition was analysed by standard methods: dry matter (EN ISO, 2004), pH (SS-3 Zeromatic pH meter, USA) and titratable acidity according to the Soxhlet Henkel method. Weight loss percentages of kashar cheese samples during storage were determined gravimetrically AOAC (2000). The thicknesses of the films were measured with a micrometer (Digimatic Micrometer / Japan). Water vapor permeability of films were determined using ASTM E96-80 (1983) method. WVP was calculated by finding the slope of weight-time line and substituting it in the following formula.

$$\textbf{Slope (C)} = \frac{\boldsymbol{WVP \times A \times \Delta p}}{\boldsymbol{X}} \qquad \boldsymbol{WVP = C\ \frac{x}{A \times \Delta p}}$$

A: Surface area (m^2)

WVP: Water vapor permeability (g mm m^{-2} h^{-1} kPa^{-1})

Δp: Partial pressure difference of the gases (kPa)

x: Film thickness (mm)

Microbiological Analysis

Samples were analyzed for *E.coli* O157:H7 using Sorbitol MacConkey Agar with Cefixime-Tellurite Supplement and incubating at 35-37°C for 24-48 hours. Samples were analyzed for*S.aureus*using Baird Parker Agar with % 5 Egg Yolk Tellurite emulsion and incubating under aerobic conditions at 35-37°C for 24-48 hours. All microbiological analyses were performed using petri films and colonies were counted (Food and Drug Administration, 2001) .

Statistical Evaluation

Statistical analyses were performed using the Statistical Package for SPPS version 15 (SPSS, Chicago IL, USA), via one-way analysis of variance. The significance level was set at $p < 0.05$.

III. Results and Discussions

Lavanderessential oil containing, camphor 39.21%, 1,8- cineole (eucalyptol) 18.47%, fenchone 15.24%, borneol 6.02%, camfen 5.16%, β-pinene 3.83% velinalool 2.15% was obtained from *Lavandula stoechas* L. *subsp. stoechas* species.

Thickness and WVP values of $EWPP_{LEO(1);}$ $EWPP_{LEO(2)}$ obtained by adding LEOs to EWPP are given in Table 4.

Presented relationship between increase in essential oil concentration and the increase in film thickness was statistically significant ($p<0.05$). Taqi et al. (2011) reported that the film thickness increased and WVP decreased in edible films containing hydrophobic agents such as wax and vegetable oils (Greener and Fennema 1989; Kester and Fennema, 1986; Kamontip and Adisak, 2001). In this study, trends in the film thicknesses were similar to those of water vapor permeability.

During storage, titratable acidity values (°SH) (decrease in pH values) increased in all samples.The expected increase in acidity of samples coated with films prepared with the addition of essential oils was higher than those with control samples. Acidity increased from the 1st day of the storage until the end of the storage. This increase was higher depending on the essential oil concentration; the highest acidity increase occurred with 2% (v/v) essential oil concentrations.The relationship between the increase in acidity and essential oil concentration was significant ($p<0.05$).

Weight loss in all cheese samples was measured and compared. Weight loss in $EWPP_{LEO(1);}$ $EWPP_{LEO(2)}$ and EWPP samples were lower compared to those with C sample. This difference was statistically significant ($p< 0.05$). Water barrier properties increased with the addition of essential oil in a dose-dependent manner.

Antimicrobial effects obtained in uncoated control samples, edible films obtained with the addition of essential oils in different concentrations to EWPP are given in Figure 1.The increase in the antimicrobial activity and the addition of essential oil at all concentrations was statistically significant ($p<0.05$). The antimicrobial effects of all LEOs supplemented films were higher than control sample. This result was associated with the high camphor 39.21%, 1,8- cineole (eucalyptol) 18.47%, fenchone 15.24%, borneol 6.02%, camfen 5.16% contents of LEOs all significantly contribute to the increased antimicrobial effects.

LEOs (Abchin et al., 2012) are essential oil with strong antimicrobial effects. Our results are consistent with the literature. Additionally, it was demonstrated that the previously demonstrated antimicrobial properties of EWPP is also true for EWPP based edible films. This antimicrobial effect was increased with the addition of LEOs to EWPP based film in a dose-dependent manner.

In addition, the antimicrobial effect of $EWPP_{LEO(2)}$ during storage was higher than that of $EWPP_{LEO(1)}$ and C sample.The antimicrobial effect of LEOs was higher on *S. aureus* than *E. coli* O157: H7.In the C sample the *S. aureus* level increased during storage and reached 8 $\log_{10}$ cfu^{-g} on days 15 and 30.The antimicrobial effect of LEO 2% (v/v) on *E. coli* O157: H7 was higher than $LEO_{(1)}$.LEOs added to EWPP at 1% (v/v) showed antimicrobial activity until the end of storage.The bactericidal effect was determined at 30 days at 2% (v/v).*E. coli* O157: H7 LEOs concentration at 1% (v/v), 5 $\log_{10}$ cfu^{-g} at 15 days, and decreased to 3 $\log_{10}$ cfu^{-g} at 30 days.But decreased to 3 $\log_{10}$ cfu^{-g} on the 15th day at 2% (v/v). In the Csample*E. coli* O157: H7 showed an increase during storage.The *E. coli* O157: H7 level detected at the 30th day in the C sample was 8,48 $\log_{10}$ cfu^{-g}.

IV. Conclusions

It was demonstrated that EWPP based films can be exclusively used in edible film production and the appearance properties are similar to other protein based films. This result is consistent with previous findings (Gennadios et al. 1998; Sothornvit, 2005; Yuno-Ohta et al., 1996). Addition of essential oils to EWPP based film improved both physico-chemical and antimicrobial properties in a dose-dependent manner. The best essential oil concentration in terms of physico-chemical and antimicrobial properties was 2% (v/v). Therefore, the present work points at new alternative to synthetic films for the cheese industry.

References

[1] Abchin, A. S., Nasrolahi Omran, A., Jafari, N., Mostafapour, M. J. and Kia, S. M. 2012. Antibacterial effects of Lavandula Stoechas essential oil, on gram positive and negative bacteria in vitro. *Medical Laboratory Journal*, 6 (2): 34-41.

[2] Abchin, A., Nasrolahi, O., Jafari, N., Mostafapour, M.J., 2013. Antibacterial effects of *Lavandula Stoechas* Essential Oil, on Gram Positive and Negative Bacteria *in Vitro*, *Medical Laboratory Journal*. 6(2):35-41.

[3] Akgun, M., Akgun, N., Dincer, S., 2000. Extraction and Modeling of Lavender Flower Essential Oil Using Supercritical Carbon Dioxide. *Industrial & Engineering Chemistry Research*. 39(2):473-477.

[4] AOAC (Association of Official Analytical Chemists), 2000. Official Methods of Analysis. Association of Official Analytical Chemists International, Association of Official Analytical Chemists (publisher), Washington, DC 20044, USA, 1018p.

[5] ASTM, 1983. "Standard Test Methods for Water Vapor Transmission of Materials, Standard E96-80", Annual Book of American Standard Testing Methods, Philadelphia, PA., 761-770.
[6] Bounatirou, S., Smiti, S., Miguel, M.G., Faleiro, L., Rejeb, M.N., Neffati, M., Costa, M.M., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., 2007. Chemical composition, antioxidant and antimicrobial activities of the essential oils isolated from Tunisian *Thymus capitus* Hoff. et Link. *Food Chemistry*, 105, 146–15.
[7] Burt, S., 2004. Essential oils their antibacterial properties and potential applications in foods-a review. *International Journal of Food. Microbiology*, 94, 223-253.
[8] Cetinkaya, F., Soyutemiz, G.E., 2004. A Study on Survival of Listeria monocytogenes during Manufacture and Ripening of Kashar Cheese.*Turkısh Journal Of Veterınary And Anımal Scıences*, 28 (2004) 927-932. TUBITAK.
[9] Coupland, J.N., Shaw, N.B., Monahan, F.J., O'riordan, E.D., O'sullivan, M., 2000. Modaeling the effect of glycerol on the moisture sorption behavior of whey protein edible films. *Journal of Food Engineering*, 43(1):25-30.
[10] Davis, P. H., (Ed.) 1982. The Flora of Turkey and The East Aegean Islands. The University Press. Edinburgh. 7: 76,77.
[11] Dutta, PK, Tripathi, S., Mehrotra, G.K., 2009.Perspectives for chitosan based antimicrobial films in food applications. *Food Chemistry*, 114(4), 1173-82.
[12] Food And Drug Administration,2001**.** In: "Bacteriological Analytical Manual Online", .www.fda.gov.
[13] Gennadios A., Rhim J.W., Handa A., Weller C.L., Hana M.A. 1998. Ultraviolet Radiation Affects Physical and Molecular Properties of Soy Protein Films, *Journal of Food Science*, 63: 225-228.
[14] Gilani, A. H., Aziz, N., Khan, M.A., Shaheen, F., Jabeen, Q., Siddiqui,B.S., Herzig, J.W., 2000. Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of *Lavandula stoechas* L. *Journal of Ethnopharmacology*, 71: 161-167.
[15] Goren, A., Topcu, G., Bilsel, G., Bilsel,M., Aydogmus,Z., Pezzuto, J.M., 2002. The chemical constituents and biological activity of essential oil of *Lavandula stoechas* ssp. *Stoechas.* Z Naturforsch [C] 57 (9-10): 797-800.
[16] Greener, IK, Fennema, O., 1989. Barrier properties and surface characteristics of edible bilayer films. *Journal of Food Science*, 54: 1393-1399.
[17] Handa, A., Gennadios, A, Froning, GW, Kuroda, N., Hana, MA, 1999. Tensile,solubility, and electrophoretic properties of egg white films as affected by surface sulfhydryl groups. *Journal of Food Science* ,64: 82-85.
[18] Kamontip, E., Adisak, A., 2001. Effect of Vegetable Oils on Physical Characteristics of Edible Konjac Films, *AU Journal of Technology*,5: 73-78.
[19] Kester, JJ, Fennema, OR., 1986. Edible films and coatings: a review. *Food Technology*, 40(12): 47-5
[20] McHugh, T.H., Krochta, J.M., 1994. Milk protein based edible films and coatings. *Food Tecnologie*, 48(1): 97-103.
[21] McHugh, T.H., Aujard, J.F., Krochta, J.M., 1994. Plasticized whey protein edible films: Water vapor permeability properties. *Journal of Food Science* ,59: 416-423.
[22] Omana, D. A., Wang, J., Wu, J., 2010. Co-extraction of egg white proteins using ion-exchange chromatography from ovomucin-removed egg white. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*., 878: 1771–1776.
[23] Padgett, T., Han, I.Y., and Dawson, P.L. 1995. Incorporation of lysozyme into biodegradabl packaging films. *Poultry Science*, 74 (Suppl. 1): 165
[24] Padgett, T., Han, I.Y., Dawson, L., 1998. Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. *Journal of Food Protection*, 61: 1330-1335.
[25] Pintado Cristina, M.B.S., Ferreira Maria, A.S.S., Sousa, I., 2010. Control of pathogenic and spoilage microorganisms from cheese surface by whey protein films containing malic acid, nisin and natamycin. *Food Control*, 21(3): 240–246.
[26] Rasouli, A., Rezaie, M., 2000. Antimicrobial activity and chemical composition of essential oils of lavender and tuberose flowers. *Journal of Kerman University of Medical Science*. 7(4):173-181.
[27] Ressouany, M., Vachon, C., Lacroix, M., 1998. Irradiation dose and calcium effect on the mechanical properties of cross-linked caseinate films. *Jounal of Agriculture Food Chemistry*, 46: 1618-1623.
[28] Sarer, E., Baser, K.H.C., Guler, N., 1993. Some *Lavandula* species, their essential oil content and the main components of the oils. *Essential oil*, 20.
[29] Sothornvit, R. 2005. Edıble Fılm Formatıon And Propertıes From Dıfferent Protеın Sources And Orange Coatıng Applıcatıon. Acta Hort. (ISHS) 682:1731-1738. http://www.actahort.org/books/682/682_231.htm.
[30] Stadelman, W. J., and O. J. Cotterill. 2001. Egg Science and Technology. 4th ed. Avi Publ. Co., Westport, CT.
[31] Tanker, M., Tanker, N., 1990. Farmakognozi, Ankara Universitesi Eczacılık Fakultesi Yayınları No. 65. Cilt-2: 301, 302.
[32] Taqi, A., Aksar, K.A., Nagy, K., Mutihac, L., Stamatin, I., 2011. Effect of different concentrations of olive oil and oleic acid on the mechanical properties of albumen (egg white) edible films. *African Journal of Biotechnology*, 10(60): 12963-12972.
[33] Quattara B., Sabato S.F., Lacroix, M., 2001. Combined effect of antimicrobial coating and gamma irradiation on shelf life extension of pre-cooked shrimp (Penaeus spp.). *International Journal of Food Microbiology*, 68: 1-9.
[34] Yetismeyen, A. 1997. Dairy Technology. Ankara University, Faculty of Agriculture. Book No : 443, pp 194-198. Ankara, Turkey.
[35] Yuno-Ohta, N., Toryu, H., Higasa, T., Maeda, H., Okada, M., Ohta, H., 1996. Gelation properties of ovalbumin as affected by fatty acid salts. *Journal of Food Science*, 61: 906-910.
[36] Zivanovic, Z., Chi, S., Draughon, A.F., 2005. Antimicrobial activity of chitosan films enriched with essential oils. *Journal of Food Science*70 (1): 45-51.
[37] Zeybek, U., 1999. Aromaterapi ve Aromakozmetikte Kullanılan Ucucu Yağlar. Defne Esensia Yayınları. 9, 31. Izmir.
[38] Zuzarte, M., Goncalves, M., Cavaleiro, C., Cruz, M., Benzarti, A., Marongiu, B., 2013. Antifungal and anti-inflammatory potential of *Lavandula stoechas* and Thymus herba-baronaessential oils. *Industrial Crops and Products*. 44(1):97-103.

Table 1 .Production of Traditional Fresh Kashar Cheese

Raw milk
Heating (32–34 °C)
Commercial liquid rennet addition
Coagulation (45 min)
Clot section (1 cm^3 in size) and rest (10 min)
Removal of a portion of the whey (approximately one-third)
Heating the curd (at 75 ± 1 °C) and mixing
keep in a 4% salt solution for an hour at room temperature
store at 5 ± 1 ^{0}C for 30 days

Table 2. Preparation of edible films solution

Egg White Protein Powder (%5 w/v) solution

↓

Sorbitol addition **(**% 3 w/v)

↓

Centrifugation (20,000 rpm / 1 min)

↓

pH adjustment (pH=8)

↓

Heating (45±2 °C/30 min)

↓

Cooling (room temperature)

↓

Filtering

↓

Essential lavander oils addition (1%;2% (v/v))

↓

Tween 20 (0.5 % (v/v)) addition

↓

Centrifugation (20,000 rpm / 1 min)

↓

Lavander essential oils fortified edible films
(LEOs) was added to the first two parts in different concentrations [1% (v/v) $EWPP_{LEO(1)}$ and 2% (v/v) $EWPP_{LEO(2)}$]. Third part was non coated control (C) sample

Table 3. Preparation and storage of kashar cheese samples

Kashar Cheese (100 g)

↓

Artificial contamination (10^6 cf/g)

↓

Immersion into inoculum (Hold for 15 minutes)

↓

First Immersion into film solution (90 seconds)

↓

Hold (for 3 minutes)

↓

Second Immersion into film solution (60 seconds)

↓

Drying (10°C/ 4-5 hours)

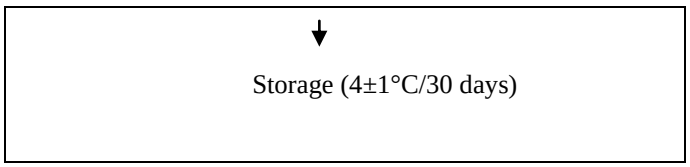

Table 4. Film thicknesses and water vapor permeability of $EWPP_{LEO(1)}$, $EWPP_{LEO(2)}$ and based films (n=3).

Sample	Thickness /mm±б	Water vapor permeability (g mm m^{-2} h^{-1} kPa^{-1})
$EWPP_{LEO(1)}$	0.169 ±0.002	6.52 g mm m^{-2} h^{-1} kPa^{-1}
$EWPP_{LEO(2)}$	0.172 ±0.004	6.49 g mm m^{-2} h^{-1} kPa^{-1}

б :Standard deviation (n=3)

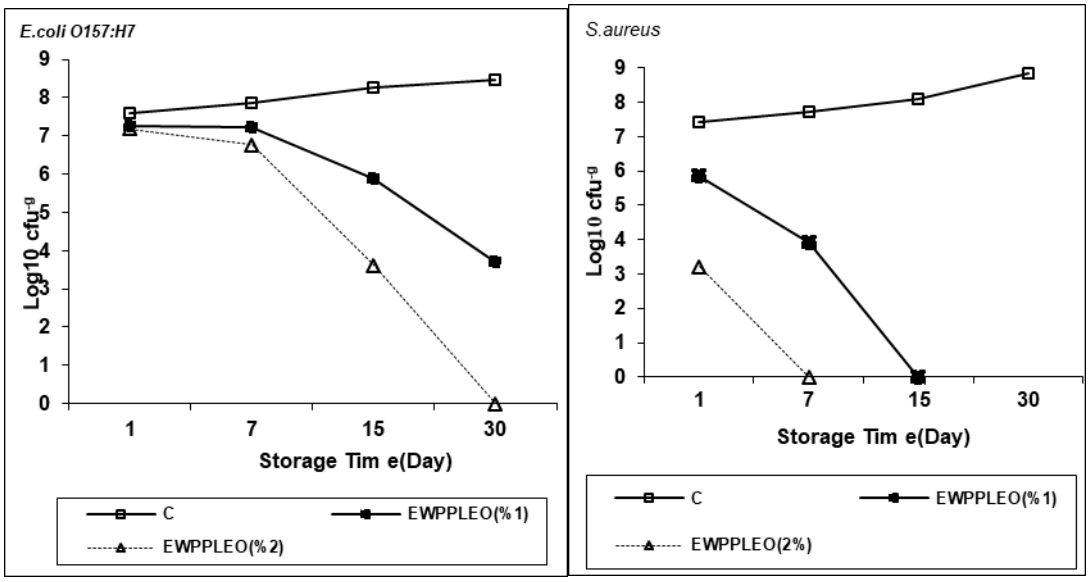

Figure 1. *E. coli* O157:H7 and *S. aureus* growth in samples coated with lavander essential oil added film and C sample.

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