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Production of biodegradable film from soy protein and essential oil of lemon peel and use it as cheese preservative

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Abstract: Soy protein with essential oil of lemon peel was used to produce a biodegradable film, which showed inhibition activity against some species of selected bacterial strains. The films were white cheese coating and studied for its biodegradation in the soil. The percentage of soy protein extract was 15% (W: W) while the percentage of lemon peel essential oil was 3% (V: W). The GC-MS result of lemon peel essential oil consisted of 38 compounds, of which the main compound is D-Limonene in 63.43%. The films produced showed inhibition activity against both Gram-positive and Gram-negative bacteria. Inhibition activity was highest against *Staphylococcus aureus* (22 mm zone), while *E. coli* O157: H7 had the lowest inhibition (18mm) when 0.1mL of essential oil with 10mL film mixed. The coating process of white cheese reduced the total microbial population (CFU/g) during 30 days of storage time. The TCB, TC, ST and MY of cheese coating were 4.28, 1.53, 1.62 and 1.33 log. CFU/g respectively after 30 days compared to the cheese non-coating samples, with 6.53, 2.51, 3.17 and 1.81 log. CFU/g, respectively. The biodegradation in soil was 97 and 72% for soy protein film and soy protein film with lemon peel essential oil after 35 days.

Key words: Film biodegradable, Soy protein, Essential oil of lemon peel.

Introduction

Among the new tendencies in food coating, many proteins and polysaccharide used to produce edible membranes, which used as the preservative coating for food. The films are natural materials such as starch films. It has become more receptive by consumers and producers compared with other packaging materials such as polyethylene. On the economic side, the using of films developed agro-industries because it leads to an increase in the consumption of materials involved in the composition of these films (Singh *et al*., 2007). Efforts to reduce environmental pollution and increase the shelf life of foods have led to further exploration of new bio-packaging materials such as edible and biodegradable films from renewable resources. Proteins films used in wrapping or coating of processed food. The Collagen films used in packaging of sausage and pharmaceutical capsules while soy protein film is called Yuba which used for wrapping of meat and vegetables before cooking in East Asian countries (Bourtoom, 2009).

Seeds of *Glycine max* L., commonly known as soybean content between 38-45% proteins, so, it can use to produce biodegradable film after mixing with some organic or inorganic materials (Xiang *et al*., 2009).

The term "effective packaging" is intended food packaging system, which possesses additional properties such as reservation of gases or moisture, oxygen scavenging, moisture control and bioactivity (Al-Bayati, 2005). Essential oils are one member from bioactivity materials. It has high antimicrobial activity against microorganisms (Niamah and Alali, 2016; Al-sahlany, 2016). The D-Limonene main compound in the essential oil of lemon peel and has antimicrobial activity and antioxidant (Djenane, 2015).

Supardan *et al*. (2016) refers to the possibility of using essential oil extracted from lemon leaves in the formation of edible films after mixing with starch. This film had inhibitory activity against *Trichoderma* and *Penicillium* and the numbers of microbial in meat reduced after coating by films (Palou *et al.*, 2015).

So, the aim of this study was to produce natural films from soy proteins and lemon peel essential oil, which have an antibacterial and biodegradable in nature.

Material and methods

Soybean seeds

Soybean seeds (Glycine max L.) Lee 74 class Argentine origin. It was obtained from the Shorja market in Baghdad, Iraq.

Bacterial isolates

Six bacterial isolates: *Bacillus cereus*, *E. coli*, *E. coli* O157: H7, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* was obtained from Department of Food Science, College of Agriculture, University of Basrah, Iraq and grown on Nutrient broth (Hi-media, India) at 35ºC for 24 hours.

Soy proteins extract

Soybeans were grinded, 100gm of soy flour put in a 1L of sodium hydroxide 0.1N and mixed by a magnetic stir plate for two hours at room temperature then centrifuged at 3000rpm/min for 20min. Then, taking supernatant and precipitate by HCl 0.1N down to tie the electric point at pH=4.5there after centrifuge at 3000rpm/min for 20min. The precipitate dried, grinded and used for film preparation (Brandenburg *et al*., 1993).

Essential oil extract

Clevenger tool was used to extract essential oil of lemon peel. 100gm of lemon peel with 500mL of distilled water was heating at 90ºC for 2hrs. The essential oil was collected and estimated by calibrated tube. It was kept in the refrigerator (Al-sahlany, 2016). The GC-MS technique was used to identify the compounds of essential oils extracted from lemon peel by GC SHIMADZU QP2010 Ultra and gas chromatograph located in Laboratories Food Research and Consumer Protection, College of Agriculture/ University of Basrah.

Films preparation

Mixed 5gm of soy protein with 100mL of distilled water and addition of 50% (W: W) glycerol as a plasticizer, pH was adjusted to 8 with sodium hydroxide 1N. Continuously stirring and heating at 70ºC for 30min. After 24hrs at room temperature, the essential oil of lemon peel 0.05, 0.07 and 0.1ml was added to 10ml of film solution with stirring for 30min. The film solution was centrifuged at 3000rpm/min for 20min and pour in plastic dish. The film solution was dried for 48hrs at room temperature (Nayak *et al*., 2008).

Inhibition activity

0.1mL (10^6-10^8 CFU/mL) of bacterial isolates was spread on Nutrient agar (Hi-media, India). The disc 6mm of soy protein within and without essential oil film was put on the surface of culture media and incubated dishes at 35ºC for 24-48 hr. Inhibition activity determination by measuring diameters of clear round the discs of the film (Supardan *et al*., 2016).

Cheese coating

The white soft cheese obtained from Basra market in Basra, Iraq. 100gm of cheese was immersed in soy protein within 0.1mL of lemon peel essential oil and drying at refrigerator temperature for 48hr (Di Pierro *et al*., 2010). Microbial tests conducted total count bacteria (TCB), Total coliform bacteria (TC), Staphylococci count (ST) and Molds and yeasts count (MY). The numbers of microbial were estimated each 10 days during storage time for 30 days. The result was compared with the control sample of no coating cheese (Harley and Prescott, 2002).

Biodegradation of film

The soy protein within 0.1mL of lemon peel essential oil film and soy protein without essential oil film was buried in the soil depth 17cm (pH=7.5) for 35 days and calculated the percentage of film weight loss each 7 days as in the following equation.

Weight Loss (%) = $[(W_0 - W_t) / W_0] \times 100$

Where W_0: weight of films before burial in the soil, Wt: weight of film after a period of burial.

Statistical analysis

Standard deviation (SD) was estimated for the results of experiments in the study. The number of replicates was three per experiment (Kerns, 2011).

Results and discussion

Extraction of Soy protein and lemon peel essential oil

The yield of soy protein exaction was 15% (W: W) while 3% (V: W) of essential oil extract from lemon peel was obtained. 38 compounds found in essential oil of lemon peel, which has been detected by GC-MS analyzes. D-Limoneneis was the main compound in essential oil of lemon peel. The percentage of D-Limonene was 63.43% (Figure 1 and Table 1). The D-Limonene compound is a circular compound, colorless or yellowish, boiling point at 176ºC and has inhibition activity against microbial. D-Limonene compound was found in the citrus peel and the percentage of D-Limonene was different among citrus peel species. Peel three types of citrus (*C. reticulate*, *C. sinensis* and *C.paradisii*) contained 64.1-71.1% of D-Limonene (Kamal *et al*., 2011). D-Limonene percentage was 41.79% in sweet lemon peel while it was 83.03% in sour lemon peel (Kamaliroosta*et al*., 2016).

Soy protein film

The film producing from soy protein with 0.0, 0.05, 0.07 and 0.1 mL of lemon peel essential oil was transparent, bright, pure and homogeneous. It can be easily removed from the template and has the lemon odor (Fig. 2).

Inhibition activity of films

Inhibition activity of soy protein with lemon peels essential oil films against six species of bacteria are shown in (Table 2). Increasing the lemon peel essential oil concentration to soy protein films was increased the diameters of the inhibition zones while no inhibitory effect of the film without essential oil of lemon peel. *Staphylococcus aureus* was more sensitive among other species of tested bacteria. The diameter of the inhibition was 22mm when used soy protein film with 0.1mL of essential oil as inhibitor factor. *E. coli* O157: H7 was the least inhibitory that inhibition zone reached 18mm (Fig. 3). Inhibition activity of films due to the addition of lemon peel essential oil, which contains D-Limonene and other compounds. These compounds have inhibition activity against microorganism and can be used as a food preservative (Espina *et al*., 2013). von Vuuren and Viljoen (2007) reported that D-Limonene had inhibition activity against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Cryptococcus neoformans* while Santos *et al.* (2016) found that cellulose acetate films with orange essential oil had inhibition activity against *Escherichia coli*, *Staphylococcus aureus* and *Penicillium* spp.

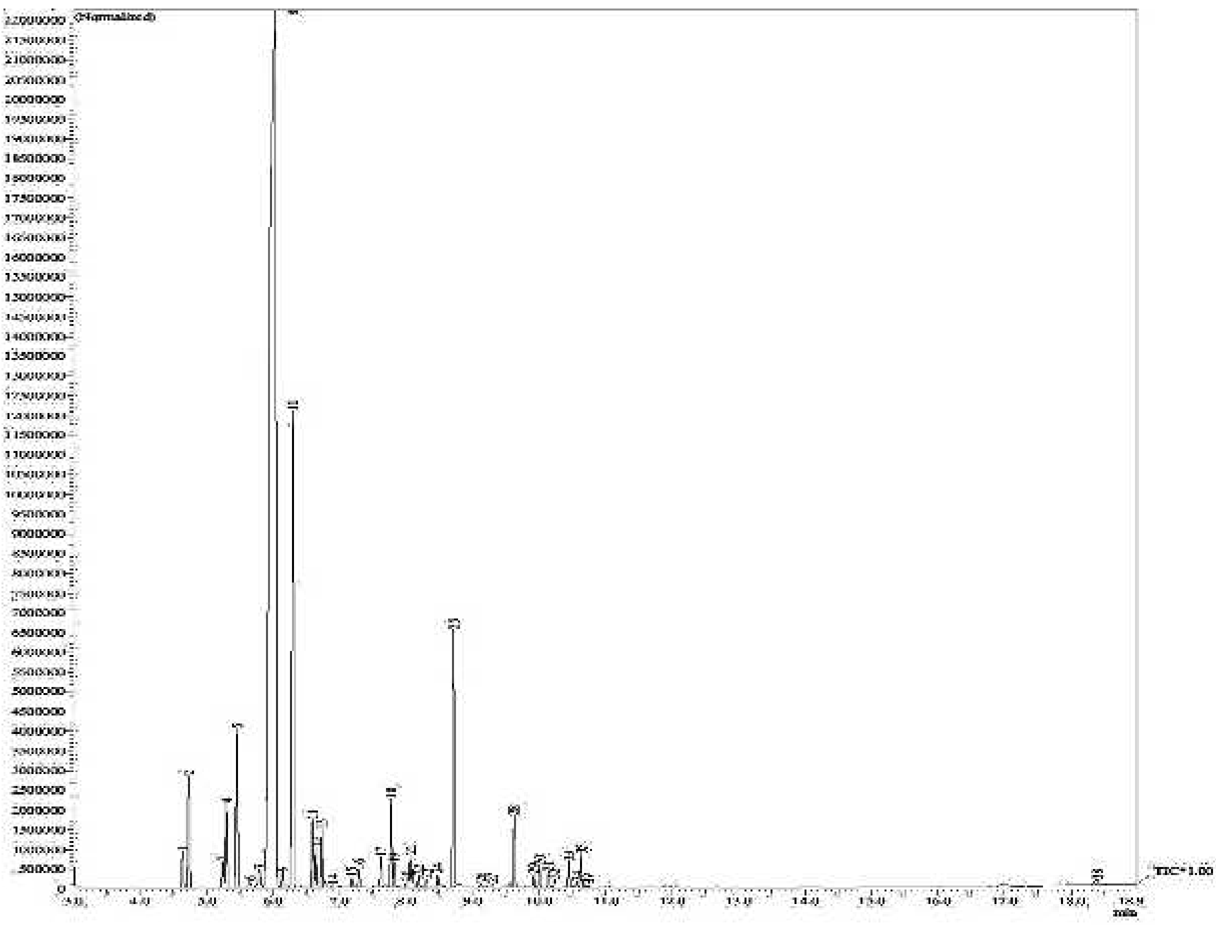

Fig. (1): GC-MS chromatogram of lemon peel essential oil.

Table (1): The compounds, concentration and retention time of essential oil extract from lemon peel.

Top No.	Compound	Concentration (%)	retention time (min)
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)	0.72	4.635
2	R-.alpha.-Pinene	2.30	4.728
3	beta.-Phellandrene	0.50	5.235
4	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene- (1S)	1.64	5.293
5	beta.-Myrcene	3.17	5.452
6	alpha.-Phellandrene	0.17	5.649
7	(+)-4-Carene	0.60	5.791
8	D-Limonene	63.43	6.024
9	1,3,7-Octatriene, 3,7-dimethyl	0.31	6.139
10	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	10.78	6.297
11	(+)-4-Carene	1.14	6.590

12	Benzene, 1-ethenyl-3,5-dimethyl	0.66	6.641
13	1,6-Octadien-3-ol, 3,7-dimethyl	0.95	6.733
14	1,4-Cyclohexadiene, 3-ethenyl-1,2-dimethyl	0.60	6.890
15	1,4-Cyclohexadiene, 3-ethenyl-1,2-dimethyl	0.15	7.187
16	7-Octenal, 3,7-dimethyl	0.31	7.292
17	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)	0.57	7.621
18	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl	1.54	7.774
19	(3S,4R,5R,6R)-4,5-Bis(hydroxymethyl)-3,6-dimethylcyclohexene	0.42	7.822
20	Tricyclo[3.3.0.0(2,8)]octan-3-one, 8-methyl	0.22	8.000
21	2-Octen-1-ol, 3,7-dimethyl	0.91	8.060
22	Cyclopropanecarboxaldehyde	0.21	8.190
23	2,6-Octadien-1-ol	0.19	8.307
24	3-Methyl-1-dodecyn-3-ol	0.19	8.479
25	Phenol, 2-methyl-5-(1-methylethyl)	0.29	8.714
26	Cyclohexene	4.90	9.129
27	2,6-Octadien-1-ol	0.08	9.300
28	1-ethenyl-1-methyl-2	0.07	9.632
29	Caryophyllene	1.20	9.925
30	Bicyclo[3.1.1]heptane	0.23	10.008
31	Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	0.39	10.143
32	1,4,7,-Cycloundecatriene	0.28	10.236
33	1H-Cyclopenta [1,3] cyclopropa [1,2] benzene,	0.15	10.450
34	1,5-Cyclodecadiene	0.44	10.573
35	Cyclohexene	0.13	10.634
36	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	0.56	10.675
37	Naphthalene	0.07	10.742
38	9,19-Cyclolanost-24-en-3-ol	0.21	18.381

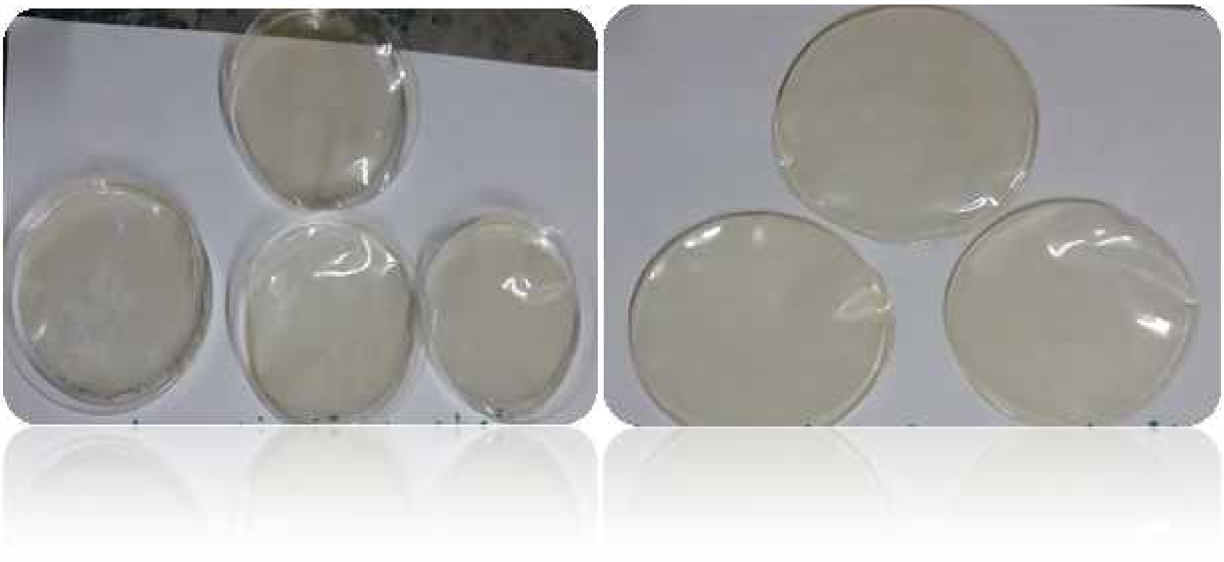

Fig. (2): Soy protein films with essential oil of lemon peel.

Table (2): Inhibition activity of soy protein with lemon peel essential oil against bacterial isolates (Mean±Sd).

Bacterial isolates	**Diameters of Inhibition zones (mm)**			
	Film without essential oil	Film 10mL with essential oil 0.05mL	Film 10mL with essential oil 0.07mL	Film 10mL with essential oil 0.1mL
Bacillus cereus	00±0.0	13±0.5	17±0.6	21±0.3
E. coli	00±0.0	11±0.3	16±0.5	19±0.5
***E. coli* O157: H7**	00±0.0	11±0.1	15±0.1	18±0.1
***Micrococcus* sp.**	00±0.0	12±0.5	17±0.2	20±0.6
Pseudomonas aeruginosa	00±0.0	11±0.1	15±0.1	19±0.2
Staphylococcus aureus	00±0.0	18±0.1	22±0.2	28±0.3

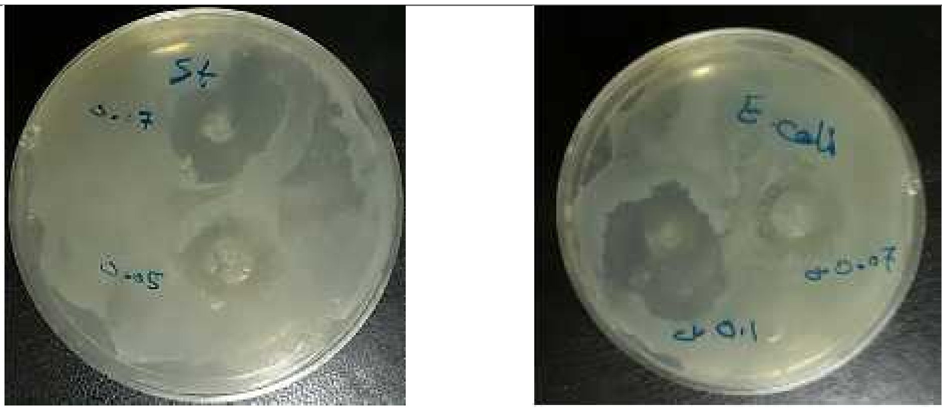

Fig. (3): Inhibition zones of soy protein film with lemon peel against bacterial isolates.

Total microbial numbers of cheese coating

Log. number of microbial of Iraqi white cheese after coating process by soy protein film with 0.1 mL of essential oil and during 30 days of storage time are shown in (Figure 4). The coating process of cheese by soy protein film with essential oil of lemon peel was reduced the numbers of microbial during storage time compared with cheese containing non-coating sample. Log. numbers of TBC, TC, and ST was 4.61,2.07 and 1.47 CFU/g and non-growth of MY in one-day storage. After 30 days of storage time, the microbial numbers of cheese coating were 4.28, 1.53, 1.62 and 1.33 Log. CFU/g for TBC, TC, ST and MY respectively while Log. numbers of cheese non-coating were 6.53, 2.51, 3.17 and 1.81 CFU/g for TBC, TC, ST and MY, respectively.

The presence of active compounds in the soy protein films due to adding essential oil of lemon peel were led to reduce the number of microorganisms and increase the shelf life of cheese. Numbers of microorganisms in cheese coating samples was within the limits allowed in the standard Iraqi standard specification for 2006 after 30 days while these numbers exceeded permissible limits during a storage time. The result of study agreed with Kavas and Kavas (2016) who found coating Kashar cheese by albumin protein film with essential oil of orange peel was reduced numbers of *E. coli* O157: H7, *Staphylococcus aureus* and *Listeria monocytogenes* during a storage time at 4ºC for 30 days.

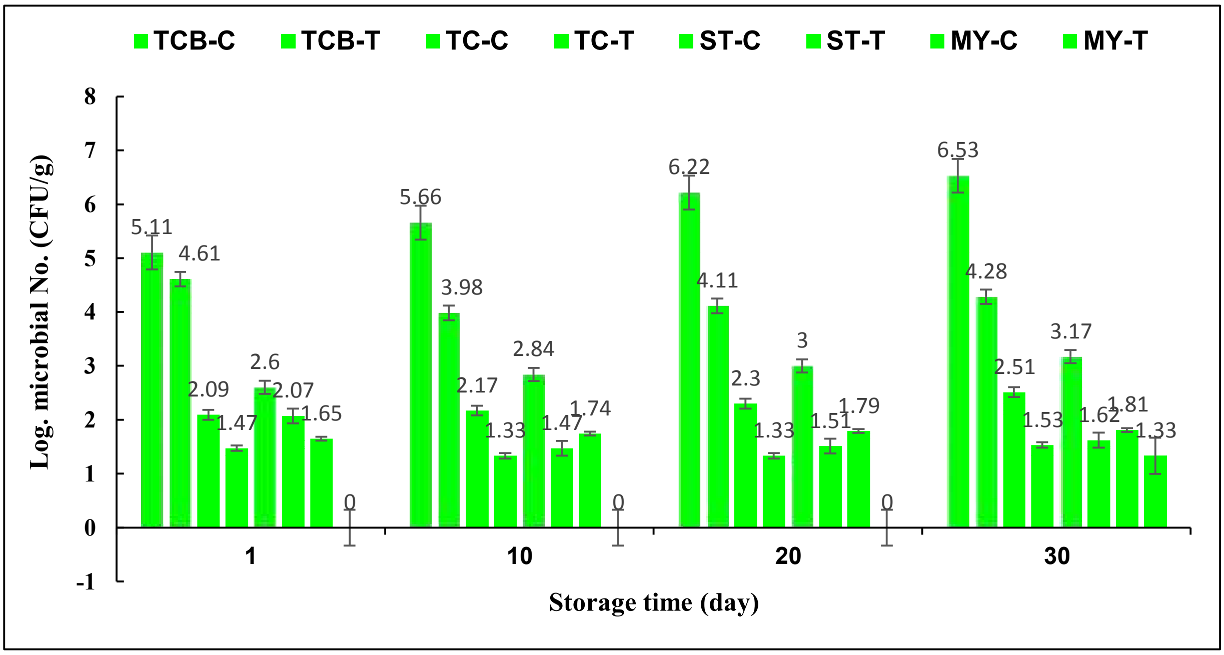

Fig. (4): Log. numbers of microbial in cheese sample, cheese coating (T), cheese non-coating (C) during storage time (30day).

Biodegradation of films in soil

Soy protein film within and without essential oil of lemon peel biodegradation percentage was 6% and 15% after 7day after burial in the soil. The loss in weight of the films was increasing during burial time. After 35 days, the percentage of biodegradation was 97% and 72% for soy protein film without essential oil of lemon peel and soy protein film within essential oil of lemon peel (Figure 5). Microorganisms in the soil and their enzymes are responsible for the degradation of films. Low percentage of the film degradation containing essential oil of lemon peel due to active compounds in essential oil which able to microbial inhibition. The result agreed with González *et al*. (2011) who pointed to biodegradation of soy protein films in the soil during 14-33day.

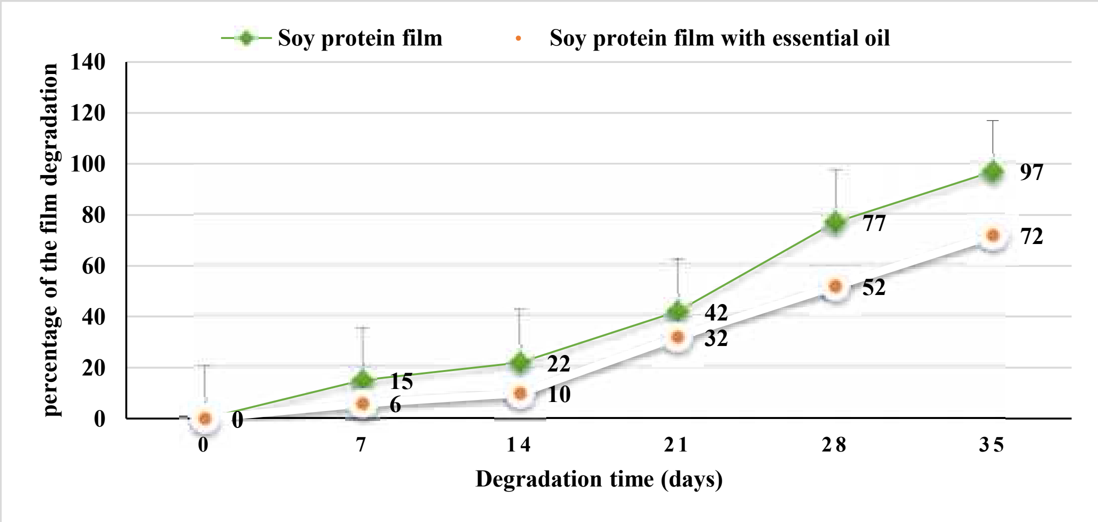

Fig. (5): Soy protein film within and without essential oil of lemon peel biodegradation in Soil.

Conclusions

The main purpose of this study was to make active biodegradable film from soy protein and essential oil of lemon peel. The use of natural inhibition activity materials as essential oil had the capability to with improve and possess inhibition activity against variety of bacteria spices. The process of biodegradation of films in the soil during short time (35 days) was improved the surrounding environment.

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References

Al-Bayati, A.A.H. (2005). Preparation of edible and biodegradable films and coatings from wheat gluten and corn zain. Ph.D. Thesis. Coll. Agriculture, Univ. Baghdad. 128pp.

Al-Sahlany, S.T.G. (2016). Effect of *Mentha piperita* essential oil against *Vibrio* spp. isolated from local cheeses. Pak. J. Food Sci., 26(2): 65-71.

Bourtoom, T. (2009). Edible protein films: properties enhancement, International Food Research Journal, 16:1-9.

Brandenburg, A.H.; Weller, C.L., & Testin, R.F. (1993). Edible films and coatings from soy protein. J. Food Sci., 58(5): 1086-1089.

Di Pierro, P.; Mariniello, L.; Sorrentino, A.; Villalonga, R.; Chico, B. & Porta R. (2010). Putrescine-polysaccharide conjugates as transglutaminase substrates and their possible use in producing cross-linked films. Amino Acids, 38: 669-675.

Djenane, D. (2015). Chemical profile, antibacterial and antioxidant activity of algerian citrus essential oils and their application in *Sardina pilchardus*. Foods, 4: 108-128.

Espina, L.; Gelaw, T.K.; de Lamo-Castellvi, S.; Pagan, R. & Garci´a-Gonzalo, D. (2013). Mechanism of bacterial inactivation by (+) Limonene and its potential use in food preservation combined processes. PLoS One, 8(2): 1-10.

González, A.; Strumia, M.C. & Igarzabal, C.I.A. (2011). Cross-linked soy protein as material for biodegradable films: Synthesis, characterization, and biodegradation. J. Food Eng., 106: 331-338.

Güven E.Ö.; Demirbilek, M. Sağlam, B.; Karahaliloğlu, Z.; Erdal, E.; Bayram, C. & Denkbaş, E.B. (2008). Preparation and characterization of Polyhydroxybutyrate scaffolds to be used in tissue engineering applications. Hacettepe Journal of biology and chemistry, 36(4): 305-311.

Harley, J.P. & Prescott, L.M. (2002). Laboratory Exercises in Microbiology. 5th ed., the McGraw-Hill Companies, USA. 449pp.

Iraqi standard specification (No.5/2270) (2006). Microbiological limits in food part 5 microbiological limits of milk and milk products. Ministry of Planning, Iraq.

Kamal, G.M.; Anwar, F.; Hussain, A.I.; Sarri, N. & Ashraf, M.Y. (2011). Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. International Food Research Journal, 18(4): 1275-1282.

Kamaliroosta, L.; Zolfaghari, M.; Shafiee, S.; Larijani, K. & Zojaji, M. (2016). Chemical identifications of Citrus peels essential oils. Journal of Food Biosciences and Technology, 6(2): 69-76.

Kavas, N. & Kavas, G. (2006). Physical-chemical and antimicrobial properties of Egg White Protein Powder films incorporated with orange essential oil on Kashar Cheese. Food Sci. Technol, Campinas, 36(4): 672-678.

Nayak, P.; Sasmal, A.; Nanda1, P.K.; Nayak, P.L.; Kim, J. & Chang, Y-C. (2008). Preparation and characterization of edible films based on soy protein isolate-fatty acid blends. Polymer-Plastics Technology and Engineering, 47: 466-472.

Niamah, A.K. & Alali, H.A. (2016). Antibacterial and antioxidant activities of essential oils extracted from Iraqi coriander (*Coriandrum sativum* L.) seeds Int. J. Sci. Eng. Res., 7(2): 1511-1515.

Palou, L.; Valencia-Chamorro, S.A. & Pérez-Gago, M.B. (2015). Antifungal edible coatings for fresh citrus fruit: A review. Coatings, 5: 962-986.

Santos, C.D.; Santos, R.R.; Ventura, L.A.F.; Melo, N.R.; Costa, B.S.; Rojas, E.E.G. & Salgado, N.L. (2016). Antimicrobial activity studies and characterization of cellulose acetate films containing essential oils. Ital. J. Food Sci., 28: 248-257.

Singh, J.; Kaur, L. & McCarthy, O.J. (2007). Factors influencing the physic chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications: A review. Food Hydrocolloids, 21 (1): 1-22.

Supardan, M.D.; Annisa, Y.; Arpi, N. Satriana, S. & Mustapha, W.A.W. (2016). Cassava starch edible film incorporated with lemongrass oil: characteristics and application. International Journal on Advanced Science, Engineering and Information Technology, 6(2): 216-220.

von Vuuren, S.F. & Viljoen, A.M. (2007). Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. Flavour and Fragrance Journal, 52(6): 540-544.

Xiang, L.; Tang, C.; Cao, J.; Wang, C.; Wang, K.; Zhang, Q. & Fu, Q. (2009). Preparation and characterization of soy protein isolate (SPI)/ montmorillonite (MMT) bionanocomposites. Chinese Journal of Polymer Science, 27(6): 843-849.