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Properties and antimicrobial activity of edible methylcellulose based film incorporated with *Pimpinella affinis* oil

Peiman Ariaii[1], Hamid Tavakolipour[1], Masoud Rezai[2] and Amir Hosein Elhami Rad[1]

[1]*Dept. of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Iran*
[2]*Dept. of Fishery Products Technology, Tarbiat Modares University, Iran*

ABSTRACT

Biodegradable polysacharyd-based film was developed by incorporating pimpinella affinis essential oil (PAO) into methylcellulose (MC) at level of 0.5%, 1% and 1.5% v/v. The effects of MC films containing PAO on physical, mechanical and antimicrobial properties of the films were evaluated. MC films containing PAO showed significant antibacterial activity against both gram-positive and gram-negative strains. Tensile strength and elongation at break were significantly ($p<0.05$) increased but water vapor permeability, and moisture were significantly ($p<0.05$) decreased with the incorporation of PAO. The contact angle water increased up to 84.40% in 1.5% PAO concentration. The color of MC films was affected by the addition of PAO; the lower transparency of the edible MC films was noticed when a greatest amount of PAO (1.5% v/v) was incorporated ($p<0.05$). MC film contains PAO provided the films with a rougher surface than pure edible film. The results suggest that, the films containing PAO can be used as a natural antibacterial agent and it has been useful for application in food preservation and packaging industry.

Keywords: methylcellulose, *pimpinella affinis*, antimicrobial activity, food packing.

INTRODUCTION

Recent food-borne microbial outbreaks are the driving force for seeking innovative ways to inhibit microbial growth in food while maintaining its quality, lipids and flavor components, freshness and safety. The development of antimicrobial packaging technologies could play a role in extending the shelf-life of food and provide microbial safety for consumers [1, 2]. Biopolymer films have received considerable attention in recent years because of their advantages over synthetic films. They are excellent vehicles for incorporating a wide variety of additives, such as antioxidants, antifungal agents, antimicrobials, colorants, flavors and fortified nutrients [3, 4].

Cellulose, the most abundant organic polymer in the world, and cellulose-derivative-based edible films are very efficient oxygen and hydrocarbon barriers, and aroma compounds and it is insoluble in water. Cellulose derivatives such as methylcellulose (MC) are of interest to researchers because they are able to form a continuous matrix. MC is the least hydrophilic cellulose ether, which shows thermal gelation and makes excellent edible films, and is used in pharmaceutical and food industries [5, 6]. MC has been combined with lipids [7- 10] and polysaccharides [6, 9, 11] to improve edible films that can serve as effective barriers to hydrocarbon, oxygen and water vapor. Polyethylene glycols are effective plasticizers for MC films [6, 12].

The antibacterial and antioxidant activities of plant extracts have formed the basis of many applications. Incorporating plant extracts and essential oils into edible films provides a novel way to enhance the safety and shelf life of foods [13]. Essential oils (EOs) have been extensively evaluated for their abilities to destroying the cell wall

and cytoplasmic membrane of bacteria and fungi, which leads to the leakage of cytoplasm, in inhibiting the synthesis of DNA, RNA, proteins and polysaccharides in bacteria and fungi, and in inhibiting the production of enzymes [14, 15].

The genus of *Pimpinella* with 23 wild species has been found in different regions of Iran, Anatolia, Jordan, Iraq, Soiree, Israel, Turkey, Afghanistan and Pakistan [16]. *P. affinis* is biennial aromatic plant, 20-110 cm, with white umbel inflorescences and ellipsoid. It grows wild in north of Iran [16, 17]. *Pimpinella affinis* oil has biological activities, such as antibacterial, antifungal properties. Major components of PAO which have showed antimicrobial properties are geijerene (17.68%), limonene (12.86%), Pregeijerene (9.92%), germacrene D (8.54%) and trans- β-cimene (4.94%) [17].

This present study was undertaken to improve the antimicrobial efficacy of edible film based on methylcellulose (MC) by incorporating *pimpinella affinis* essential oil (PAO). The physical, mechanical and antibacterial properties of edible films were also evaluated.

MATERIALS AND METHODS

Materials

Commercial MC was purchased from Sigma Aldrich (amrica). Polyethylene glycol and tween 80 was acquired from Merck (Frankfurt, Germany). The fruits *pimpinella affinis* were collected in March 2012, from the north of Iran. Essential oils were extracted by hydro-distillation from the dried samples by the Clevenger type apparatus, and the obtained oils stored in a dark container at 4°C until used.

Film Preparation

The MC films were prepared by the method of Turhan and sahbaz. [6]. 3% MC was dissolved into the ratio of 2:1 distilled water and ethanol and rotary shaking was undertaken concurrently for 30 min. As the edible MC film was brittle, 33% of Polyethylene glycol (PEG 400) was added to the edible film solution. Then Tween 80, at a level of 0.2% v/v of essential oil, was added as an emulsifier to aid essential oil dissolution in the MC film-forming solution, After 30 min of stirring, food grade PAO at 0.5, 1 and 1.5% v/v concentration was added to the MC film-forming solution. The solution was homogenized with homogenizer Model D500 (Wiggenhauser Machinenbau, 10965 Berlin, Germany) at room temperature for 2 min at 7000 rpm [18]. The solution was kept overnight at 4°C in order to remove all bubbles. Fourteen grams of the every solution were cast on the glass plates then dried in room temperature. Dried films were peeled from the plates and stored in a desiccator at 25–27°C and 50±2% relative humidity until evaluation.

Antibacterial activity

Antibacterial properties of edible film-forming solution and disks were studied using the agar diffusion method [19]. Seven different pathogenic and spoilage bacteria including *Staphylococcus aureus* (Persian Type Culture Collection (PTCC 1431), *Pseudomonas aeruginosa* (PTCC 1151), *Pseudomonas putida* (PTCC 1694), *Esherichia coli* (PTCC 3315), *Listeria monocytogenes* (PTCC 1163), *Bacillus subtilis* (ATCC 465), *Vibrio parahaemoliticus* were used for testing. Microorganism strains were cultured overnight in Brain Heart Infusion Broth (Scharlua, Spain) at 37°C. 70 ml of different film-forming solutions were poured into Mueller Hinton (Scharlua, Spain) agar wells (7.9 mm diameter).Their plates had been seeded with 0.1 ml of inoculums by swab containing approximately 10^6–10^7 CFU/ml of the indicated bacteria. In the same way, films were punched into discs of 13.4 mm diameter, and then placed on the plates. Next, the plates were incubated in desiccator at 37°C for 48 h. After incubation, the inhibitory zone was calculated, and then subtracted from the film disks' diameters. This difference was reported as the inhibitory zone of the film-forming solutions [19].

Determine of Physical Properties of Film

Film thickness

Thickness of the films was determined using a manual 0.001 mm digital micrometer (Mitutoyo, Mizonokuchi, Japan) at five random locations of the film sheets. Mean thickness values for each sample were calculated and used in water vapor permeability and tensile properties calculations.

Contact angle

Contact angle (CA) measurement was determined by a Goniometer (PG-X, Thwing-Albert Instrument Co., NJ) in a conditioned room. A small drop of distilled water was dropped on to the surface of the film. At least five replicates were made per formulation [18].

Moisture content
Moisture content of films was determined using about 50 mg of film were dried at 110°C during 24 h. The weight loss of each sample was determined, and the moisture content was calculated as the percentage of water removed from system [20].

Water vapor permeability (WVP)
The water vapor permeability (WVP) of the films was measured gravimetrically based on ASTM E96-95 method [21]. The tested film was sealed to the glass dish containing anhydrous calcium cholorid, 0% RH. A desicator maintained RH gradient across the film at 75%. In order to keep uniform RH throughout the desicator, the air was stirred in it. The RH inside the cell was always lower than outside. Transported water vapor was determined from the weight gain of the diffusion cell at a steady state of transfer. Weight changes of cells were recorded to the nearest 0.0001 g and plotted as a function of time. The slope of the weight loss v.s time was divided by the effective film area (m2) to obtain the water vapor transmission rate (WVTR). The WVP was then calculated as follow:

WVP= (WVTR×L)/ΔP)

Where L is the mean film thickness (mm), ΔP is the water vapor pressure difference (kPa) between two sides of the film.

Surface colour measurement
The color of the films was determined by colorimeter (BYK Gardner, MD). Film samples were placed on a standard plate (L^* = 93.49, a^* = -0.25 and b^* = -0.09). . Measurements are expressed as lightness (L) and chromaticity parameters a^* (red – green) and b^* (yellow – blue) were measured. Total color differences (ΔE) were calculated with respect to standard plate parameters by using following equation:

$$\Delta E = \sqrt{(L* - L)2\ (a* - a)2\ (b* - b)2}$$

Where L^*, a^* and b^* are the color parameter values of the standard and L, a and b are the color parameter values of the sample.

Determine of Mechanical properties of film
Tensile strength (TS) and elongation at break (E %) was measured according to ASTM standard method D 882–91 [22] with an Instron Universal Testing Machine (Model 200, Hiwa Engineering Co., Iran). The films were cut in rectangular specimens (2.54 × 10 cm). Initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively. This measurement was repeated

Film Microstructure
Control film and MC film incorporated with 1.5% concentrate PAO were examined with Philips XL 30 scanning electron microscope (SEM) (Philips Research, Eindhoven, the Netherlands) under high vacuum condition and at an electron voltage of 20.0 kV. The samples were mounted the specimen holder with aluminum tape and then sputtered with gold in BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein).

Statistical Analysis
One-way ANOVA was used and mean comparison was performed by Duncans' new multiple range test. Statistical analysis was prepared using the SPSS statistical software, (release 16.0) for Windows (SPSS Inc. Chicago, IL).All data are presented as mean ±SD.

RESULTS AND DISCUSSION

Antibacterial Properties
Effects of PAO on antimicrobial properties of MC based films are shown in Table 1. Film without PAO (control film) was not effective against any of the microorganisms used in the tests, which coincides with results obtained by Nonsee et al [23] about hydroxypropyl methylcellulose based films.

Films containing PAO showed considerable inhibition against most of the microorganisms. This can be explained by the fact that the addition of PAO into MC resulted in diffusion through the agar gel and provided a clear zone surrounding the film. Increasing the concentration of PAO in the film increased the diameter of inhibition zones ($p< 0.05$). Only between these microorganisms, *P. putida* and *Ps. Aeruginosa* are resistant to PAO and clear zone of inhibition was not observed with them. The same results have been reported by Verdian-Rizi et al (2008) their findings showed that PAO did not have any activity against *Ps. Aeruginosa.*

L. monocytogenes was the most sensitive bacteria at a level of 1.5% of PAO; the inhibition zone was 34.44. Gram positive bacteria were more susceptible than gram negative bacteria strains [24]. The existing inhibition zone diameter of tested microorganisms can be attributed to the fact that the mode of action of monoterpene competent especially, Limonene disintegrates the outer membrane of bacteria, releasing lipopolysaccharides and increases the permeability of the cytoplasm membrane to adenosine tri-phosphate (ATP) [25, 26].

Table1 Antibacterial activity (inhibitory zone) of MC films incorporated with PAO against gram-positive and gram-negative bacteria

Bacteria	PAO conc. (v/v) in film solution (%)	Inhibitory zone* (mm2)
V. parahaemoliticus (Gram -)	Control	0^{c}
	0.5	15 ± 1.73^{b}
	1	22.66 ± 1.52^{a}
	1.5	26.33 ± 1.52^{a}
B. subtilis (Gram +)	Control	0^{c}
	0.5	11.66 ± 1.52^{c}
	1	14.66 ± 1.15^{b}
	1.5	18 ± 1.01^{a}
E. coli (Gram -)	Control	0^{c}
	0.5	14 ± 1.00^{b}
	1	17.33 ± 1.15^{b}
	1.5	22.33 ± 1.52^{a}
P. putida (Gram -)	Control	0
	0.5	0
	1	0
	1.5	0
Ps. Aeruginosa (Gram -)	Control	0
	0.5	0
	1	0
	1.5	0
L. monocytogenes (Gram +)	Control	0^{d}
	0.5	19.00 ± 2.00^{c}
	1	26.00 ± 1.00^{b}
	1.5	34.33 ± 1.15^{a}
St. aureus (Gram +)	Control	0^{d}
	0.5	14.66 ± 1.52^{c}
	1	21.00 ± 3.00^{b}
	1.5	31.33 ± 1.52^{a}

**For each microbial species, different letters in columns indicate a significative difference ($p < 0.05$). Control is a film disc containing no essential oil.*

Physical Properties of films

The effects of incorporating PAO on the physical properties of MC films are shown in Table 2. Thin films were easily removed from the cast plate.

The thickness of films did not change significantly from incorporating PAO, ranging from 0.35 to 0.41 mm as shown in Table 2. The same results observed by Bahram et al [27] on whey protein incorporated with CAO.

Water sensitivity is one of the major problems of polysaccharide-based films, and is evaluated by different methods such as monitoring moisture content, solubility, contact angles and through the measurement of the water vapor permeability [24, 28]

CA of a water droplet on packing film indicates the hydrophobicity surface of the film. This is generally used to estimate the resistance of the film against water [29]. It is well-known that the water CA will increase with decreasing surface hydrophilicity. Table 2 shows the CA value for MC based films. The control film had low water CA, 36.33°. Adding PAO increased water CA significantly ($P < 0.05$) to 84.40% at a level of 1.5% v/v PAO and resulted increasing the hydrophobicity of the MC film, which might be due to the loss of free functional groups (amino and hydroxyl groups) [18].

The moisture content value decreased as PAO was incorporated into MC based film, which is attributed to compactness of film network. As PAO concentration increased (1.5% v/v), the moisture content of films decreased

significantly ($p < 0.05$). Ojagh et al [18] reported that cinnamon essential oil decreased moisture content of chitosan-based films.

The WVP is the most extensively studied property of biodegradable films mainly because of the importance of water in deteriorative reactions in foods. A main function of biodegradable films is often to impede moisture transfer between food and the surrounding atmosphere, or between two components of a heterogeneous food product. Therefore WVP should be as low as possible. WVP of MC-based films is reported in Table 2. The control film was 1.90 (g/msPa) 10^{-10}. Incorporating of PAO into MC based film formulation at level of 1.5% v/v led to 1.00(g/msPa) 10^{-10}reducing in WVP. Cellulose derivatives are relatively hydrophilic, but MC is more hydrophobic than common cellulose films such as cellulose acetate or cellophane [30, 31]. The incorporation of EOs into a polymeric matrix can improve the WVP of the films by increasing the hydrophobic compound in the film [24]. A permeability decrease with increase in PAO concentration could be related to the hydrophillicity of PAO. Introducing hydrophillic additives, favorable to adsorption and desorption of water molecules, is known to enhance the water vapor permeability of hydrocolloid biodegradable films [2, 32]. The same results were found by Nonsee et al [23] in encapsulated clove oil incorporation into HPMC films.

Color of the packaging is an important factor because they influence the consumer's perception of acceptability [33]. Edible MC films without the incorporation of PAO appeared clear and transparent and it had a slightly yellow appearance. The addition of PAO affected the appearance of the color of edible film and its transparency was reduced. The results of ΔE of MC films are shown in Table 2. Control film had ΔE 19.06 As PAO concentration increased, ΔE value increased significantly ($P < 0.05$), with the highest ΔE observed at a level of 1.5% v/v MC (30.23). The results are in agreement with the results of Abdollahi et al [4] and Bahram et al [27].

Table 2 Physical properties of MC films incorporated with PAO

*Physical properties	PAO conc. (v/v) in film solution (%)	
Thickness (mm)	Control	0.041±0.001[a]
	0.5	0.040±0.001[a]
	1	0.038±0.001[a]
	1.5	0.035±0.005[a]
Contact angle	Control	36.33±6.38[c]
	0.5	63.51±3.24[b]
	1	65.00±1.80[b]
	1.5	84.43±0.72[a]
Moisture content (%)	Control	17.18±2.13[a]
	0.5	13.90±0.56[ab]
	1	13.13±0.52[ab]
	1.5	12.66±0.80[b]
WVP (g/msPa) 10^{-10}	Control	1.90±0.11[a]
	0.5	1.80±0.17[a]
	1	1.20±0.21[b]
	1.5	1.00±0.03[b]
Total color difference (ΔE)	Control	19.06±0.05[d]
	0.5	25.72±0.18[c]
	1	26.85±0.10[b]
	1.5	30.23±0.02[a]

** Means in each column with different superscript letters are significantly different ($p < 0.05$).*

Mechanical Properties of films

Mechanical properties reflect the edible film's ability to protect the integrity of foods. Table 3 shows the mechanical properties of MC films. MC control film had tensile strength value 14.95 MPa. Incorporation of PAO into MC films decreased tensile strength values, but As PAO concentration increased (1.5% v/v), tensile strength values significantly increase ($P<0.05$) (19.50 MPa). It could be caused a strong interaction between the polymer and the PAO produced a cross-linker effect, which decreases the free volume and the molecular mobility of the polymer. A similar result was reported by Hosseini et al [20] on chitosan films incorporated cinnamon essential oil. The elongation value (E %) in control film was 25.6% as shown in Table 3. Incorporation of PAO (1.5% v/v) into MC films increased E% values significantly ($p < 0.05$) (50.0). A similar result was reported by Osés et al [34] on WPI film incorporated mesquite gum.

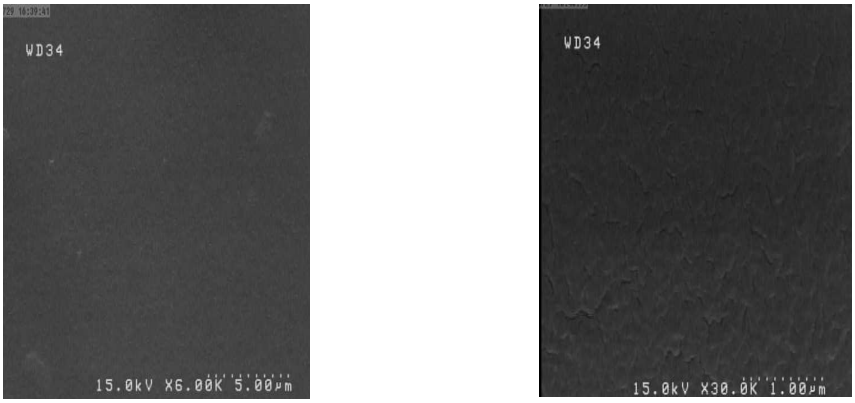
Table 3 Mechanical properties of MC films incorporated with PAO

*Mechanical properties	PAO conc. (v/v) in film solution (%)	
Tensile strength (MPa)	Control	14.95±2.16[b]
	0.5	14.09±1.27[b]
	1	10.61±1.59[b]
	1.5	19.50±0.44[a]
Elongation at break (%)	Control	25.62±0.91[b]
	0.5	24.93±0.12[b]
	1	23.47±0.53[b]
	1.5	50.0±0.10[a]

** Means in each column with different superscript letters are significantly different ($p < 0.05$).*

Film Microstructure

Figure 1 shows SEM micrographs of the MC based films. Control films were compact, and the film surface had a smooth contour without pores or cracks (Fig. 1a). The microstructure of the film containing PAO (1.5 % v/v) showed many pores and a cracked structure (Fig. 2b), which might be attributed to the evaporation of the EO during the drying process of the film. Bahram et al [27] reported the same structure for whey-based film incorporated with CEO.



(A) **(B)**

FIG 1 SEM microstructure of (A) MC film (B) MC film incorporated PAO (1.5 % v/v)

CONCLUSION

The edible MC films incorporated with *pimpinella affinis* oil exhibited antibacterial activity against the gram-positive and gram-negative bacteria tested. Increasing the *pimpinella affinis* oil in the edible MC films yielded a higher inhibition of tested pathogenic bacteria. Increasing PAO in the edible MC films improved WVP and the films' Moisture content. The color of edible MC films was darker and more yellowish as *pimpinella affinis* oil was increased. The CA test revealed that the improvements were attributed to hydrophobicity of the film when PAO was added. PAO incorporated in edible MC films provided the films with a rougher surface than that of pure edible MC films. Our results pointed out the films containing PAO (1.5% v/v) had unique properties that are useful for coating of highly perishable foods such as fish and poultry.

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REFERENCES

[1] Appendini P, Hotchkiss J H, *Food Sci EmTechnol*, **2002** 3:113–126.
[2] Thaworn J C, Chanthachum S, Wittaya T, *Food Science and Technology*, **2004** 44:284-292.
[3] Rhim J, Hong S, Park H, Ng P, *J Agricultural and Food Chemistry*, **2006**, 54 (16):5814–5822.
[4] Abdollahi M, Rezaei M, Farzi G, *J Food Eng*, **2012**, 111:343-350.
[5] Donhowe I G, Fennema O, *J Food Processing and Preservation*, **1993**, 17:247– 257.
[6] Turhan K, Sahbaz F, *J Food Engineering*, **2004**, 61:459–466.
[7] Greener I K, Fennema O, *J Food Science*, **1989,** 54(6):1393–1399.
[8] Kamper S L, Fennema O, *J Food Science*, **1984**, 49:1478–1481.
[9] Kester J, Fennema O, *J Food Science*, **1989**, 54(6):1383–1389.

[10] C Koelsch C M, Labuza T P, *Lebensmittel-Wissenschaft und-Technologie*, **1992**, 25(5):404–411.
[11] Park H J, Chinnan M S, *J Food Process Engineering*, **1995**, 25:497–507.
[12] Debeaufort F, Voilley, A, *J Agricultural and Food Chemistry*, **1997**, 45:685–689.
[13] Cagri A, Ustunol Z, Ryser E T, *J Food Sci*, **1991**, 66: 865–870.
[14] Burt S, *International Journal of Food Microbiology*, **2004**, 94 (3):223–253.
[15] Kraśniewska K, Gniewosz M, *Pol J Food Nutr Sci*, **2012**, 62 (4):199-206.
[16] Mozaffarian V, *Farhang Moaser Tehran Iran*, **1996**,412-414.
[17] Verdian-Rizi M, *Research journal of biological sciences*, **2008**, 3 (8): 913-915.
[18] Ojagh S M, Rezaei M, Razavi S H, Hosseini M H, *Food Chemistry*, **2010**, 122 (1): 161–166.
[19] Gómez-Estaca J, López de Lacey A, López-Caballero M E, Gómez-Guillén M C, Montero P, *Food Microbiology*, **2010**, 27 (7): 889–896.
[20] Hosseini M.H, Razavi S H, Mousavi M A, *J Food Process preserv*, **2009**, 33: 727-743.
[21] ASTM, *Annual book of ASTM Philadelphia PA*, American Society for Testing and Material, **1995**.
[22] ASTM, *Annual book of ASTM. Philadelphia PA*, American Society for Testing and Material, **1996**.
[23] Nonsee K, Supitchaya C, Thawien W, *International Food Research Journal*, **2011**, 18(4):1531-1541.
[24] Sánchez-González L, Vargas M, González-Martínez C, Chiralt A, Cháfer Mt, *Food Rev Eng*, **2011**, 3:1-16.
[25] Oussalah M, Caillet S, Saucier L, Lacroix M, *Food Control* , **2007**, 18:414-420.
[26] Askari F, Sefidkon F, Teimouri. M, Youse f, Nanaei S, *J Agr Sci Tech*, **2009**, 11:431-438.
[27] Bahram S, Rezaei M, Soltani S, Kamali A, Ojagh M, Abdollahi M, *J Food Processing and Preservation*, **2013**, 1745-4549.
[28] Joana T, Miguel A, Cerqueira António A, *Food Hydrocolloids*, **2012**, 27:220-227.
[29] Phan T D, Debeaufort F, Luu D, voilley A, *J Agric and Food Chem*, **2005**, 53:973-981.
[30] Hoeve J, *American Chemical Society, Washington, DC*, **1980**, 134- 146.
[31] Debeaufort F, Voilley A, Meares P, *J Membrane Science*, **1994**, 91:125-133.
[32] Gontard N, Duchez C, Cuq J L, Guilbert S, *J Food Science*, **1993**, 49:1482-1485.
[33] Bourtoom T, Chinnan M S, *LWT - Food Science and Technology*, **2008**, 41:1633-1641.
[34] Osés J, Fabregat-Vázquez M, Pedroza-Islas R, Tomás S A, Cruz-Orea A, Maté J I, *J Food Engineering*, **2009**, 92:56-62.