ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal
www.orientjchem.org
Est. 1984

ISSN: 0970-020 X
CODEN: OJCHEG
2021, Vol. 37, No.(2):
Pg. 362-367

Physico-chemical Characterization of Edible Packaging Film Supplemented with *Ocimum* (Tulsi) Essential oil

SIMRANPREET KAUR and CHAYANIKA PUTATUNDA*

Department of Microbiology, DAV University, Jalandhar, Punjab, India.
Present Address- Om Sterling Global University, Hisar, Haryana, India.
*Corresponding author E-mail: putatunda7@gmail.com

http://dx.doi.org/10.13005/ojc/370214

(Received: December 24, 2019; Accepted: March 16, 2021)

ABSTRACT

The concern about the possible adverse health effects of plastic based food packaging as well as the environmental pollution caused by plastics has lead to efforts in looking for alternative, eco-friendly and biocompatible food packaging materials. In the present investigation, a composite edible film was prepared from whey, pectin and gelatin, which was supplemented with *Ocimum* essential oil. These films were characterized for thickness, transparency towards visible and UV-light, and total moisture content. Antimicrobial activity of these films were examined against *Escherichia coli* MTCC 118, *Pseudomonas aeruginosa* MTCC 741, *Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 1272 and *Pseudomonas fluorescens* MTCC 103. The film activity was found to be maximum against *B. cereus* with 8 mm zone of inhibition on addition of 6% of *Ocimum* essential oil. The films were also active against all other pathogens except *P. aeruginosa*.

Keywords: Edible packaging, Whey, Pectin, Gelatin, *Ocimum*, Anti-bacterial.

INTRODUCTION

The numerous benefits of edible packaging such as biodegradable nature, cost effectiveness, being pollution free, potential carrier for antioxidants or antimicrobials, nutritional, safe to consume, good barrier to gases and moisture, shelf life enhancement etc. has increased adoption of edible films as an alternative method of packaging by manufacturers and this factor heightens growth of the global edible films and coatings in the market. The edible films can be prepared from polysaccharides, proteins, and lipids; the polysaccharides used are starches and modified starches, chitosan, alginates, gums, cellulose derivatives and pectin[1]. These edible films prevent moisture loss and causes controlled exchange of gases, which are necessary for respiration. Normally proteins and polysaccharides derived edible films are fragile and thus, plasticizers are to be integrated in order to increase its pliability. Commonly used plasticizers are monosaccharides, oligosaccharides, polyols, lipids, and their derivatives.

On the other hand, use of preservatives has increased in the food industry to protect food from getting damaged by microbes and increasing the food shelf life. However, many of the commonly used chemical preservatives have been found to have detrimental effects on the human beings[2]. So, now more stress is being laid on the usage of naturally derived antimicrobial agents. According to Burt[3], the secondary metabolites of plant especially essential oil (EO) is odorous, volatile product, normally formed in special cells or groups of cells, are well known for its antimicrobial potential that could be used to control food spoilage and food-borne pathogenic bacteria. EOs consists of number of small terpenoid and phenolic compounds which exhibit antimicrobial activity if present in pure forms. Plant EOs and their derivatives are becoming more popular now a days because of consumer demands for less chemical used food or minimally processed food. These antimicrobials such as plant extracts, spices, organic acids provides organoleptic and antioxidants properties to the packaged food[4]. The use of these antimicrobial compounds can replace use of preservatives making food more nutritional and healthy.

Pectin has properties like reducing water permeability[5]. Pectin forms gel under certain conditions this can be used as edible film packaging material[6].On the contrary, whey comes up with better gas and mechanical barrier than polysaccharides and show higher intermolecular binding potential[7]. Higher intermolecular bonding aid functional properties to proteins. It was observed that proteins are poor water vapor barrier and this ultimately affects its mechanical strength. Plasticizer such as glycerol, sorbitol etc. are able to ameliorate the conditions and help preparing protein films with better mechanical strength. Thus, they are well known in preparation of edible films. *Ocimum* is commonly called “Queen of Herbs” because of providing many clinical and medicinal benefits. Ocimum oil and extract contain linalool, the eugenol, carvacrol, camphor, methyl cinnamate and β-caryophyllene as their active components[8]. These compounds aid antimicrobial activity to *Ocimum* essential oil.

Nowadays, the awareness has been observed in consumers for demanding healthy, nutritional, food without plastic or PVC packaging, preservatives free food etc. Increasing awareness is resulting in increasing market for edible films. Thus, on this basis pectin, whey protein, gelatin integrated with *Ocimum* essential oil were utilized for synthesizing the composite edible biofilm. Although, earlier many sets of combinations to develop edible films have been studied but, pectin film, whey protein film along with *Ocimum* have not been reported yet. The main objective to conduct this study was to prepare and characterized edible biofilm made from the edible source containing *Ocimum* essential oil as antimicrobial agent.

MATERIALS AND METHODS

Materials

Ocimum essential oil of therapeutic grade was procured from local market. As, *Ocimum* essential oil is GRAS qualified thus, is food grade also. Whey used was of food grade; the rest of the chemicals like Pectin, gelatin, glycerol, Cween 20, media components etc. used in present study were of analytical grade. The bacterial strain of *Escherichia coli* (MTCC 118), *Pseudomonas aeruginosa* (MTCC 741), *Staphylococcus aureus* (MTCC 96), *Bacillus cereus* (MTCC 1272) and *Pseudomonas fluorescens* (MTCC 103) used for determination of antibacterial activity were obtained from MTCC i.e. Microbial Type Culture Collection and Gene Bank, IMTECH, Chandigarh, India.

Preparation of edible film solution

Whey protein (8%), Pectin (1%) and Gelatin (15%) were first dissolved in distilled water and pH of the solution was adjusted to 8.0. After adjusting pH, the mixture was heated to 90°C for half an hour with shaking in water bath. Plasticizer i.e. glycerol in the ratio of 1:1 and Cween 20, an emulsifier at a level of 0.2% v/v, was added to the solution to help dissolution of film. Then, mixture was placed for magnetic stirring for 30 minutes. The resulting solution was the control film. Five grams of the solution was cast on 90 mm plastic Petri plates then placed in Vacuum desiccator for drying at room temperature for 72 hours. Dried films were then peeled off from the plates and stored at ambient temperature as stated by Seydim and Sarikus (2006). This resulted in standalone film (C). For preparation of Essential supplemented film, *Ocimum* essential oils were added to the control film forming solution at 1%, 2%, 3%, 4%, 5% and 6% v/v concentration. After addition of oil another round of magnetic stirring was carried out for 30 min at room temperature (Sharma *et al.,* 2017). Five grams of the solution was cast on 90 mm plastic Petri plates then placed in Vaccum dessicator for drying at room temperature for 72 hours. Dried films were then peeled off from the plates. These were designated as C+1 (with 1% *Ocimum* essential oil), C+2 (with 2% *Ocimum* essential oil), C+3 (with 3% *Ocimum* essential oil), C+4 (with 4% *Ocimum* essential oil), C+5 (with 5% *Ocimum* essential oil) and C+6 (with 6% *Ocimum* essential oil).

Assessment of Antimicrobial Activity

Agar well diffusion was carried out on Muller Hinton agar plates to analyse the antimicrobial activity of the composite film prepared from whey, pectin, gelatin and essential oil of *Ocimum*[9]. The anti-microbial activity was assessed against *Escherichia coli* MTCC 118, *Pseudomonas aeruginosa* MTCC 741, *Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 1272 and *Pseudomonas fluorescens* MTCC 103.

Characterization of film

Film thickness

Films sections were cut from different positions. Thickness of these cut pieces were measured using micro meter of resolution 0.01mm. Films were placed in the two pieces of plastic strips and then thickness of strips was subtracted from the total thickness. After having all the thickness values of the sections, the mean value was calculated[10].

Moisture content

To know moisture content of films, films were exposed to temperature of 60°C in a hot air oven till appearance of constant weight. The moisture content of films by following formula viz. Moisture Content (%) = (I.W.–F.W.)/ I.W. X 100 where, I.W. = Initial Weight and F. W = Final Weight.

Transparency determination

Double beam UV-Vis spectrophotometer (UV-Vis 1800, Shimadzu) was used to measure transparency. Long rectangular thin pieces of the films were cut and stacked in quartz cuvette to take readings at 550nm and 280nm. According to Han *et al.,*[11], formula used to calculate transparency (T) is $T = A_{550}$/film thickness, where, A = absorbance of the film at a wavelength of 550 nm. To calculate the transparency in UV range formula used, $T = A_{280}$/ film thickness, where, A = absorbance of the film at a wavelength of 280nm.

RESULTS AND DISCUSSIONS

Antimicrobial potential of composite edible film

Antibacterial potential was analysed for Control film and *Ocimum* essential oil supplemented films against *Escherichia coli* MTCC 118, *Pseudomonas aeruginosa* MTCC 741, *Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 1272 and *Pseudomonas fluorescens* MTCC 103. Ampicillin (1µg/mL) was used as control. The aim of our study was to prepare edible packaging which may enhance shelf life of food. So, it is necessary to protect food product from physical as well as biological damage. Packaging must prevent microbial attack on food. Control films showed no inhibitory activity on the test microorganisms. Thus, it needed to be added with essential oil as antimicrobial agent to make them active. *Ocimum* essential oil has been reported to show good inhibitory effects against many gram negative and gram positive bacteria[12-13]. At 1% supplementation concentration however no inhibitory effect was observed. On increasing the concentration, increase in the inhibitory effect was observed in case of all the test microbes except *Pseudomonas aeruginosa* MTCC 741 (Table 1). C+6% was found to result in zone of inhibition (8 mm) comparable with that of ampicillin (8.4 mm) in case of *B. cereus. P. fluorescens* was found to be susceptible at 3% onwards and exhibited equal zone of inhibition i.e. 1.4mm at C + 5% and C + 6%. It may not be affected further on increasing oil concentration to the control films. In case of *P. aeruginosa* even at 6% addition of *Ocimum* essential oil to the film, no anti-microbial activity was observed. It may be effected by control film with higher concentration of essential oil[14]. The extraction methods or stage of extraction of essential oil affects its antimicrobial activity[14].

Table 1: Antimicrobial activity of edible films with different concentration of *Ocimum* essential oil against different bacteria

	Zone of inhibition (in mm)							
MTCC cultures	Ampicillin	C	C+1	C+2	C+3	C+4	C+5	C+6
Pseudomonas aeruginosa MTCC 741	-	-	-	-	-	-	-	-
Staphylococcus aureus MTCC 96	8.4	-	-	0.5	1	1.6	1.8	3
Bacillus cereus MTCC 1272	8.4	-	-	0.9	1.4	4.6	5.4	8
Pseudomonas fluorescens MTCC 103	8.2	-	-	-	1.2	1.3	1.4	1.4
Escherichia coli MTCC 118	7.8	-	-	-	1.1	1.6	1.8	2

Characterization of films

The effect of supplementation of *Ocimum* essential oil on the color of the edible film was assessed by visual comparison of the film colors. Film solution and standalone film so produced was pale yellow in colour, homogenous and transparent. The change in colour was observed by addition of *Ocimum* essential oil to the film. The colour of film changed to yellow and went on acquiring darker hue of yellow with increasing concentration of oil. Rhim *et al.,*[15] reported that the addition of any compound that have capability of binding structurally to the film forming solutions changed the native color of soy film. Du *et al.,*[10] reported that darker films produced with addition of cinnamon, allspice, and clove bud oils to the film forming solution which means that color of the film was directly proportional to concentration of oil used.

Estimation of thickness of an edible film

The film sections were cut from different positions. Thickness of these cut pieces were measured using micro meter. The thickness of all the films were observed to be less than 0.3 mm with maximum value of 0.214 shown by C + 6% and minimum value of 0.12 shown by C. As per Pavlath and Orts[16] (2009) the thickness of edible films should be less than 0.3 mm, hence thickness-wise all the films were found to be suitable for being used as edible films for food packaging. Essential oil is hydrophobic in nature. According to Bertan *et al.,*[17], increase in thickness of biofilm is resulted by addition of hydrophobic substance. It is observed that as we go on increasing oil concentration to the film solution, thickness and tensile strength of film also goes on increasing as shown in Table 2. An addition of essential oil to an edible film resulted in increase in its tensile strength[9]. In similar research by Du *et al.,*[18], an edible film prepared was incorporated with polyphenols from apple skin contributing strengthening to the edible films. It was also concluded that thickness of film increases with increase in concentration of polyphenols.

Estimation of transparency of film

Films can be transparent or opaque. Transparency is another interesting factor that may decide the cost of food treated with an edible film. Transparent films will be more attractive than thick films and hence may change appearance of food. UV transmittance has significance in arresting oxidative degradation of fat-based food products. On absorbing UV-light, the films are expected to help in increasing shelf life of fat based food items. Films were analysed by using UV-Vis spectrophotometer (double beam spectrophotometer) at wavelength of 280nm and 550nm for measurement of UV opacity and transmittance respectively. The opaqueness of the edible films was found increasing on increasing the concentration of *Ocimum* essential oil. The increase in opacity causes more light to be absorbed and less light to be transmitted through the film. The C + 6% film showed minimum transmittance (%) of 1.840 and 0.0086 at 550nm and 280nm respectively. However control film showed maximum transmittance (%) of 56.104 and 0.0176% at 550nm and 280nm respectively. Thus, it was found that on increasing concentration of essential oil, transmittance goes on decreasing. In the similar findings by Atares and Chiralt[19], it was found that on increasing concentration of essential oils to the films transparency goes on decreasing. Control film showed transmittance of 56.104% and UV opacity i.e. 0.0176. It showed sharp decline in transmittance when control film was added with 1% essential oil (37.239%). However, after addition of essential oil transmittance dropped to 1.840% on 6% addition of *Ocimum* essential oil (Table 2). It showed sharp decline when control film was added with 1% essential oil (37.239%).

Estimation of moisture content of the film

Moisture content was calculated to know the presence of water in the film. Moisture content of edible films may cause variation in taste, weight, shelf life of food materials etc. The edible film must retain water content and must acts like a good water barrier in order to prevent water loss from the film. On the contrary high moisture level can enhance the chance of spoilage by food borne pathogens. Moisture content was found to be decreased by increasing concentration of essential oil (Table 2). A sharp decrease in moisture content was observed with addition of 1% of oil (C + 1) to the control film (C). From Table 2, it is concluded that on addition of essential oil to film, moisture content goes on decreasing, although the decline of moisture content was relatively lesser in C +2% to C + 6%. But all edible films containing essential oil of *Ocimum* were found to have less moisture content as compared to control films. This is possibly associated with the hydrophobic nature of essential oil. In a research conducted by Ariaii *et al.,*[20], it was observed that addition of *Pimpinella affinis* oil to methylcellulose based film caused decrease in moisture content of film. It aids in compactness of film network. A significant decrease in moisture content was observed with increase in concentration of oil. Ojagh *et al.,*[21] made similar observations on addition of cinnamon essential oil to chitosan based films.

Table 2: Characteristics of the edible films containing different concentration of *Ocimum* essential oil

Sr. No.	Edible Films	Thickness (mm)	Film opacity at (550nm)	Film opacity at (280nm)	Moisture Content (%)
1	C	0.12	56.104	0.0176	36.87
2	C +1 %	0.155	37.239	0.0112	21.46
3	C + 2%	0.17	36.728	0.0103	21.32
4	C + 3%	0.177	10.839	0.0086	21.28
5	C +4%	0.194	5.88	0.0086	20.53
6	C +5%	0.207	3.935	0.0086	20.09
7	C+ 6%	0.214	1.84	0.0086	18.83

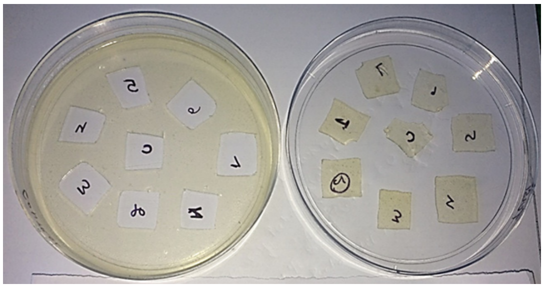

Fig.1. Control film showing pale yellow color

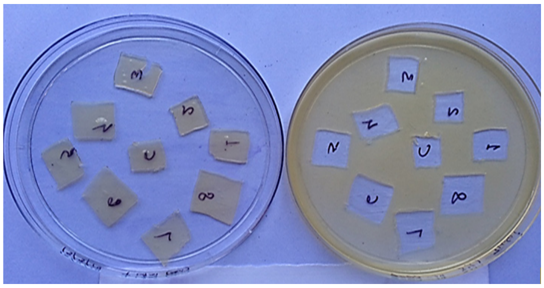

Fig. 2. C+6% edible film showing colour change to dark yellow C+6%

CONCLUSION

The widespread use of plastic wrapping leads to accumulation of plastic waste in the environment. Extensive use of such wrapping causes many health issues to arise. Motive of our study was to prepare an edible film from natural sources as an alternative packaging. The composite film consisting of whey (8%), pectin (1%) and gelatin (15%) incorporated with *Ocimum* essential oil to provide it with antimicrobial properties. The antimicrobial activity of edible films containing 1% to 6% essential oil against five pathogens i.e. *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Pseudomonas fluorescens* was observed. The composite film incorporated with *Ocimum* essential oil was found to be more effective against *Bacillus cereus.* Zone of inhibition was found to be comparable with the zone of inhibition formed by ampicillin i.e. 8mm. However, no antimicrobial effect was observed against *Pseudomonas aeruginosa* even at 6% addition of *Ocimum* essential oil. It may be affected at high percentage of essential oil. Thus, it is necessary to keep going with this research to obtain composite edible film with better physical properties which can be used for making edible packaging material in the near future.

ACKNOWLEDGEMENT

The authors acknowledge the DAV University, Jalandhar, Punjab, India for providing the necessary facilities for carrying out the present work.

Conflict of Interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Krochta, J.M.; Mulder-Johnston, C.D. *Food Technol.*, **1997**, *51*, 61-74.
2. Linke, B.G.O.; Casagrande, T.A.C.; Cardoso, L.A.C. *Afr. J. Biotechnol.*, **2018**, *17*, 306-310.
3. Burt, S. *Inter. J. Food Microbiol.*, **2004**, *94*, 223-253.
4. Bourtoom, T. *Inter. Food Res. J.*, **2008**, *15*, 237-248.
5. Xu, Y.X.; Kim, K.M.; Hanna, M.A.; Nag, D. *Indus. Crops Prod.*, **2005**, *21*, 185-192.
6. Han, J.H. Innovations in food packaging. USA: Elsevier Academic Press., **2014**.

7. Jolie, R.P.; Duvetter, T.; Van Loey, A.M.; Hendrickx, M.E. *Carbohydrate Res*., **2010**, *345*, 2583-2595.
8. Kayastha, B.L. *J. Med. Plants Studies*., **2014**, *2*, 1-8.
9. Sharma, D.; Dhanjal, D.S.; Mittal, B. *J. App. Pharma. Sci*., **2017**, *7*, 160-164.
10. Du, W.X.; Olsen, C.W.; Avena-Bustillos, R.J.; McHugh, T.H.; Levin, C.E.; Friedman, M. *J. Food Sci*., **2009**, *74*, M372-M378.
11. Han, J.H.; Floros, J.D. *J. Plastic Film Sheet.,* **1997**, *13*, 287-98.
12. Kpadonou Kpoviessi B.G.; Ladekan, E.Y.; Kpoviessi, D.S.; Gbaguidi, F.; Yehouenou, B.; Quetin-Leclercq, J.; Figueredo, G.; Moudachirou, M.; Accrombessi, G.C. *Chem. Biodiver*., **2012**, *9*,139-150.
13. Yamani, H.A.; Pang, E.C.; Mantri, N.; Deighton, M.A. **2016**, *7*, 681.
14. Saharkhiz, M.J.; Kamyab, A.A.; Kazerani, N.K.; Zomorodian, K.; Pakshir, K.; Rahimi,, M.J. Jundhishapur *J. Microbiol*., **2014**, *8*, e13720.
15. Rhim, J.W.; Gennadios, A.; Handa, A.; Weller, C.L.; Hanna, M.A. *J. Agric. Food Chem*., **2000**, *48*, 4937–4941.
16. Pavlath, A.E.; Orts, W.Edible Films and Coatings: Why, What, and How? In Embuscado M, Huber KC eds. Edible Films and Coatings for Food Applications. New York: Springer-Verlag., **2009**, 1-23.
17. Bertan, L.C.; Tanada-Palmu, P.S.; Siani, A.C.; Grosso, C.R.F. *Food Hydrocolloid*., **2005**, *19*, 73-82.
18. Du, W.X.; Olsen, C.W.; Avena-Bustillos, R.J.; Friedman, M.; McHugh, T.H. *J. Food Sci*., **2011**, *76*, M149-55.
19. Atarés, L.; Chiralt, A. *Trends Food Sci. Technol*., **2016**, *48*, 51-62.
20. Ariaii, P.; Tavakolipour, H.; Rezaei, M.; Elhami Rad, A. H.; Bahram, S. *J. Food Process. Pres*., **2014**, *39*, 1647-1655.
21. Ojagh, S.M.; Rezaei, M.; Razavi, S.H.; Hosseini, M.H. *Food Chem*., **2010**, *122*, 161–166