Effect of Carom Seed Oil on the Antimicrobial, Physicochemical and Mechanical Properties of Starch Based Edible Film

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***Abstract*— *Packaging material is necessary in the preservation process. Edible films containing essential oils can be incorporated into the conventional food packaging systems with a dual purpose, edible and natural preservative, that can maintain quality, extend the shelf life and reduce the risk of pathogen growth specifically in unprocessed or minimally processed foods like fruits and vegetables. In present study, pumpkin-arrowroot starch based edible film incorporated with carom seed oil at 0.5%, 1% and 1.5% were prepared and studied for the antimicrobial properties. Film with 1.5% Carom seed oil showed exceedingly good antimicrobial activities against E. coli, Staphylococcus and Aspergillus. The films were further studied for physical, mechanical and water vapour transmission properties. The results indicated that the film with 1.5% carom seed oil did not alter the mechanical properties of the film significantly, compared to control film and is ideal for coating to extend the shelf life of food products.*

***Keywords*— Edible film, carom seed oil, antimicrobial activity, mechanical properties.**

I. INTRODUCTION

Edible films are the films made using edible material and can be used on various food products to achieve their role in preservation of products (Fakhouri*etal*. 2015). The function of edible films is to provide mechanical integrity or handling characteristics to the food. These films can also act as carriers of active ingredients, such as antioxidants, flavours, fortified nutrients, colorants, anti-microbial agents, or spices (Regalado *et al*. 2006). Novel edible materials have been derived from many natural sources that have conventionally been regarded as discarded materials or even the low-cost sources which are yet to be regarded as a potential base for edible films (Shit and Shah 2014). Pumpkin is one of the well-known edible plants that is grown all year round. India is the second largest producer of pumpkin producing 35,500,000 metric tonnes per year (FAO, 2008) after China. It contains several phyto-constituents belonging to the categories of alkaloids, flavonoids, and palmitic, oleic and linoleic acids (Yadav *et al*. 2010). It can be used as a low-cost raw material for edible film preparation. Plasticizer such as glycerol is added to edible film to prevent from becoming brittle while aiding in the extensive and flexible properties (Wiset*et al*. 2014). Pectin is often added to films as it provides the properties of gel formation and selectivity to gas permeation through the film along with providing stability and thickening.

Essential oils are volatile complex compounds synthesized during the secondary metabolism of plants and impart antimicrobial properties due to the presence of alkaloids, phenols, terpenes and other derivatives(Akthar*et al*. 2014). Extensive research has shown that essential oils of oregano (*Origanum vulgare*),cinnamon (*Cinnamon casia*) and clove (*Eugenia caryphyllata*) are among the most active against strains of *E. coli*, yeasts and moulds (Du *et al*. 2011). Whey protein films with 2% oregano oil was effective against several microbes including *E. coli* O157:H7, *Staphylococcus aureus*, *Salmonella enteritidis*, *Listeria monocytogenes*, and *Lactobacillus plantarum* (Ravishankar *et al*. 2009). Carom (*Trachyspermumammi*) or ajwain seed is an aromatic and medicinally important seed spice used as flavouring agent and as a digestive stimulant to cure liver disorders. The active compounds present in the carom seed oil are the phenols especially thymol and some carvacrol that provide the antimicrobial properties by obstructing the peroxidation of liposome phospholipids in a concentration dependent manner (Prashanth *et al*. 2012). Carom seed oil showed antimicrobial activity against *S. aureus* at 1-8 µL/ml, *P. aeruginosa* at 8-32 µL/mland *E.*

coli at concentrations of 2-32 µL/ml (Zomorodian*et al*. 2011). The aim of this work was to develop an edible film using carom seed having anti-microbial properties. This prepared film can be used to increase the shelf life of food products such as fruits, fish, cheese etc.

II. MATERIALS AND METHODS

2.1 Materials: Pumpkin, Arrowroot starch were purchased from the local market in Delhi. Carom seed oil was obtained from Mohan Oil Mills, INA market, Delhi. Glycerol and Citric acid were obtained from Molychem, Delhi. Pectin and L-ascorbic acid were obtained from Central Drug House Private Ltd. (CDH), Delhi. Pure cultures of *E. coli*, *Aspergillus* and *Staphylococcus* were grown and maintained in the microbiology laboratory of Institute of Home Economics, University of Delhi, Hauz Khas, New Delhi, India.

2.2 Preparation of the film solution

2.2.1Pumpkin puree preparation: Pumpkin was washed and cut, seeds and peels were removed and then it was pressure cooked until soft. It was then pureed using a mixer grinder.

2.2.2 Film preparation: Thirty grams of prepared pumpkin puree was taken and diluted with 60 ml distilled water to form the desired concentration for the film formation.The flowsheet for preparation of film is depicted in fig.1.

Prepare 100 ml of 12.5% arrowroot solution at 70-80ºC
↓
Add 4g pectin to the starch solution
↓
Add 0.25g each of ascorbic and citric acid to the above mixture
↓
Add 10 ml glycerol and pumpkin puree (30g in 60 ml water)
↓
Add different concentrations (0.5%, 1% and 1.5%) of oil to the film mixtures
↓
Homogenize whole mixture for 15 minutes at 8000 rpm
↓
Pour the solution on Teflon plates (20x20 cm)
↓
Dry at room temperature (25-27ºC) for 48 hours
↓
Peel off the dried films

Fig.1: Flowchart for preparation of edible film

2.3 Microbiological Analysis: Microbiological analysis was done by agar well diffusion method given by Abbas *et al*. 2016. The test compound was introduced into the well and the plates were incubated at 37 ºC for 24 h for bacteria and for 5 to 7 days at 28ºC-30ºC for fungi. After incubation, the zone of inhibition was calculated and then subtracted from the diameter of the agar well. This difference was reported as the zone of inhibition of the film-forming solutions.

2.4 Physico-chemical and Mechanical Properties

2.4.1Thickness: Thickness of the prepared films was evaluated by the method given in ASTM D 6988 – 03, 2004. Measurements were done using manually operated thickness gauge. Samples measuring 10 x 10 cm were used. The sample was kept on an anvil. The press foot was raised and then gently lowered on to the sample. The reading on the dial gauge was recorded as the thickness of the sample. The above procedure was repeated to at 20 different locations on the sample to obtain the values of thickness. Readings were taken in triplicates for each sample and the results were expressed in mm.

2.4.2 Ash Content and Moisture content: Ash content (%) and moisture content (%) of the films were analyzed using the methodology given in AOAC (2000).

2.4.3 Film Solubility: Solubility of the films was determined according to the method mentioned by Romero-Bastida*et al*. 2005.

2.4.4 Tear strength and bursting strength: Tear strength and Bursting Strength of the films was evaluated using method given in Rangana (1999).

2.4.5 Tensile strength: It was conducted in accordance with IPC-TM-650 Test Methods Manual, using a Paramount digi strength tensile strength tester (capacity: 250 Kg, sensitivity: 100 grams). Triplicate readings were taken for each film and tensile strength was expressed as kg/cm^2.

2.4.6 Water vapor transmission rate (WVTR): It was evaluated using the dish method given in Rangana (1999).

2.5 Statistical Analysis: The obtained data was subjected to statistical analysis using one-way ANOVA (Post-hoc Duncan's test) to arrive at meaningful inferences at a significant level of ($p<0.05$) using the IBM SPSS Statistics 22 software.

III. RESULTS AND DISCUSSION

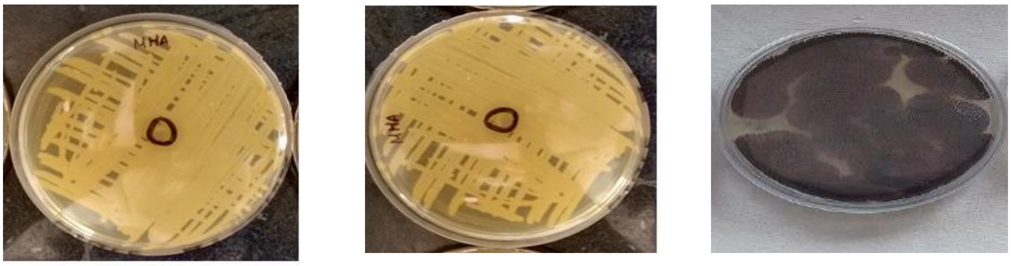
3.1 Antimicrobial activity: *E. coli*, *Aspergillus* and *Staphylococcus* are the most common food spoilage micro-organisms and thus were tested for executing the antimicrobial properties of the film by the agar well diffusion method. The control film solution without any carrom oil did not show any inhibitory effect against any of the microorganisms (Fig. 3) which confides with the results obtained by (Ravishankar *et al*. 2009) in apple puree edible films.

Film solution incorporated with 1.5% carom seed oil showed inhibitory effect against *E. coli*, *Staphylococcus* and *Aspergillus*. The film solution showed inhibition zone of 11.667 mm against *Staphylococcus* and 12.667 mm against *E. coli*. (Fig. 4). Film forming solution with 0.5% and 1% carom seed oil also showed inhibitory effect against *E. coli* and *Staphylococcus*, but the zone of inhibition was not as prominent. For *Aspergillus*, there was inhibitory action observed at 1.5% carom seed oil concentration (Fig. 4), but not for 0.5% and 1%. The activity can be explained by the fact that the addition of carom oil into the film solution resulted in diffusion of oil through the agar gel and provided a clear zone surrounding the film solutions.

Pure carom oil was also tested for its antimicrobial properties. The results showed that pure carom oil has inhibitory effect against *E. coli*, *Staphylococcus* and *Aspergillus* as no growth of microbial colonies were observed on the agar surface (Fig. 2).The strong antimicrobial potential of the carom oil is due to thymol and its precursors, cymene and terpinene. The results of the study are in accordance with the results obtained by Zomorodian*et al*. (2011) which showed the antimicrobial activity of carom seed oil (*Carumcopticum* oil) in concentration ranging from1-8 µL/ml for *S. aureus* and2-32 µL/ml for *E. coli*.As film with 1.5% carom seed oil showed better antimicrobial activity than film incorporated with 0.5% and 1% carom seed oil, therefore films prepared with 1.5% carom seed oil were further studied for physical, mechanical and water vapor transmission rate properties.

E. coli *Staphylococcus* *Aspergillus*

Fig.2: Inhibition zone exhibited by pure carom seed oil



E. coli *Staphylococcus* *Aspergillus*

Fig.3: Inhibition zone exhibited by control film solution

E. coli *Staphylococcus* *Aspergillus*

Fig.4: Inhibition zone exhibited by film solution containing 1.5 % carom seed oil

3.2 Film Thickness: Thickness is an important parameter in determining the workability of edible films as packaging materials for food products because the thickness of the films affects other characteristics of the films, such as tensile strength, elongation, and water vapor permeability and gas transmission rate (GTR) etc. GTR is inversely proportional to the thickness. The film thickness is dependent on both the composition of film and processing conditions (Arham*et al*. 2016). The film prepared without any carom seed oil (control) had thickness of 0.237 mm and the film incorporated with 1.5% carom seed oil had the thickness of 0.236 mm. The results depicted that there was no significant difference ($p<0.05$) in the films incorporated with carom seed oil in comparison to the control films. The plasticizer binds with starch to form starch-plasticizer polymer as a result, the starch-starch bond is replaced by the starch-glycerol-starch bond which leads to improvement in the thickness of film. Therefore, addition of glycerol in the manufacture of edible film resulted in increasing the film thickness (Arham*et al*. 2016).

3.3 Ash content and Moisture content: It was observed that bothcontrol film and film with 1.5% carom seed oil had similar ash content of 1%. Moisture content is important for the processing and handling of food. The moisture content of control film (15.96%) was found out to be higher than the film containing 1.5% carom oil (14.84%). Other studies also showed similar results as the incorporation of the hydrophobic essential oils can affect the ability of the film to retain water leading to the decrease in moisture content (Ghasemlou*et al*. 2013).

3.4 Film Solubility: Solubility is a physical property related to the ability of edible films to dissolve in water so that when ingested it can be digested properly, or if discharged into the environment it can decompose naturally. It is important that the film has low solubility, so that it cannot dissolve on the surface of product and retain high water resistance property. And on the other side, film with low solubility cannot protect the product from humidity and water loss (Arham*et al*. 2016). The film with 1.5% carom seed oil (6.420%) showed slightly higher solubility than the control film (6.324%). But there was no significant difference ($p>0.05$) between the control film and film incorporated with oil.

3.5 Tear Strength and Bursting Strength: Higher tear values may be needed for machine operations or for the package strength while low tear values are necessary and useful for the easy opening of some package types (Rangana 1999). Films containing 1.5% carom seed oil had higher tear strength i.e. 19904 g than the control film i.e. 18474.66 g. The results also indicated that there was a significant difference ($p<0.05$) between the films prepared with oils.

Bursting Strength is measure of resistance to rupture and primarily as an indication of the suitability of certain fiber material and the extent of processing (Rangana 1999). After the addition of carom seed oil, the bursting strength of the prepared edible film was reduced. The control film had the bursting strength of 1.733kg/cm^2 and the film with carom seed oil had 1.466 kg/cm^2.

3.6 Tensile Strength and % Elongation: Tensile Strength (TS) and Elongation at break (EB) are key indicators of a films strength and flexibility. The estimated values of tensile strength showed that control had higher value i.e. 0.139 kg/cm^2 and the film incorporated with 1.5% carom seed oil had lower value i.e. 0.008 kg/cm^2. The incorporation of essential oils into the film network caused a decrease in tensile strength because the stronger polymer-polymer interactions are replaced by weaker polymer oil interactions and hence the network structure gets weakened (Noshirvani*et al*. 2017). Other studies also showed similar results as film without oil had maximum tensile strength i.e. 38.238 kg/cm^2 and minimum for film containing cinnamon oil i.e. 10.706 kg/cm^2 (Souza *et al*. 2013). There was a significant difference ($p<0.05$) between the control sample and the film containing 1.5% carom seed oil. Elongation of the film decreases with addition of oil as control film showed higher Elongation at break value.

3.7 Water Vapor Transmission Rate (WVTR): Controlling moisture migration is crucial for maintaining the taste, texture and overall quality of packaged food products. By knowing the WVTR, the initial and critical moisture contents of food and the humidity gradient between the inside and the outside of the package, the shelf life of the product could be predicted to a fair degree (Rangana, 1999). The WVTR of packaging material is usually determined by the dish method. The film containing carom seed oil (148.59 g/m^2/24 hours) showed higher water vapour transmission than control film sample (117.89 g/m^2/24 hours).This phenomenon can be explained by the decrease in intermolecular forces of attraction between the polymer chains due to the increase in plasticizer concentration. The oil, being a plasticizer as well, along with glycerol and a firm gel structure producing pectin increased the hydrophilic character in the film, possibly because of more terpene than fats. The increase of hydrophilic to hydrophobic ratio in turn increased which promoted the dissemination of water molecules through the film. The unsaturated fatty acids in carom seed oil decreases the melting point of lipid compound, thus explaining the increase in water vapour transmission of the film as diffusion increases several folds in lipids than in saturated fatty acid or lipids (Adjouman*et al*. 2017). Moreover, due to double bonded linoleic acid (57-66%) being the major component present in the carom seed oil (agris.fao.org), as oleic acid aided in increasing melting of components, decreasing the density of the macromolecular network and thus effectiveness against the water transmission (Adjouman*et al*. 2017).

IV. CONCLUSION

Film with 1.5% Carom seed oil showed extremely good antimicrobial activities against *E. coli, Staphylococcus and Aspergillus.*Carom oil decreased the tensile strength by reducing the intermolecular interaction between the polymer chains. The film with 1.5% carom seed oil exhibited low moisture content which is ideal for a packaging material to extend the shelf life of food products. Other properties of the film were not significantly affected by the incorporation of carom seed oil. Our ultimate goal was to develop a commercially viable technology for the manufacture of edible films with cheaper raw materials incorporated with antimicrobial components in an efficient manner for protection of food products. Further studies could be conducted to assess the sensory characteristics and stability of the films incorporated with these essential oils.

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