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INCORPORATION OF ANTIMICROBIAL EXTRACT INTO PACKAGING MATERIALS, A CASE STUDY: STARCH ANTIMICROBIAL FILM AGAINST *Bacillus cereus*

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ABSTRACT

Today there is increasing interest in the safety of food stocks and uses edible and biodegradable films. Starch can be used to formulate edible films which must be completely neutral with reference to color, taste and odor. Wheat starch based active films were developed by dispering wheat starch in dH_2O and glycerol and polyethylene glycon were added as a plasticizer. To prepare the antimicrobial film, extract was added at different concentration (1,5,10 and 20%).The agar diffusion method was used for determining the antibacterial effects films on bacterial strain (*Bacillus cereus*). In films containing 1% and more concentration of extract clear inhibitory zone by the absence of bacterial growth was observed. As the concentration increased, the zone of inhibition also increased significantly ($p<0.05$). The edible films containing 20% extract clearly presented higher antimicrobial activity and the less active concentration was 1%

Keywords: Packaging, starch antimicrobial film, pomegranate rind extract, *Bacillus cereus*

INTRODUCTION

Today there is increasing interest in the safety of food stocks, the food industry currently uses techniques to control microbial growth that improve the quality and shelf life of the majority of food products. One of these techniques is the use of antimicrobial agents. The most commonly used antimicrobial compounds are both synthetic and natural in origin. The natural antimicrobial compounds can be enzymes, antibiotics or plant extracts [1]. The use of medicinal plants in the world

contributes significantly to primary health care. Species and herbs have been added to food since ancient time, not only as flavoring agents but also as folk medicine and food preservatives [2].

It has been reported that pomegranate peel and pomegranate peel extract have significant free radical scavenging, antimicrobial, antiatherogenic and antimutagenic properties these findings have led to increased interest in pomegauate peel extract [3].

Different methods may be used to apply plant extract on foods so that they can perform their antimicrobial function. For example, they can be applied directly on food using a spray. The problem with this technique is that one must use a large quantity of extract because the exact coverage and release rate cannot be controlled. Another possibility is to use the extract as an active compound in active antimicrobial packaging. The extract with its active compounds is generally introduced into the polymeric plastic during the extrusion process. Theoretically, the active compounds should be liberated inside the packaging, thereby creating an internal antimicrobial atmosphere. One of the major problems of using extract in polymeric plastics obtained by extrusion is the high temperature used in the process. Recently, extract have been incorporated in edible food coating formulations. Edible films are defined as a thin layer of material which can be consumed and provides a barrier to moisture, oxygen and solute movement for the food. Edible films can be produced from materials with film forming ability. During manufacturing film materials must be dispersed and dissolved in a solvent and plasticizers, antimicrobial agents, colors or flavors can be added in this process [4].

Starch is a polymeric carbohydrate composed of anhydroglucose units. Starches are often used in industrial foods, they have been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renew ability and it has good mechanical properties [5].

Bacillus cereus is a large, Gram-positive, rod-shaped, endospore forming, facultative aerobic bacterium. *B. cereus* is mesophilic, growing optimally at temperatures between $20^{\circ C}$ and $40^{\circ C}$, and is capable of adapting to a wide range of environmental conditions. It is distributed widely in nature and is commonly found in the soil as a saprophytic organism. As a soil bacterium, *B. cereus* can spread easily to many types of foods such as plants, eggs, meat, and dairy products, and is known for causing 2-5 % of food-borne intoxications due to its secretion of emetic toxins and enterotoxins.

Food poisoning occurs when food is left without refrigeration for several hours before it is served. Remaining spores of contaminated food from heat treatment grow well after cooling and are the source of food poisoning [6].

The objective of this research was to identify the antimicrobial activity of starch films when different pomegranate rind extract concentrations were incorporated. The microorganism targeted in the study was *B. cereus*.

MATERIALS AND METHODES

Plant material and preparation of extract – *Punica granatum* (Punicaceae) was obtained from Agriculture Research Institute of Yazd (Iran). Fruits were peeled and oven dried at 50^{oc} and then ground with an electric grinder in to fine powders. Dried powdered plant material was extracted with methanol 80% according to Mathabe *et al.* [7] method. 2 grams of plant sample was mixed with 50 ml of solvent. The mixture was left on a mechanical shaker at 150 rmp for 24h at room temperature and then faltered whatman No .1. The extract was further concentrated using a rotary evaporator. The yield was weighted and dissolved in dH2o to final concentration of 100 mg/ml. The sample was then stored at 4^{oc} and further used for antibacterial test.

Microorganism and growth condition – *B. cereus* (PTCC 1015) was obtained from Persian Type Culture Collection (Tehran, Iran). The bacteria was grown in nutrient broth at 37^{oc} and maintained on nutrient agar slants at 4^{oc}.

Preparation of antimicrobial films – 4gr of wheat starch was dispered in 100 ml dH_2O, moderately stirred for 20 min at room temperature the heated to 95^{oc} for over 15min. Glycerol and polyethylene glycon were added as a plasticizer at a concentration of 2:0.4 (v/v % of starch solution) and the resulting dispersion was subjected to further mixing for 5min. To prepare the antimicrobial film, extract was added at different concentration (1,5,10 and 20%) in an emulsifying equipment at 13000 rpm for 2 min. 12ml of film forming solution was casted on every circular glass plate and then dried at 20^{oc} for 48h. Starch film without antimicrobial agents was also prepared in the same way and used as a control.

Film thickness and disc weight- Film thickness were measured with a digital micrometer to the nearest 0.01 mm. Measurement were taken at five random locations of the films sheets. Average film thickness was 0.18 mm. Circular discs were cut from the edible films using a cutting well (diameter = 10 mm). Disc weight were measured at five random locations of film on each film making, average disc weight was 0.01 gr.

Determination of antimicrobial effects of films – The agar diffusion method was used for determining the antibacterial effects films on bacterial strain and the zone of inhibition assay on solid media was used for determination of the antimicrobial effects of films. During tests, 3 discs were placed carefully in to each petridish containing solid medium (Brain Heart Infusion Agar) where 0.1ml seeding culture had been spread. The concentration of bacterial seeding culture was 0.5 Mcfarland (1.5×10^8 CFU/ml) respectively. The petridishes where then incubated at 37^{oc} for 24h. The plates were examined to find the diameter of the inhibition zone, It was measured with a caliper to the nearest 0.01mm in triplicate.

Statistical analysis – Three observations were performed at each level (1,5,10 and 20%) of extract treatment to starch films and each experiment was replicated 3 times. Microsoft Excel and spss were used for the statistical analyses. Data were subjected to analysis of variance (ANOVA) and comparison of means was carried out by Duncan's multiple range tests.

RESULTS AND DISCUSSION

A visual examination of the films was per formed. All films were transparent, flexible and homogenous. Their surfaces were soft and smooth without any evidence of pores and crakes.

Control discs are necessary in order to have a reference for microbial development when using inoculated films without pomegranate rind extract enrichment. As expected no inhibition halo indicating bacterial development was observed in any case.

As shown in Table 1 the edible film with extract of pomegranate showed antibacterial activity against the tested microorganism with the diameters of zone of inhibition ranging between 4.33 and 13.83 mm. There was significant differences ($P<0.05$) in the antibacterial activity of different concentration of extract. The edible films containing 20% extract clearly presented higher antimicrobial activity and the less active concentration was 1%

The agar diffusion test is a method commonly used to examine antimicrobial activity regarding the diffusion of the compound tested through water – containing agar plate. The diffusion itself is dependent on the size, shape and polarity of the diffusing material [8]. The chemical structure and the crosslinking level of the films also affect this phenomenon [9]. When antimicrobial agents are incorporated, there will be diffusing material through agar gel and further more resulting clearing zone on the bacterial growth.

Results obtained in this study on antibacterial activity of *P. granatum*, seen to agree with those obtained by Melendez & Capriles [10] who reported that alcohol extracts of pomegranate showed antibacterial activity when tested against *B. cereus* Shan *et al*. [11] also reported the alcoholic extract of pomegranate rind to be active against *B. cereus*. Dahham *et al*. [12] showed that *P. granatum* extracts hare positive antibacterial activity against *B. cereus*. In other study extract was found to be effective against *B. cereus* [13]. Also it has been reported that pomegranate rind extract exhibited antibacterial activity against *B. cereus* by Hayrapetyan *et al*. [14].

Antibacterial activity of plant extract may be indicative of the presence of several metabolic toxins or broad – spectrum antibiotics. Several metabolic from herb species, including alkaloids, tannins and sterols, have previously been associated with antimicrobial activity [15].

In order to investigate components from ethanolic extract of pomegranate peel, the HPLC analysis among some other minor constituents mainly shows some major phenolic compounds, gallic acids and ellagic acids in addition to punicalagin as a major ellagitannin [15]. Gallic acid was reported to have antibacterial activity against some intestinal bacteria [16], ellagic acid has antimicrobial activity [17] and punicalagin was reported to show anti – food borne pathogens [18]. This suggests that these components may also provide antibacterial activity against *B. cereus* and provide a plausible explanation for the higher antibacterial activity of extract. The mechanism responsible for antibacterial activity on microorganisms was related to reaction with sulfohydryl groups of proteins and unavailability of substrates to microorganism [19].

In the current study, this inhibitory effect was incorporated and expressed in a bio–based film and pomegranate rind extract maintain its known antimicrobial activity in wheat starch based edible film.

Table1. Antimicrobial activity of starch film incorporated with pomegranate rind extract

Content (%)	Diameter of inhibition zone (mm)
	B. cereus
0	.00^{e}
1	4.33^{d}
5	6.23^{c}
10	8.06^{b}
20	13.83^{a}

Different lower case letters in the column indicated significant differences (P<0.05)

CONCLUSION

In food applications the use of edible antimicrobial films could potentially allow control of the migration of antimicrobial agents from the film to the food surface, thereby having a continuous effect on it.

Concentration of the antimicrobial agent and the compositions of film material have crucial effect on their biological activity in edible films. In this study the potential application of natural antimicrobial ingredients to biodegradable film based on wheat starch was investigated. Pomegranate rind extract added films exhibited inhibitory zones on *B. cereus* at all level of extract (1% and more). As the concentration in creased, the zone of inhibition also increased significantly.

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