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Characterization of novel basil-seed gum active edible films and coatings containing oregano essential oil

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ABSTRACT

Basil seed gum (BSG) edible films containing oregano essential oil (OEO) (1–6%) were fabricated and evaluated for antibacterial activity against *E. coli*, *S.* Typhimurium, *P. aeruginosa, S. aureus* and *B. cereus*, as well as antioxidant activity, film thickness, moisture content, transparency, swelling index, contact angle and water vapor permeability (WVP). The thickness of BSG based film was recorded as 0.06 mm; the increase in OEO concentration did not change the thickness significantly ($p > 0.05$). The WVP was decreased significantly by incorporation of OEO while the moisture content, contact angle, transparency and swelling index ($p < 0.05$) of edible films were increased. All films containing 2–6% OEO presented a significant antibacterial activity against the examined pathogens. The DPPH and ABTS radical scavenging activities and ferric reducing ability of BSG films were enhanced considerably with increasing OEO concentration. According to results, the fabricated edible films with BSG and OEO can be considered for further approaching as an edible food packaging.

1. Introduction

With an ever increasing demand of consumers for high level of quality for food products in addition to raised environmentally concerns regarding the adverse effect of plastic packaging, food industry drived to develop and implement of new type of the edible films [1]. Edible films comprised of various edible substances such as polysaccharides, lipids proteins, or their combinations which are classified based on used composition [2]. Approaching the edible films as food packaging could provide several benefits such as biocompatibility and environmentally friendly, extended shelf life [3], economically affordable, good barrier properties to gasses and carriers of other foods additives such as vitamins, antimicrobial and antioxidants agents [4].

Basil (*Ocimum basilicum L.*) as a pharmaceutical plant which atracted a high level of interest to be used as an edible film is grown mainly in Iran and widely used to treat different diseases (*e.g.* colic ulcer, diarrhea, and dyspepsia) [3]. Also, basil contains notable amounts of gum −while soaked in water- could provide some of favored technological functions for several food products [5]. Some functional characteristics of the extracted gum that commonly known as Basil seed gum (BSG) including biodegradable properties, low production cost, heat-resistant and hydrophilic nature and acceptable rheological properties caused great interest to further application as film-forming material [3,6,7].

To produce safer products with longer shelf life, control of microorganisms by incorporation of antimicrobial agents into the packaging films has drawn considerable attention. The capability of extracted essential oils from plants to retard the spoilage process has been proven by previously conducted investigations [8–10]. Among the essential oils, Oregano (*Origanum vulgare*) essential oil (OEO) can be considered as one of the most useful antimicrobial agents which have been successfully approached against a broad spectrum of microorganisms including bacteria, yeasts, and fungi [11,12]. The antimicrobial and antioxidant activity of OEO can be attributed to the high amount of phenolic compounds mainly carvacrol and thymol which constitute about 78–85% of proximate composition of OEO [13]. Other responsible components for antibacterial activity can be summarized as monoterpene, hydrocarbons, γ-terpinene and *p*-cymene [14]. Although some studies have been conducted on the application of OEO into edible films and coating [15–17], as far as we know the incorporation of OEO into BSG based film has not been investigated yet. Therefore, the aim of present study was to develop a novel edible film based on OEO incorporated BSG and further characteristics evaluation of fabricated film including antibacterial activity and different physical properties.

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2. Materials and methods

2.1. Plant materials and chemicals

The air-dried *Origanum vulgare* subsp. *viride* (13% $MC_{db,}$ EO: 13.7% (v/w)) was obtained from agriculture research fields belongs to Ferdowsi University of Mas3% $MC_{db,}$ EO: 13.7% (v/w)) was obtained from agriculture research fields belongs to Ferdowsi University of Mashhad (Mashhad, Khorasan Razavi, Iran). Basil seeds were purchased from a local grocery, Shiraz, Fars province, Iran and identified by an expert at the Botany Department of the Faculty of Sciences of Shiraz University. All used chemicals were supplied by Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

2.2. Bacterial strains and condition of culture

Five bacterial strains (*Salmonella* Typhimurium ATCC 14028, *Bacillus cereus* ATCC 10876, *Escherichia coli* O157:H7 ATCC 35150, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853) were obtained from the culture collection of Veterinary School of Shiraz University, Iran. The bacterial cells were recovered from long-term storage at −80 °C by using 20% glycerol. Bacteria were reactivated in Trypticase Soy Broth (HiMedia, India) at 37 °C overnight and inoculated into Trypticase Soy plate to check the purity before the experiments.

2.3. Extraction process of essential oil

Extraction of OEOs from the dried plant was accomplished using the Ultrasonic-Ohmic apparatus [18]. An all-glass Clevenger-type apparatus was attached 30 g of plant material in dried form was loaded into the chamber, furthermore the sufficient electrical conductivity between the two electrodes was provided by the addition of 500 mL NaCl solution (**2.8%, w/v).** Extracted OEO stored in sealed vials at 4 °C for further experiments (Maximum one wk).

EO extraction process was conducted in triplicate.

2.4. Essential oil analysis

The extracted EO was analyzed by gas chromatography − mass spectrometry (GC–MS), (6890N, Agilent Technologies, Palo Alto, California, USA) equipment which was equipped with an oven (HP-5MS Agilent Technologies, Palo Alto, California, USA), a capillary column was employed (30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness). The *n*-hexane (1/10, v/v), was used for prepared diluted samples and one microliter was injected with a split ratio of 10. The injector was set at 290 °C, and the carrier gas (helium) velocity was adjusted at 0.8 mL/min [18].

2.5. Extraction of basil seed gum

The BSG gum was extracted applying the described procedure by Khazaei et al. [3]. Briefly, basil seeds (50 g) were sieved and then added to its triple weight of ethanol to wash under constant stirring for 10 min. Ethanol was removed from seeds by filtration followed by drying in the oven at 60 °C. BSG was separated from the swollen seeds using filtration (cheese cloth). Basil seed gum extraction was conducted in triplicate.

2.6. Preparation of edible coating solution

In order to prepare the film forming solution, aqueous hydrocolloid solution containing 5% BSG with the appropriate amount of glycerol (30% w/w) were warmed up to 35 ± 1 °C for 15 min under constant stirring at 600 rpm according to the described method by Hashemi et al. [19]. OEOs were incorporated into film solution to reach final proposed concentrations (0, 1, 2, 3, 4, 5 and 6% (v/v) dry basis). The obtained dried film was peeled off and stored for further evaluations (Maximum one wk).

All the experiments were established in triplicate.

2.7. Determination of physical characters of films

2.7.1. Film conditioning

Before determining film properties, all films were stored in a storage room at the adjusted temperature (25 °C) and humidity (53% RH) for 48 h.

2.7.2. Film thickness

Film thickness was measured by a digital micrometer (Mitutoyo No. 293–766, Tokyo, Japan) with an accuracy of ± 0.001 mm.

2.7.3. Moisture content

Moisture content (MC) of films were calculated by determining the weight loss of films, which have been left for drying in an oven at 90 °C for 24 h [3]. The following equation was used for MC (%) calculation:

$$MC\ (\%) = (M_i\text{-}M_0/M_i) \times 100$$

Where; M_i and M_0 can be defined as the initial and final mass of the film, respectively.

2.7.4. Transparency

The film transparency was determined with consider to the described method by [20] by applying a spectrophotometer (UV/Visible Philips Cambridge, UK). The transmittance was recorded at 600 nm, and the film transparency was assessed as follow:

$$T\ (\%) = \log (T_{600}/b)$$

Where T_{600} is measured transmittance at 600 nm, and b is film thickness.

2.7.5. Determination of water vapor permeability (WVP)

WVP index was assessed using the described method [21,22]. The sealed cells which contain film specimens were stored in desiccator cabinet at 30 °C. The cups were analyzed by interval weighting of 1 h during 24 h period and the reported WVP as WVP as g s^{-1} $m^{-2}$$Pa^{-1}$ was calculated as the following equation:

$$WVP = {}^{w.\ b}\Big/_{A.\ t.\ (P_2 - P_1)}$$

Where w is assessed weight of cups (g), b is the determined thickness of film (mm), A is the examined area of film (m^2), t is the applied time in order to determine the weight (s), ΔP is the difference of vapor pressure through the film (Pa). Three replicates for each type of film were analyzed for WVP test.

2.7.6. Determination of swelling index (SI)

SI was indicated as described by Mayachiew and Devahastin [23]. The film was cut into sizes of 15 × 15 mm and incubated at RH of 45–50% and 25 ± 1 °C for one day. This measurement with a microbalance periodically was repeated until reaching to equilibrium. The SI (%) of the films was reported by using the following equation:

$$SI\ (\%) = [(W_w - W_d)/W_d] \times 100$$

Where W_w and W_d can be considered as the wet and dry weights of the films, respectively (g), respectively.

2.7.7. Contact angle measurements

The contact angles of prepared films were assessed by the recommended procedure by Ojagh et al. [24]. Water drop (5 μL) were deposited on the surface of films (5.0 cm × 5.0 cm).Then by means of an optical goniometer the dynamic contact angles were automatically