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The preparation, characterization and in vitro application evaluation of soluble soybean polysaccharide films incorporated with cinnamon essential oil nanoemulsions

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ABSTRACT

In this study, we developed the new bioactive film from soluble soybean polysaccharide (SSPS) incorporated with different concentrations of cinnamon essential oil nanoemulsions (CNO) and the functional properties of them were evaluated. Then CNO-SSPS film was applied on the meat during refrigerate for 8 days. The use of CNO in film production has reduced thickness, water vapor permeability, water solubility, lightness (L*), redness (a*) and whiteness (WI) and increased antioxidant activity of SSPS-films. Also, the SSPS- film containing 0.6% CNO activity only on gram-positive bacteria (*Staphylococcus aureus and Streptococcus pyogenes*) and SSPS- film containing 0.8% CNO had antimicrobial activity on gram-positive and gram-negative bacteria. In vitro application, the pH of the meat treated with CON remained in the natural pH of meat during storage (8 days). Based on the results, the highest and lowest hardness values were for the samples of CNO- 0.8 and control, respectively. Incorporating CON at 0.6 and 0.8% concentration of cinnamon reduced 4.14 and 5.71 log cycle in the total aerobic viable count compared to uncoated and decreased of yeast and molds on 8th day by 1 log cycle compared uncoated. These resulted showed CNO-SSPS film can be used as a good preservative in meat products.

1. Introduction

During the past 10 years, food antimicrobials technology among other preservation methods has played a crucial role in habiting growth of pathogenic and spoilage microorganisms [1]. They can be classified as synthetic or natural antimicrobials. Natural antimicrobials extracted from various parts of plants such as leaf, seed and fruit compared to synthetic antimicrobials are more desirable to satisfy consumer demand for "natural" food [2]. Essential oils (EOs) such as cinnamon (*Cinnamomum zeylanicum* L.) have been studied among all natural preservatives as antioxidants or antimicrobial agents to preserve and improve the quality of food products [3–5]. However, the use of EOs as a preservative in the food industry is limited due to its low solubility in the aqueous phase, interactions with food components, high volatile character and sensitivity to oxygen and light [6,7]. One of the most effective technologies to improve the solubility and stability of EOs in adverse environmental is nanoemulsions [8–10]. Nanoemulsified EO components (terpenes mixture and D-limonene) indicated improved antimicrobial efficacy against *Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, *Escherichia coli* in fruit juice [11]. Due to the low interfacial tension between aqueous and oil phase, emulsifiers and surfactants play an important role in forming nanoscale oil droplets. In recent years, due to the good emulsifier, natural food biopolymers such as soy protein isolate and lecithin are used in preparation of nanoemulsions [12]. Nowadays, the use of nanoemulsions has been studied as a new technology in producing edible films such as incorporating ginger essential oil nanoemulsion in gelatin film [13], and incorporating carvacrol nanoemulsion in modified chitosan [8]. Soluble soybean polysaccharide (SSPS) has a structure similar to pectin. It is composed of a galacturonan backbone of homogalacturonan and rhamnogalacturonan branched by β-1,4-galactan and α-1,3 or α-1,5- arabinan chains. It is also suitable to develop edible films owing to its good biodegradability, biocompatibility and rheological properties [14,15]. To our knowledge, the effect of cinnamon nanoemulsion (CON) on the properties of SSPS-based films has not been studied yet. Therefore, the current work was conducted to develop CON- loaded SSPS films and to study the effectiveness of CON as a new edible film on meat physicochemical properties during 8-day storage at the refrigerator temperature.

2. Material and methods

Soy protein isolate (SPI) and Lecithin of food grade were purchased from Safir gosht Co (Shiraz, Iran) and Daity Co (Shiraz, Iran), respectively. Cinnamon oil was purchased from Zarband Co (Tehran, Iran). Soluble soybean polysaccharide (SSPS) was kindly provided by Fuji Oil

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Chemical Co., Ltd. (Osaka, Japan). Analytical grade hydrochloric acid and sodium hydroxide purchased from Merck (Damstadt, Germany). To prepare of all solutions, de-ionized and distilled water was used.

2.1. Microorganisms and culture media

The Persian type culture collection of the Iranian Research Organization for Science and Technology (IROST, Tehran, Iran) were provided the bacteria used in this research (Gram-negative (*Escherichia coli* PTCC1554, *Pseudomonas aeruginosa* and *Salmonella typhi* PTCC1609) and Gram-positive (*Enterococcus faecium*, *Bacillus cereus* and *Staphylococcus aureus* ATCC25923)). Culture media of nutrient agar (NA) and Mueller Hinton agar (MHA) and peptone water were purchased from Merck Chemical Co. (Darmstadt, Germany).

2.2. Solution preparation

By dispersing SPI powder in deionized water and stirred for 5 min, the stock solution of SPI was prepared. Then the solution exposed to ultrasonic 500 W (Elma, s60 h, Germany) for 5 min at 50 °C and then centrifuged at 5000*g* for 10 min. This solution was stored at room temperature for 24 h to ensure complete dissolution of SPI.

2.3. Nano emulsions preparation

Method of [16] was used to prepare the cinnamon oil-nanoemulsion (CON). The briefly, first homogenizing 2 wt% cinnamon oils with 98% aqueous emulsifier solution (1% w/v SPI and 0.05 wt% lecithin) in high- speed blender (UltraTurrax T-25, IKA Instruments, Germany) followed by sonication for 2 min at a frequency of 25 kHz, amplitude of 60% (UP 200H, Germany). A series of preliminary experiments were carried out to select this formulation [16].

2.3.1. Droplet size measurement

Droplet size and particle size distribution of nanoemulsions were measured by the Dynamic Light Scattering (DLS) instrument (ZEN3600, England). To have a specified particle count range, the samples were diluted with deionized water.

2.4. Preparation of nanoemulsion-SSPS film

SSPS film-forming solutions (3% w/v) were prepared under magnetic stirring (1000 rpm), followed by heating to 85 °C on a hot plate for 30 min. The solution was then mixed with a plasticizer (glycerol, 50% w/w of the polysaccharide) [15]. CON was mixed with this stock dispersion so that the final concentration of CO reached to 0, 0.2, 0.4, 0.6 and 0.8% (v/v). For uniform distribution of nanoemulsions, film-forming solutions were blended at 3000 rpm for 1 min. Solution films were cast by pouring the mixture onto polystyrene Petri dishes placed at room temperature and room relative humidity for 20 h. Dried films were peeled off the casting surface and stored inside desiccators at 25 °C and 53% RH until evaluation.

2.5. Analysis of the nanoemulsion-SSPS film

2.5.1. Thickness of the film

A manual micrometer was used to evaluate the thickness of the films at eight random positions around the films.

2.5.2. Water vapor permeability of film

A modified method [17] based on ASTM Standard Methods E96-05 [18] was used to determine the water vapor permeability (WVP) [17,18].

2.5.3. Moisture content of film

The moisture content was determined by heating in a drying oven at 105 °C until a constant weight was reached. Four replication of each film treatment were used for calculating the moisture content.

2.5.4. Water solubility of film

The methods of [19] were used to determine the solubility of nanoemulsion-SSPS films in water. To determine initial solid content, film samples were dried at 105 °C for 24 h in a laboratory oven. Then, film sample (1 cm × 3 cm) was immersed under constant agitation in 50 ml of distilled water for 5 h at 25 °C. At the end, the remaining pieces of films were filtered and dried at 110 °C to constant weight. According to the Eq. (1), the water solubility (%) of the films [19].

$$WS(\%) = \frac{Wi - Wf}{Wi} \times 100 \quad (1)$$

where W_i is the initial weight of the film and W_f is the weight of the undissolved film.

2.5.5. Color values of film

The Konica Minolta chroma meter CR-200 instrument based on Hunter color scale was used to analyze the color of films. CIE "L*" lightness, "a*" redness, "b*" yellowness was determined from five random different surfaces of the samples. Also, the following Eq. (2) was used to determine the whiteness (WI).

$$WI = 100 - \sqrt{(100 - L*)2 + a*2 + b*2} \quad (2)$$

2.5.6. Antioxidant activity of film

DPPH (2,2, -diphenyl-1-picrylhydrazyl) free radical scavenging method was used to determine the antioxidant activity of the films. Briefly, 5 ml of film extract solution was mixed with 1 ml of the 1 mM methanolic solution of DPPH. At the room temperature, the mixture was vortexed and incubated for 45 min. Then, the absorbance of the solution was evaluated at 517 nm. The following equation (Eq. (3)) was used to calculate the DPPH radical- scavenging activity [20].

$$\text{DPPH scavenging activity } (\%) = (Abs_{DPPH} - Abs_{extract}/Abs_{DPPH}) \times 100 \quad (3)$$

where Abs_{DPPH} and $Abs_{extract}$ are the absorbance value of the methanolic solution of DPPH and the sample extract at 517 nm, respectively.

2.5.7. Antimicrobial activity of film

The diffusion method was used to evaluate the antimicrobial activity of the films. The treatment films (6 mm diameter) placed on the plates containing Mueller Hinton agar which seeded with 0.2 ml of an overnight broth culture approximately 10^5–10^6 CFU/ml of the test bacteria. The plates were incubated at 37 °C for 24 h. The area of an inhibition zone was measured and subtracted from the film disk area, and the difference in the area was reported as the zone of inhibition. The tests were done in triplicate for each formulation.

2.6. In vitro application

2.6.1. Preparation of meat treatments

The fresh beef meat was purchased from the local market and transported to the laboratory under refrigerated condition within 15 min. The meat was cut into approximately 5 × 5 cm pieces using stainless steel knife after initial washing with tap water and rinsing. In this study, 4 treatments of meats were prepared (Table 1). The dipping method was used to coat the treatments. The meat samples were immersed into the different solutions (Table 3) at room temperature, held 20 s and then left to dry 1 min. All the treatments were packaged