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Introducing a Novel Composite Polymer for Food Packaging

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ABSTRACT

Introduction: Safety and quality control of foods have attracted significant global attentions. Intelligent and active packaging materials are emerging areas of food technology, which is attracting a lot of attention in the food industry. The present study with the aims of incorporating *Rosa damascena* extract (RDE) into the chitosan-gum Arabic (CH-GA) through the casting method and investigating its antimicrobial property as potential application in the food packaging were conducted.

Methods: Preparation of films based on CH-GA containing RDE was conducted via casting method. The antimicrobial activity of the designated films was investigated by the disk diffusion assay. The morphology of the fabricated films was determined under the field emission scanning electron microscopy.

Results: The prepared films had antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*, and *Bacillus cereus* ranging from 5.50 mm to 9.33 mm inhibition zone diameter. The film containing the RDE has a rough surface and the pure film exhibited a smooth, dense surface, a uniform structure, and no cracks.

Conclusion: This result showed that the CH-GA film containing RDE can be used as an active packaging material in the food industry for enhancing the freshness of the protein-rich foods.

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Introduction

Global concerns, including environmental and climatic changes are forced researches and policies on sustainable economic cultures (1, 2). Numerous methods have been recommended as a part of the sustainable development to keep natural resources, decrease pollution, and inhibit global waste accumulation (3). Biodegradable based packaging materials can be used for reducing this concerns owing to their biocompatibility, biodegradability, non-toxicity, antioxidant, and antibacterial properties (4). In the last decades, the biodegradable films/nanofibers are produced from the wastewaters and by-products of numerous food sectors, suggesting an alternative approach to natural food packaging materials (5). Chitosan (CH) and gum Arabic (GA) has been extensively applied in the food packaging applications among the numerous natural polymers due to its easy film-forming capacity, biodegradability, appropriate oxygen and water vapor barrier ability, and excellent mechanical characteristic (6). These are utilized in many different applications, including food and medical formulations, chemistry, and other biological properties. However, the low antioxidant and antibacterial potentials of CH and GA films, which are critical parameters for an active food packaging film, limit their potential application in the food packaging (7, 8).

Moreover, important attention has been paid to the incorporation of natural extracts and essential oils into the polymeric nanofibers/films in the food industries. Essential oils (EOs) and extracts have always been regarded as trustworthy and safe food preservatives in the food based products, which can limit lipid oxidation and delay the spoilage microbial growth (9). *Rosa damascena* Mill. (Damask rose, oil-bearing rose, and pink rose), is a native aromatic plant of the Middle East that is extensively utilized in a wide variety of food products, especially Iranian yogurt-based drink and pickles regarding its flavoring and high levels of phenolic compounds and anthocyanins (6). It has broad antimicrobial activity against molds/yeasts and Gram-negative and Gram-positive bacteria without adverse effect on sensory attributes of food products (10). The leaves of *R. damascena* belonging to the *Rosaceae* family, has traditional applications owing to its

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biological properties, such as antidiabetic, antiatherosclerotic, anti-inflammatory, anticonvulsant, carminative, digestive, and analgesic effects (11). The food safety (GRAS-Generally Recognized as Safe) of plant essential oils and extracts has been completely confirmed through the Food and Drug Administration (FDA) of the USA (12). Based on our findings, there is no information regarding the possibility of incorporation of *R. damascena* extract (RDE) into the CH-GA based films. Therefore, the present study with the aim of incorporating RDE through the casting method and investigating its antimicrobial property were conducted.

Materials and Methods

R. Damascena Extract Preparation

Fresh Damask rose (*R. damascena* Mill.) flowers were supplied from a local market in Kermanshah, Iran. The obtained plants were identified at the Faculty of Agriculture, Razi University (Kermanshah, Iran). The preparation of the extract was conducted by 20 ml extraction solution (ethanol: water, 20:80 volume/volume) mixture and concentrating using a rotary evaporator (Heidolph, Germany) (6). Moreover, all culture media and chemicals were purchased from the Merck, Germany.

Fabrication of CH-GA based film containing R. damascena extract

CH (medium molecular weight = 250 KDa, 75-85% deacetylated) and GA spray dried from acacia tree were purchased from Sigma-Aldrich (UK) and Merck (Germany), respectively. In order to prepare film based on CH-GA, 2 g of CH was dissolved in 100 ml of 1% acetic acid solution. To achieve proper distribution of CH, the CH solution was stirred for 3 h at room temperature (24 ± 1 °C) on a heater stirrer (IKA, Germany). Then, glycerol at the rate of 0.75 ml/g was added as a plasticizer to the CH solution and stirred for another 30 min (13). The amount of 14 g of GA was mixed in 100 ml of distilled water and stirred for 5 h at 50 ± 1 °C. Then, glycerol was added at the rate of 0.75 ml/g as a plasticizer and stirred for 30 min. CH and GA were mixed in a ratio of 40:60 and stirred for 30 minutes at room temperature (14). Then, 5% RDE was added and mixed for another 30 min at room temperature (24 ± 1 °C). Finally, the final solution was homogenized with a homogenizer at a speed of 12600 × g for 1 min. After evaporating the solvent at room temperature for 48 h, the prepared films were separated from glass molds (diameter = 12 cm) and used for other experiments in this study.

Antimicrobial activity of CH-GA based film containing R. damascena extract

Antimicrobial property of the prepared films against *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (ATCC 19118), *Bacillus subtilis* (ATCC 6633), and *Bacillus cereus* (ATCC 11774) were investigated by the disk diffusion method (15). The preparation of the mentioned bacteria has been conducted by activating in tryptic soy broth plus 0.6% yeast extract for 24 h at 37 ± 1 °C (16). The prepared films were cut into 6 mm diameter and placed on Mueller Hinton agar culture medium containing 7 log CFU/ml of bacteria. After incubating at 37 ± 1 °C for 24 h, the diameter of the growth inhibition zone was measured (17).

Scanning electron microscopy of CH-GA based film

The morphology of the fabricated films was determined under TeScan MIRA3 field emission scanning electron microscopy (FE-SEM) after affixing to sample stubs and sputter-coating with a thin layer of gold at an accelerating potential of 15 kV with a working distance of 10 mm (18).

The release of R. damascena extract from CH-GA based film

The release rate of RDE from designed films was investigated by dissolving 0.1 mg of the sample in 100 ml of 85% ethanol-water solution and stirring at 100 rpm for 84 h under dark conditions at 4 ± 1, 25 ± 1, and 37 ± 1 ºC. At any specified time, 1 ml of the solution was taken and its concentration was measured at a wavelength of 530 nm using a UV-visible spectrophotometer based on the method of Guo et al., (2020) (19).

Statistical Analysis

The experiment was repeated three times. Data analysis was conducted using the SPSS program (version 21 for Windows, Chicago, IL, USA). The results were presented as mean ± standard deviation. A statistical significance was considered as $P < 0.05$.

Results and Discussion

The release of R. damascena extract from CH-GA based film

The amount of RDE cumulative release from CH-GA film at 4, 25 and 37 ºC is shown in Fig. 1. Based on the findings of this study, the amount of cumulative release of RDE from CH-GA film increased significantly with increasing

temperature ($P < 0.05$), which can be due to the increased mobility of macromolecule chains (20). Moreover, 100% of the RDE was released from the CH-GA film at 37 °C after 72 h. Similar findings have been found by Wu et al., (2015) who evaluated the diffusion of cinnamon essential oil in nanoliposome incorporated into the gelatin film (21). Ghadetaj et al. (2018) also indicated a same effect of nanoemulsion formation on the release kinetics of *Grammosciadium ptrocarpum* Bioss. essential oil from whey protein isolate film (22). Same findings are also in agreement with those reported by Aziz1 & Almasi, (2018) for releasing *Thymus vulgaris* L. extract from whey protein isolate film (2). Li et al., (2021) also found a similar abrupt release (about 25% at 24 h) for encapsulated eugenol from gelatin nanofibers (23). Maroufi et al., (2021), indicated that that the thyme essential release curve from poly (lactic acid) nanofibers displayed a 62 h-plateau period (24).

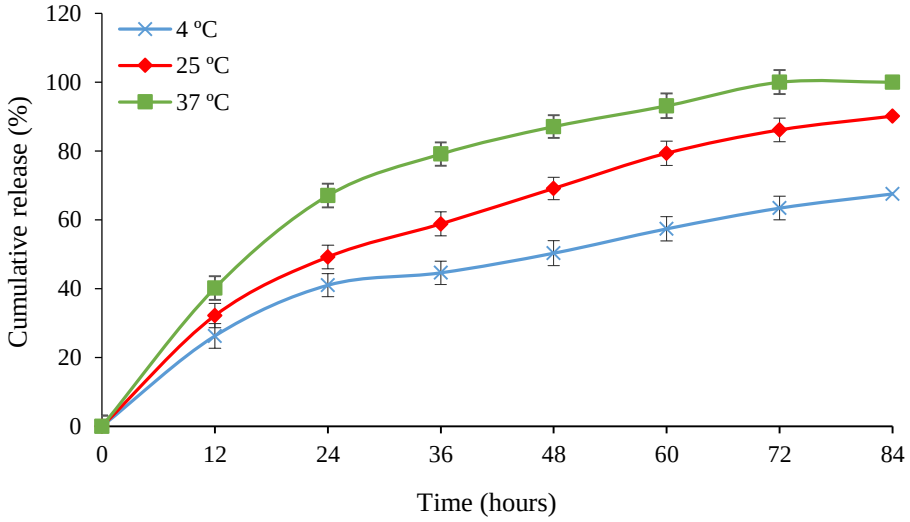

Figure. 1. The release of *R. damascena* extract from CH-GA based film.

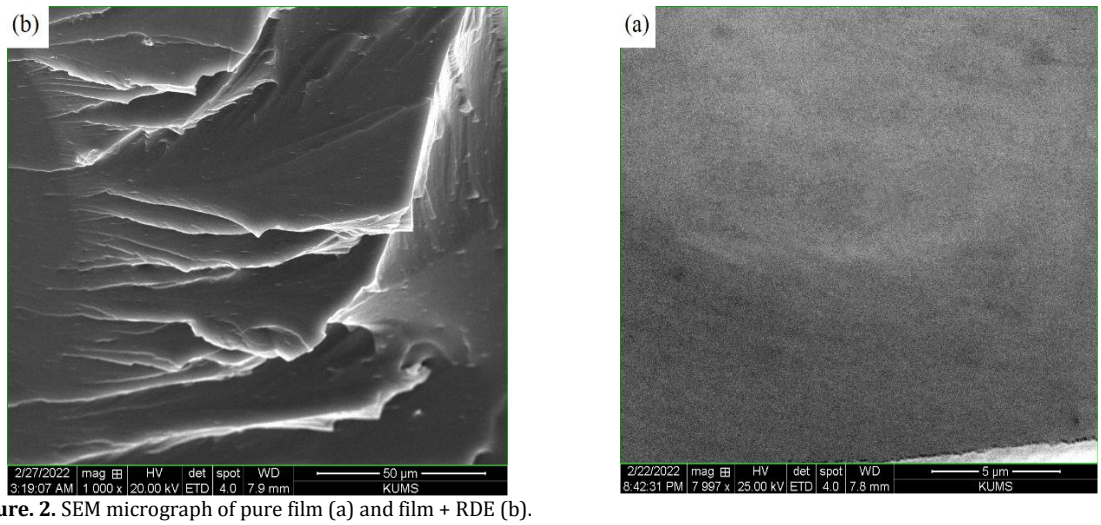

Figure. 2. SEM micrograph of pure film (a) and film + RDE (b).

Scanning electron microscopy of CH-GA based film

The FE-SEM micrographs of pure films and those containing RDE are presented in Fig. 2a and 2b, respectively. As shown in Fig. 2a, the pure film had a smooth, dense surface, a uniform structure, and no cracks. The film containing the RDE has a

rough surface probably due to the protruded structures mediated by the various chemical constituents of the RDE compounds (18). The results of the present study exhibited a homogeneous and dense microstructure without phase separation, and this suggested that there was good biocompatibility between the extract and CH-GA matrix (25).

Antimicrobial activity of CH-GA based film containing R. damascena extract

The antimicrobial property of the prepared film against *S. aureus*, *L. monocytogenes*, *B. subtilis*, and *B. cereus* are shown in Table 1. After removing the straight CH-GA polymer from the agar plate, the growth of bacteria did not find and a growth inhibition zone did not observe. This antimicrobial activity is in agreement with the findings of other researchers regarding CH-methyl cellulose nanofiber (26), CH nanoparticles (27), and CH-gelatin film (28). In the present study, the film containing RDE had antimicrobial properties against all investigated microorganisms (Table 1). It is to be noted that RDE components may lead to membrane disintegration in bacteria, hydrolysis of membrane components, and leakage of intracellular electrolytes and proteinaceous constituents, and therefore RDE had the potential to be used to control spoilage and pathogenic microorganism's growth for extending shelf-life of perishable food (12, 29). Previous studies also have indicated that RDE could inhibit the growth of pathogenic bacteria, such as *B. cereus*, *E. coli*, *Salmonella* Typhi, and *S. aureus* (10, 30).

Table 1. Antibacterial activities (inhibition zone diameter; mm) of chitosan-gum Arabic film containing *Rosa damascena* extract (RDE).
ND: not detected

Formulation	*S. aureus*	*L. monocytogenes*	*B. subtilis*	*B. cereus*
Pure chitosan-gum Arabic	ND	ND	ND	ND
Chitosan-gum Arabic + RDE 5%	8.49 ± 0.06	9.33 ± 0.08	6.78 ± 0.02	5.50 ± 0.01

Conclusion

In the current study, we considered the fabrication of antimicrobial packaging with RDE anthocyanins and CH-GA film. The results of the present study showed that RDE successfully incorporated into the CH-GA film via casting method, as the SEM micrograph confirmed this phenomenon, to prepare an antimicrobial film. This result showed that the CH-GA film containing RDE can be used as an active packaging material in the food industry for enhancing the freshness of the protein-rich foods.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

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