Journal of
Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2023-162

Original Article

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Physical Properties, Antioxidant Activity, and Antimicrobial Properties of Edible Film Prepared from Black Plum Peel Extract as a Valuable By-product of Plum Processing

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Received: October 03, 2023
Accepted: November 13, 2023
Published: November 17, 2023

Citation: Mohammadi-Moghaddam T, Kariminejad M, Hadad S, Jafarzadeh S, Shahrampour D, et al. 2023. Physical Properties, Antioxidant Activity, and Antimicrobial Properties of Edible Film Prepared from Black Plum Peel Extract as a Valuable By-product of Plum Processing. *J Food Chem Nanotechnol* 9(4): 156-162.

Published by United Scientific Group

Abstract

Black plum peel (BPP) as a by-product of plum processing, has nutritional, antimicrobial, and antioxidant properties. In this study, the effect of black plum peel extract (BPPE) (0, 0.5, 1.5, and 2%) on physical (thickness, water solubility (WS), and water vapor permeability (WVP)), mechanical (tensile strength (TS) and elongation at break (EAB)), structural (FTIR; Fourier transform infrared spectroscopy) properties, antioxidant activity, and antimicrobial characteristics of chitosan/gelatin edible film was investigated. Antioxidant activity and total phenolic compound of BPP were 87.63% and 104.03 mg GA/g, respectively. Increasing the concentration of BPPE increased the WS, WVP, and antioxidant activity of chitosan/gelatin edible film, however reduced EAB and TS ($p < 0.05$). The results of FTIR presented the intramolecular and intermolecular interaction between chitosan, gelatin, and BPPE. By increasing the black plum peel extract, antimicrobial activity of films significantly improved which could be because of antioxidant and phenolic compounds of BPP. According to the results of this study, BPPE can be used to improve the quality of chitosan/gelatin edible film.

Keywords

Black plum peel, Chitosan, Gelatin, Edible film, Antioxidant activity, Antimicrobial properties

Introduction

These days, there is an increasing demand for preservation and extended shelf life of industrial food products, as well as a growing concern for environmental issues. Therefore, producing biodegradable packaging based on natural renewable polymers has received valuable attention in the food industry [1, 2]. There are a bunch of biopolymer sources that can be useful for production of edible film in food technology, such as proteins, lipids, carbohydrates, or combination of them [3]. One of these polymers is bovine gelatin. Due to containing myofibrillar proteins, gelatin is a source of suitable film forming materials [4]. Furthermore, due to its disulfide bonds, hydrogen bonds and hydrophobic interactions, gelatin could produce a brittle film with lower WVP [4, 5]. Combining different polymers is a good strategy that could produce packaging with higher and improved mechanical and structural properties. Chitosan, derived from chitin through deacetylation, is a plentiful natural polymer characterized by linear polysaccharide structures. It is composed of poly-(1/4)N-acetyl-d-glucosamine. In addition, chitosan is nontoxic, biocompatible, biodegradable and it is known for its antimicrobial properties [1, 4, 6]. Therefore, compounding both chitosan and gelatin can improve structural and physicochemical characterization of the films [7]. Lately, there has been a growing interest in using antioxidants for food packaging. Phenolic compounds (catechin, caffeic acid, gallic acid, tannic acid, etc.) as natural compounds are available in many plants at low cost [2, 8-10]. These compounds are non-toxic, food grade, and have antioxidant and antimicrobial activities.

Plum (*Prunus* subg. *Prunus*) is a single seed fruit with different sizes, colors, and flavors. This fruit contains a variety of vitamins and minerals, antioxidants, phenolic compounds, anthocyanins, carotenoids, and edible fibers, and it is considered a wonderful food. Due to its special compounds, this fruit has anti-constipation, nausea, fever, blood pressure, cholesterol, cardiovascular diseases, and diabetes effects. During the processing of plum in Neyshabur (Iran), a large volume of plum peel is produced (about 10000 tons). So far, no application has been stated for this valuable substance and its residues in the environment and also its pollution. According to the report, BPP has the following nutritional composition: 3.4% protein, 0.66% fat, 13.73% total sugar, and 2.5% crude fiber. Additionally, BPP is considered to be a rich source of calcium (201.29 mg/100 g), phosphorus (88.60 mg/100 g), zinc (0.97 mg/100 g), and ascorbic acid (25 mg/100 g). Antioxidant activity and phenolic compounds were significant (88.59% and 105.91 mg/g GA). Heavy metals, including mercury, cadmium and lead, and organophosphorus pesticides were insignificant, and the amount of mold was <10 CFU/g [11]. The nutritional properties of BPP, which is similar to plum fruit, make it possible to use it as a functional ingredient for active packaging to extend the shelf-life of food products. While various sources discuss the utilization of different fruits in edible films [12-14], there has been a lack of research on incorporating BPPE into chitosan/gelatin edible film to enhance the utilization of natural antioxidants in food packaging. Therefore, the aim of this study is to attempt to produce a blend film using chitosan/gelatin containing BPPE (0, 0.5, 1.5, and 2%) and investigate its (thickness, WS, and WVP), structural properties (FTIR), and mechanical (TS and EAB), antioxidant activity and antimicrobial properties of chitosan/gelatin composite edible film.

Material and Methods

Materials

Gelatin (bloom gel 240 - 270, water ≤ 12%), chitosan (medium molecular weight, 75 - 85% deacetylate), citric acid was purchased from Biobasic (Markham, Canada) and Merck company (Darmstadt, Germany), respectively. Plum peel for the study was obtained from plum processing factories located in Neyshabour (Iran). The bacterial strains used in the experiments were sourced from the microbial collection maintained by the Iranian Research Organization for Science and Technology.

Preparation of BPPE

First, BPP was washed and cleaned to remove impurities. After being crushed using an industrial crusher, the BPP was stored in a freezer until further experimentation. A modified method, based on Zhang et al. [2] was employed to extract phenolic compounds of BPP in order to prepare BPPE. Specifically, 10 g of BPP was extracted in 100 ml of 96% (v/v) ethanol solution at 4 °C for 24 h. After filtering the extraction with Whatman paper, it was then centrifuged at 10,000 xg for 20 min using a refrigerated centrifuge (KavoshMega, Iran), and the supernatant (BPPE) was collected to produce an aqueous solution of BPPE at concentrations of 0.5, 1.5, and 2%.

Total phenolic compounds of BPPE

The Folin-Ciocalteu reagent was utilized to assess the total phenolic content present in BPP [15]. Initially, 50 µl of BPPE was combined with approximately 3 ml of distilled water. Then, the solution was subjected to the addition of 250 ml of Folin-Ciocalteu reagent, which was subsequently mixed with 750 µl of sodium carbonate and 950 µl of distilled water. After vigorous shaking of the solution, the resulting mixture was placed in a dark room for a duration of 15 min. Following the incubation period, the absorbance was measured at 760 nm. The experiment was repeated five times to ensure accuracy. The obtained results were determined by constructing a calibration curve using gallic acid as a standard, with the values expressed as micrograms (µg) of gallic acid per gram of the dry sample.

Preparation of polymer solutions and composite films

The film solutions were prepared using a modified method based on Tang et al. [16] and Kariminejad et al. [17]. To start, 1.5 g of gelatin was accurately measured and dissolved in 100 ml of distilled water. The resulting mixture was then stirred for a period of 2 h at a temperature of 80 °C, resulting in the preparation of the gelatin solution (G). Following that, 1.5 g of chitosan was precisely weighed and dissolved in 100 ml of 1% acetic acid. The solution was subsequently heated to a temperature of 50 °C and continuously stirred for 1 h. The gelatin and chitosan solutions were then mixed in a 1:1 ratio to prepare the control film (CG), and each solution was stirred for 1 h at room temperature. BPPE was added to the solutions in four levels (0, 0.5, 1.5, and 2%). All samples containing BPPE were stirred individually for 2 h and at 60 °C. To enhance the flexibility and pliability of the solutions, glycerol was introduced as a plasticizer with a quantity of 25 wt.% (based on dry matter). Next, 50 ml of each solution was cast into roundish glass plates with a diameter of 15 cm, separately and was allowed to dry at 25 °C for 48 h. After drying, the films were peeled away from the plates and placed in desiccators containing magnesium nitrate saturated solution ($Mg(NO_3)_2.6HNO_3$) to achieve a relative humidity of 52 ± 1% for at least 72 h before analysis.

Antioxidant activity of BPPE and composite films

To evaluate the antioxidant activity of the films, 1 ml of 1 mM methanol solution of DPPH was mixed with each film sample (1 x 1 cm). The mixtures were stirred and placed in a dark location for 1 h. The UV absorbance of each solution was then measured at 517 nm using a spectrophotometer [18]. The test was performed in five repetitions. The results were calculated using the following equation 1:

$$DPPH\ scavening\ activity\ (\%) = \frac{Abs(DPPH) - Abs(extract)}{Abs(DPPH)} \times 100 \quad (1)$$

Thickness and WS

The thickness and WS of all composite films were determined following the methodology established by Liu et al. [19]. The thickness of the films was determined by a digital micrometer (Mitutoyo, Japan). The average thickness of the films was measured at 10 random locations around each film (Accuracy 0.001 mm).

To determine the WS, a square piece (2 x 2 cm) of each film was dried at 105 ˚C and immersed and stirred in 50 ml of distilled water for 24 h at 25 ˚C, following the method reported by Mohammadi et al. [20]. After 24 h, in order to reach a constant weight, the remaining films were dried again at 105 ˚C. The WS was calculated using the following equation 2:

$$WS\% = \frac{(Mi - Mf)}{Mi} \text{ x } 100 \quad (2)$$

Where, Mi: initial weight and Mf: final dry weight.

WVP analysis

The WVP was gravimetrically measured at 25 ˚C using the method described by Zhang et al. [2]. Circular test cups with a diameter of 5 cm were filled with 5 g of $CaCl_2$, and each film sample was placed into each cup. Para film sealant was utilized to tightly attach the film sample to the cup mouth. The cups were then positioned in desiccators which contained a saturated solution of NaCl, providing a relative humidity of 74.0 ± 2%.

Following a duration of 10 h, the cups reached a point where their weight gain stabilized. The cups were then weighed five times at 2-hour intervals to ensure accuracy. The WVP ($gms^{-1}m^{-2}.Pa$) was calculated using the equation 3:

$$WVP = \left[(m/t) \text{ x } (L/A) \text{ x } (1/\Delta P)\right] \quad (3)$$

Where, m: the weight of water vapor transmitted (g), t: the time (s), L: the thickness of the film (m), A: the area of the cup (m^2), and ΔP: the water vapor pressure gradient across the film (Pa).

Mechanical properties

The mechanical properties TS and EAB% of the prepared films, were measured at room temperature (25 ˚C) according to the ASTM standard method D882-09 (2009) (standard). The test was performed by using a microcomputer-controlled electronic tensile machine (SMT-20, Santam, Tehran, Iran), and the applied tensile rate during the experiment was set at 5 mm/min. Film samples, measuring 100 x 10 mm, were prepared, and then placed in a desiccator for a period of 2 days to ensure proper conditioning before testing. Each sample's mechanical properties were assessed through a minimum of three measurements, and the obtained average values were used for analysis.

FTIR analysis

The structural interaction of the film samples was assessed using the method developed by Mohammadi et al. [20]. Each sample was conditioned into a desiccator with silica gel for at least 2 weeks to ensure moisture removal. Then, the structural interaction of the composite films was analyzed using FTIR spectroscopy in transmittance mode (Irprestige-21, Shimadzu, Japan) between wavenumbers of 400 - 4000 cm^{-1}. Each film sample was placed into the spectroscope's crystal cell and inserted into the FTIR spectrometer. The spectrum was measured using an automatic signal over 16 scans. The results of the FTIR analysis were used to evaluate the structural changes and interactions between the components of the films.

Antimicrobial properties

The antimicrobial properties of the films against *Escherichia coli* (PTCC 1399) and *Staphylococcus aureus* (PTCC 1431) as Gram-negative and Gram-positive bacteria were evaluated, respectively. First, 50 µl of an overnight culture of each bacterium (10^8 CFU/ml) in nutrient broth was inoculated on petri dishes containing nutrient agar. Then, a disk of each composite film (6 mm diameter) was placed on the inoculated solid form of growth medium and incubated for 24 h at 37 ˚C. After incubation time, each inhibition zone that was formed around the films was measured and reported as antagonistic activity of active films [2].

Statistical analysis

The statistical analysis was performed using SPSS 25.0 for Windows (SPSS Inc., Chicago, IL, USA). To determine significant differences among the means, analysis of variance was used. To identify differences between means, Tukey's test was performed, and the level of significance was set at $p < 0.05$. In order to plot the curves, GraphPad Prism (Version 8.0.1, USA) was used.

Results and Discussion

Antioxidant activity and total phenolic compound of BPP

BPP is a valuable source of antioxidant activity and phenolic compounds. It's reported that the amount of these compounds in plum peel is 5 times that of plum fruit [21]. The results showed that antioxidant activity and total phenolic compounds were 104.03 mg GA /g, and 87.63%, respectively. In a study, Mohammadi-Moghaddam et al. [11] reported that BPP pure exhibited antioxidant properties with a phenolic compound content of 105.91 mg/g GA and an antioxidant activity of 88.59%.

Thickness and WS

The results of the thickness of the films are presented in figure 1. The highest value was for the control film (CG). Adding the BPPE to the films up to 0.5% decreased ($p < 0.05$) the thickness of films. By increasing the concentration of BPPE from 0.5 to 2.5%, the thickness increased. However, any significant difference was not observed between 1.5 and 2% ($p > 0.05$).

The WS considered as one of the great factors that could affect the quality of the packaged food product during storage [8]. The result of the WS of the films presented in figure 1. The WS of the films increased as the BPPE concentration increased. The highest amount of solubility was for CS2 (51.37 ± 0.140).

This could be due to the high hydrophilic nature of anthocyanins, which is responsible for the dark red color in BPP [2] and also the hydrophilic functional groups of gelatin. Similar results were investigated by Zhang et al. [2] who reported that by adding BPPE to chitosan/TiO_2 film, the solubility of the films was increased. Rui et al. [8] showed that by increasing the gallic acid amount, WS of the films generally increased. In another study, a reduction in the solubility of gelatin/pomegranate peel powder film was reported, which was due to the insolubilized parts of the pomegranate powder [4].

WVP analysis

One of the important barrier factors which has a vital role in the stability and resistance of the film against bad tensions and situations is WVP. Figure 1 shows the WVP of the composite films contained BPPE. The lowest value was for the control film. By increasing the percentage of BPPE, WVP of the films was significantly increased. This could be because of the hydrophilic interaction between gelatin and BPPE. It seems that the higher hydrophobicity of gelatin due to its lower proline and hydroxyproline contents, and the hydroxyl group of hydroxyproline, which is regularly available to form hydrogen bonding, tended to interact with hydrophilic anthocyanin groups of BPPE more than chitosan, especially at a higher percentage of BPPE [22]. Ashrafi et al. [12], reported after the addition of kombucha tea extraction, the WVP of chitosan/ kombucha films decreased significantly, which was claimed to be due to the covalent interaction between chitosan and kombucha extract. The results obtained in this study align with the findings of Zhang et al. [2] where it was observed that increasing the BPPE on chitosan/TiO_2 film increased the WVP of the films, which is similar to the obtained results of this study.

Mechanical properties

For resistance of the packaging to external tension and stress and maintaining the integrity of food, sufficient mechanical properties such as good EAB and TS, are required. Figure 2 presents the mechanical results of the films. By the addition of 0.5% of BPPE caused a significant ($p < 0.05$) increase in TS, however by adding more, TS value of the films was decreased. The highest value of TS was for the CG0.5 (60.14 MPa). The better and stronger interactions between the functional groups of both polymers and BPPE may have happened in 0.5% of BPPE. Both TS values of CG1.5 and CG2 films were lower than the control film. The EAB of the films was significantly decreased by different amounts of BPPE. The highest level of EAB was for the control film. According to the study conducted by Bao et al. [22], the TS value of gelatin, tea-polyphenol loaded, and chitosan nanoparticle films was decreased as polyphenol extraction was raised. Hanani et al. [4], found that the percentage of pomegranate peel powder to fish gelatin could improve the TS value and reduce the EAB of the films. This could be due to the difference in the base polymers and the different antioxidant capacity of the extracts. To increase in the TS and decreased in the EAB values of chitosan/gelatin/garlic acid films were reported in a study on chitosan/protocatechuic acid film [8]. Xu et al. [23], in the study of chitosan/gelatin films containing hop plant extract, reported that hop α-acid and hop β-acid improved the TS value and reduced the EAB value of chitosan/gelatin films.

FTIR analysis

The FTIR analysis was used in order to identify the structural properties of the composite films. The FTIR spectra results were shown in figure 3. Some main bands which detected in the control film (CG) were at about 3300 cm^{-1} (N-H stretching vibration, stretching vibration of hydroxyl and primary amino groups, amide A), 2942 cm^{-1} (asymmetric stretching vibration of C-H and NH_3^+, Amide B), 1635 cm^{-1} (C=O stretching, hydrogen bonding and amide I), 1550 cm^{-1} (N-H bonding and stretching of aromatic groups and amid II), and 1242 cm^{-1} (aromatic primary amine and C-N stretching of amide III) which have been previously reported by other researchers [2, 20, 21]. The main peaks in the composite films with BPPE were at around 1500 cm^{-1}, indicating the aromatic C=C bands [24] and peaks at around 2800 - 2950 cm^{-1} assigned to the stretching of CH_3 and CH_2 [25]. C-O stretching was detected at 1000 - 1100 cm^{-1} [26]. At around 1500 - 1600 cm^{-1} that indicated the bands of C-O stretching and N-H bending [2] were less sharp in those films that con-

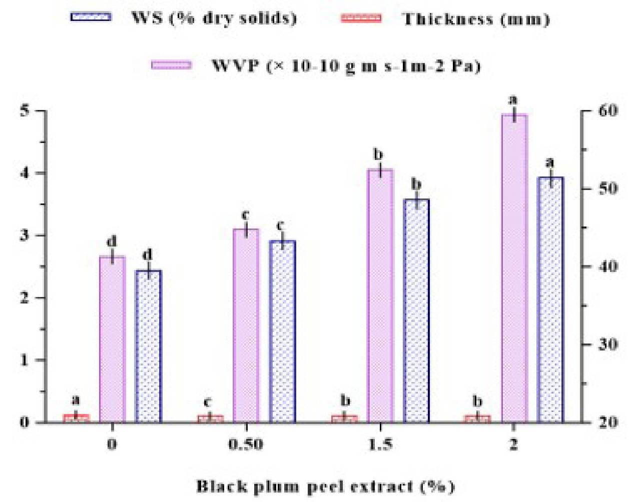

Figure 1: The effect of BPPE on physical properties of composite film of chitosan/gelatin.

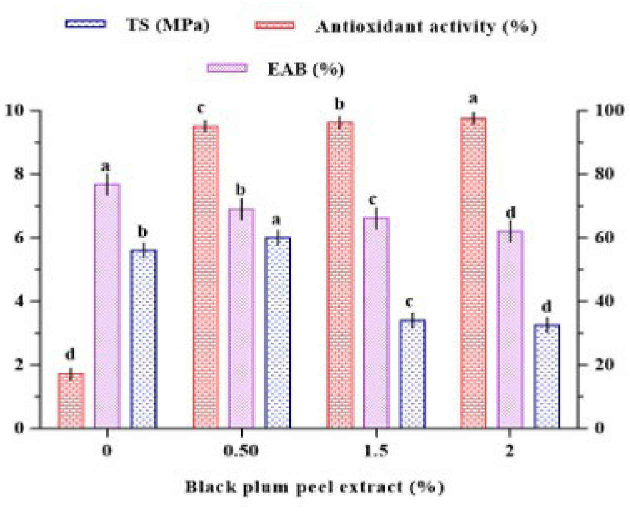

Figure 2: The effect of BPPE on antioxidant activity and mechanical properties of composite film of chitosan/gelatin.

tained BPPE compared to the control film (CG) which could express the intermolecular interaction between both polymers and the natural antioxidant agents [2]. Moreover, there were some peaks at around 650 - 1100 cm^{-1}, contributed to CH and CH_2 bonding, aromatic CH bond, C-O and COC stretching vibration which were increased in the composite films with BPPE [25, 26]. These results express the intramolecular and intermolecular interaction between both polymer and BPPE which was illustrated in the antioxidant composite films.

Antioxidant activity of composite films

As figure 2 illustrated, the CG film presented the lowest antioxidant properties and by increasing the concentration of BPPE, the antioxidant activity of the films significantly ($p < 0.05$) improved. The highest antioxidant activity was for CG2 (97.60 ± 0.049[a]). In a similar investigation, the amount of BPPE increased the chitosan film [2]. Other studies reported the improvement and increase in antioxidant activity such as pomegranate peel powder in fish gelatin films, a phenolic compound in zein/chitosan films, chitosan/gelatin films conging hop plant extract, fish gelatin film with Curcuma extract, plant extract in chitosan/gelatin films and gelatin films with natural antioxidant [4, 23, 27].

Antimicrobial activity of composite films

During preservation and storage, food-borne microorganisms and environmental pollution could cause an unpleasant quality of packaged food. Therefore, the antimicrobial properties of packaging have an important role in food packaging [2]. As indicated in table 1, a slight antimicrobial activity was observed in the control film that can be attributed to chitosan. Moreover, by increasing BPPE in film structure, its antimicrobial activity significantly grows ($p < 0.05$). This may be due to the high antioxidant activity and phenolic compounds of BPPE that have been widely approved by other investigations [2, 4]. A higher inhibitory effect was observed against *E. coli* as a Gram-negative bacterium than *S. aureus* as a Gram-positive bacterium. The different antimicrobial activities of the films against two tested bacteria could be due to differences in the cell wall structure of these microorganisms. A thick cell wall structure with multilayers of peptidoglycan is a sign of Gram-positive bacteria that separates them from Gram-negative bacteria which are with a thin peptidoglycan layer [28]. Recently, Nejad and Najafian [29] reported similar results about the antibacterial activity of date plum (*Diospyros lotus*) syrup against Gram-negative and Gram-positive bacteria. Contrary to the results of this study, Hanani et al. [4] observed stronger antibacterial activity of fish gelatin film containing pomegranate peel powder against *S. aureus* (Gram-positive bacterium) than *E. coli* (Gram- negative). These observations were attributed to the outer membrane of Gram-negative bacteria (lipopolysaccharide layer). The presence of thymol as a phenolic compound in the gelatin film in Kavoosi et al. [30] study led to significant antimicrobial properties against a wide range of food-pathogenic bacteria. In another research, the inhibitory activity against *S. aureus* and *E. coli* was obtained by adding the plant ethanolic extracts of cinnamon, rosemary, guarana and boldo-do-chile separately in chitosan/gelatin films [27]. Moreover, the enhancing of antibacterial potential was reported after encapsulating *Allium ursinum* L. extract in polylactide by Radusin et al. [13]. In another research, the more inhibitory activity against *E. coli* (21 to 24 mm) and *S. aureus* (23 to 26 mm) was obtained in disk diffusion test by adding of cinnamon, rosemary, boldo-do-chile and guarana ethanolic extracts separately in chitosan/gelatin films in comparison to this study [27]. Also, the inhibition zones of alginate-based film containing 3% (w/v) of Roselle extract against *S. aureus* and *E. coli* were reported 11 and 19.33 mm, respectively [31]. Due to the difference in type and chemical composition of plant extracts used in the structure of active films, it can be expected that their antimicrobial activity against food microorganisms will be different. The antimicrobial characteristics of antioxidant and phenolic compounds have been proven in many previous studies. The antimicrobial activity of phenolic compounds is influenced by the number and position of hydroxyl groups. Phenolic compounds inhibit the growth of bacteria through reaction with sulfhydryl group and inactivation of membrane enzymes) non-specific binding with proteins) [32].

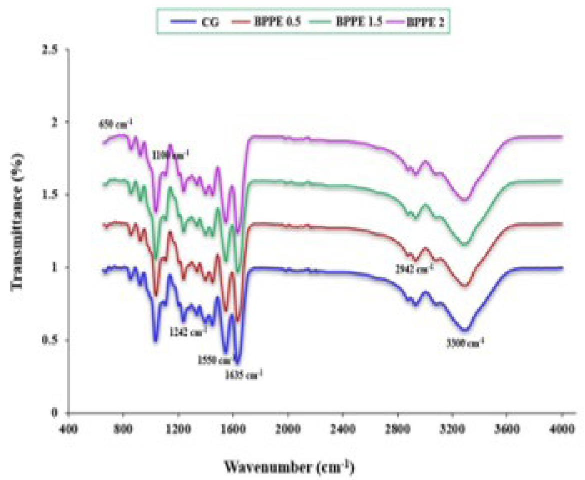

Figure 3: FTIR spectra of the films based on chitosan/gelatin and BPPE.

Table 1: Antimicrobial activity (diameter of inhibition zone in mm) of composite films of chitosan/gelatin and BPPE.

BPPE (%)	Gram negative *Escherichia coli* (PTCC 1399)	Gram positive *Staphylococcus aureus* (PTCC 1431)
0	3.1 ± 0.45[d]	0.54 ± 0.057[d]
0.5	5.27 ± 0.46[c]	3.20 ± 0.056[c]
1.5	7.30 ± 0.47[b]	4.17 ± 0.055[b]
2	8.50 ± 0.45[a]	5.45 ± 0.054[a]

Conclusion

In this study, the impact of incorporating BPPE, a valuable by-product, on various aspects of chitosan/gelatin edible film such as physical, mechanical, and structural properties, antioxidant activity and antimicrobial characteristics of chitosan/gelatin edible film was examined and studied. The highest value of WS, WVP, antioxidant activity and antimicrobial properties obtained for the sample contained 2% BPPE, though, this sample had the lowest TS and EAB. FTIR spectroscopy showed the intramolecular and intermolecular interaction between both polymer and BPPE. Consequently, the results of the research indicate that BPP exhibited a high potential for application in edible films intended for food packaging. This is primarily due to its significant antioxidant activity and abundant presence of phenolic compounds.

Acknowledgements

None.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Funding

We thank the Neyshabur University of Medical Sciences for funding the project (Project number 97292).

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