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Properties of Active Levan-Bitter Vetch Protein Films for Potential Use in Food Packaging Applications

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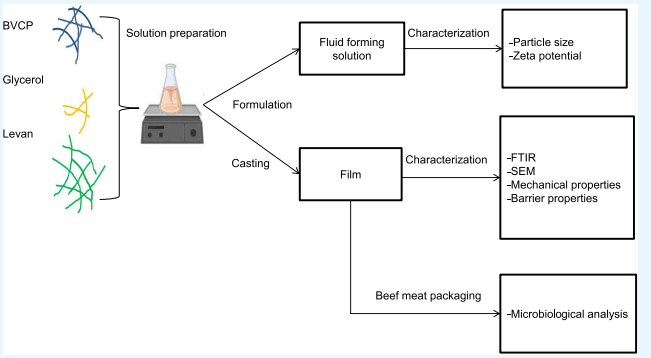
ABSTRACT: ζ-potential and *Z*-average were determined on film-forming solutions of bitter vetch−levan-based films prepared at different ratios in the absence and presence of glycerol as a plasticizer. The casting method was used to obtain manageable films. The results revealed that levan increases the elongation at break of bitter vetch protein films and reduces the tensile strength. The optimal result was obtained through the film that was prepared with the ratio of 50% bitter vetch proteins and 50% levan, in terms of mechanical properties. The surfaces of the prepared films appeared to be more compact and smooth. On increasing the glycerol concentration in the bitter vetch protein−levan films, the oxygen and water vapor permeability increased compared to the control ($P < 0.05$). Based on the overall results, the reinforcement of bitter vetch proteins with levan at a ratio of 1:1 represents optimal film properties in the presence of a low concentration of glycerol. The proposed film is suggested as an innovative packaging system for beef meat to preserve its quality over time.



INTRODUCTION

Basically, there has been a spate of interest in petroleum-based synthetic polymers used as packaging materials, raising environmental concerns due to their toxicity and non-biodegradability.[1] From this perspective, several research works have been particularly oriented toward overcoming this problem through focalizing on eco-friendly packaging materials by using biodegradable polymers from renewable sources.[2−4] These films can be made by using biopolymers such as polysaccharides, lipids, and proteins or their combinations.[5] Such polymers sometimes act as additives, which can ameliorate the functional properties of the films, making them suitable for the production of edible films as food packaging materials.[6,7] Within this framework, several polysaccharides from different bacteria have been explored to form these films, garnering significant interest in the packaging industry. Indeed, they rest upon a unique colloidal nature, abundance, and low cost as well as good film-forming property.[8]

Bacillus subtilis AF17 produced a high amount of exopolysaccharide-type levan.[9−11] Levan is made up of repeating fructose subunits, which form a main chain of β-(2 → 6) fructofuranosidic with some β-(2 → 1) linked branched chains. Levan is a high-cost polymer, and the use of low-cost fermentation substrates can reduce its production costs.[21]

Yet, edible films created from natural polymers like pure levan display poor mechanical properties as well as weak resistance.[12,13] As a matter of fact, multiple strategies have been set forward to enhance their functional and mechanical properties, including blending with natural polymers such as alginate, chitosan, cassava starch[14,15] cross-linking, and combination with other compounds, such as proteins.[16]

Proteins are a very good substrate for the production of biopolymer packaging because the films made of them are flexible, transparent, tasteless, and odorless.[5] Indeed, proteins may allow film preparation demonstrating enhanced mechanical or permeability characteristics compared to those produced from proteins or polysaccharides solely.[17] However, the ability of proteins to form edible packaging is highly dependent on their molecular characteristics.[18] Recently, proteins derived from bitter vetch (*Vicia ervilia*, BV) seeds have been suggested as a cheap and promising source to produce both edible films and biodegradable containers.[19,20] Therefore, it could be a worthy candidate to be used in combination with levan to result in improved materials.

A few studies have dealt with the application of levan as a matrix for blending films. First, Bostan et al.[21] focused on the

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blending films of chitosan and levan. Despite the promising results, no mechanical property conditions were evaluated. Second, Mantovan et al.[14] applied levan in edible films based on cassava starch to obtain new biopolymer blends with functional properties. It is remarkable that for these experiments there was a gap in characterizing the fluid-forming solution.

To the best of our knowledge, this research is the first one to study the effect of levan on bitter vetch (BV, *V. ervilia*) protein property films. The basic objective of this research is to characterize both the film-forming solutions and films made with the BV protein concentrate (BVPC) containing different amounts of levan in the presence or absence of glycerol (GLY). The *Z*-average and ζ-potential of film-forming solutions were determined, and the obtained films were characterized. As a possible application, casting BVPC films with varying amounts of glycerol and levan, the potential of BVPC−levan as an additive in packaging was investigated. Its mechanical and optical properties, as well as its ability to extend the shelf life of beef meat, were examined.

MATERIALS AND METHODS

Materials. BV seeds were purchased from a local market in Gallicchio (PZ), Italy. GLY (about 87%) was supplied by the Merck Chemical Company (Darmstadt, Germany). All other chemicals and solvents used in this work were analytical-grade commercial products.

Levan Production. Levan was produced and purified as described by Bouallegue et al.[10] Briefly, levan was produced in a fermentation medium, containing (g/L) tryptone 10, yeast extract 5, gelatin 2.5, glucose 5, sucrose 100, sodium citrate 1, and sodium azide 0.075 with an initial pH of 7 at 150 rpm. After 72 h of incubation at 30 °C, cells were removed by centrifugation (9000 rpm, 4 °C). Two volumes of absolute ethanol were added to the obtained supernatant and kept overnight at 4 °C. Crude levan was harvested by centrifugation (9000 rpm, 20 min, 4 °C), dissolved in ultrapure water, and dialyzed for 2 days at 4 °C using a dialysis membrane (Medicell International, Ltd., London, U.K.) having a cutoff of 12−14 kDa. After the dialysis, the solution was lyophilized. The carbohydrate content was evaluated by the phenol-sulfuric acid method,[22] whereas the protein content was determined by the Lowry method.[23]

Titration of Levan Solution. Levan solution (1 mg/mL) was prepared at pH 2.0 using 1.0 N HCl. Subsequently, the titration was carried out from pH 2.0 to pH 12.0 under constant stirring at 25 °C by using an automatic titrator unit MPT-2 (Malvern, Worcestershire, U.K.) containing as titrant solutions NaOH 1.0, 0.5, and 0.1 N. ζ-potential and *Z*-average values were determined by a Zetasizer Nano-ZSP (Malvern, Worcestershire, U.K.) equipped with a helium−neon laser of 4 mW output power operating at the wavelength of laser red emission of 633 nm. The software Zetasizer Nano-ZSP was used to calculate the ζ-potential by applying a voltage of 200 mV using the Henry equation. The values at each pH were measured in triplicate.

Preparation of BVPC. Proteins contained in BV seeds were extracted as previously described.[17,20] Briefly, BVPC was prepared by using the acid−base method (1:10, w/v) using distilled water. Primarily, the BV seeds were milled through the use of a rotary mill (Grindomix GM200, Retsch GmbH, Haan, Germany) at 1200 rpm for 5 min. The first step was to bring the solution to pH 11.0 with NaOH 1.0 N and stir it at medium speed for 1 h at room temperature. After centrifugation at 3200*g* for 10 min, the supernatant was collected and the pH was adjusted at 5.4 by 0.1 N HCl addition to form a precipitate, which was then separated by a new centrifugation at 3200*g* for 10 min. Finally, the pellet was poured, uniformly distributed on a plastic plate, and dried at 37 °C and 25% relative humidity. The obtained BVPC was finally ground, and its protein content (77%) was determined by Kjeldahl's method,[24] using a nitrogen conversion factor of 6.25.

Film Preparation. BVPC was dispersed in distilled water (2 g/100 mL), and the pH value was adjusted to pH 12.0 by using NaOH 0.1 N under constant stirring until the powder was completely solubilized. Levan (pH 12.0) was added drop by drop to BVPC with continuous agitation at medium speed. 2 hours later, GLY was added at different amounts (5−30% v/400 mg BVPC or levan alone or at different ratios between BVPC and levan) to obtain manageable films as depicted in Table S1 (Supporting Provided). All of the FFSs (50 mL/400 mg total mass of the solution) were finally cast.

All FFSs were characterized for their ζ-potential, *Z*-average, and conductivity by a Zetasizer Nano-ZSP (Malvern, Worcestershire, U.K.) as stated above. All different FFSs were poured into 8 cm diameter polystyrene Petri dishes (7.8 mg BV proteins/cm^2 correspond to 100% BVPC) and were finally allowed to dry in an environmental chamber at 25 °C and 45% RH for 48 h. The manageable dried films were peeled intact from the casting surface and then stored in controlled conditions at 25 °C and 50% RH for 2 h by placing them in a desiccator over a saturated solution of $Mg(NO_3)_2{\cdot}6H_2O$ before being tested.

Film Characterization. An Fourier-transform infrared (FTIR) spectrophotometer (PerkinElmer Spectrum BX FTIR) was used to record the spectra and analyze functional groups of the composite films. The characterization was performed in the range of 4000−400 cm^{-1} wavenumber at a resolution of 2 cm^{-1}.

For SEM, the different films were coated with gold using a sputter coater (JFC1100, JEOL, Japan) and then examined by a scanning electron microscope system (JSM-5400, JEOL, Japan) under high vacuum conditions.

Film thickness was measured using a digital micrometer (DC-516, sensitivity 0.1 μm) for nonconductive materials on nonferrous substrates. Six different values were taken at different locations for each specimen, and the mean value was calculated.

The mechanical properties tensile strength (TS), elongation at break (EB), and Young's modulus (YM) were measured according to ASTM D882[25] by using a universal testing instrument model no. 5543A (Instron, Norwood, MA). Different film strips (1 cm wide and 5 cm long) were obtained by using a sharp pair of scissors and were conditioned in an environmental chamber at 25 °C and 53% RH for 2 h. Finally, six samples of each film type were tested, and the speed was 5 mm/min in the tension mode.

The opacity of the films was calculated as the ratio of absorbance (*A*) (in nm) to the film thickness (in mm) using a UV−vis spectrophotometer. According to Galus and Kadzińska,[26] film strips cut in dimension (1 cm × 4 cm) were placed directly into a glass cuvette; an empty glass cuvette was used as a reference. The film's opacity was measured at 600 nm wavelength as in the following formula.

$$\text{opacity (mm}^{-1}) = A_{600\text{nm}}/X$$

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where O is the opacity, A_{600nm} is the absorbance of the sample at 600 nm, and X is the thickness of the film (in mm). The analyses were repeated 6 times, and a mean value was reported.

A differential scanning calorimeter was used to investigate the differential scanning calorimetry (DSC) test by heating the sample in the temperature range of 35−200 °C at a 10 °C min^{-1} heating rate. The film sample was cut to 10 mm^2 and conditioned at 25 °C and 60% relative humidity.

To determine film permeabilities to O_2 and WV aluminum, masks were used with the aim of reducing the film test area to 5 cm^2. The measurements were carried out in triplicate at 25 °C and 50% RH by using a TotalPerm apparatus (Extra Solution s.r.l., Pisa, Italy).

Application of Active Films for Minced Beef Meat Packaging. Fresh beef minced meat samples, purchased from a local supermarket in Sfax (Tunisia), were prepared as 25 g square pieces under aseptic conditions. Then, the samples were wrapped with the prepared film samples (70 mm × 70 mm). All beef meat samples were stored for 7 days at 4 °C and analyzed at 1, 3, 5, and 7 days.

Microbiological Analyses of Beef Meat during Storage. Microbiological testing was conducted on the whole minced meat. Samples of each minced meat were suspended in a sterile peptone−water solution and homogenized for 2 min at room temperature. Total aerobic counts were counted on plate count agar (PCA), which was incubated at 30 °C for 48 h. Coliforms were counted on Violet Red Bile Lactose agar (VRBL) and incubated for 24 h at 37 or 44 °C for total and fecal coliforms, respectively. Psychrophilic bacteria were evaluated by using PCA after incubation at 4 °C for 5 days. Salmonella was detected by a presence−absence test. Initially, 25 g of the sample was homogenized in 225 mL of buffered peptone water and left to incubate for 24 h at 37 °C. After incubation, 1 mL was transferred to 10 mL of selenite−cystine broth and incubated at 37 °C for 24 h. Then, a loop full of broth was plated onto a Hektoen medium and incubated at 37 °C for 24 h.

Statistical Analysis. JMP software 10.0 (SAS Institute, Cary, NC), one-way ANOVA, and the least significant difference test for mean comparisons were used. Differences were considered to be significant at $p < 0.05$. All experiments were carried out using at least three freshly prepared solutions, and results were reported as the mean ± one standard deviation.

RESULTS AND DISCUSSION

Levan, of high-purity grade, contained less than 0.5% protein expressed per dry matter and did not contain mono- and disaccharides.[27] The exopolysaccharides (EPS) proved to be a levan containing a backbone of 6-substituted β-fructoses, with a low grade of branching at position 1 (linear/branched ratio 20:1). The relative molecular mass obtained by gel permeation chromatography was more than 20 MDa.[9] As the ability of levan to form films[14,28] as well as BVPC only in the presence of a plasticizer[29] has been recently demonstrated, we were highly motivated to investigate the effects of levan on the properties of BVPC films in the presence or absence of GLY.

Levan Titration. Therefore, preliminary experiments aiming to find suitable conditions for the development of stable FFSs were carried out by determining the ζ-potential of levan aqueous solutions as a function of pH using a device that is able to separate by microelectrophoresis and measuring dynamic light scattering. The results are illustrated in Figure 1.

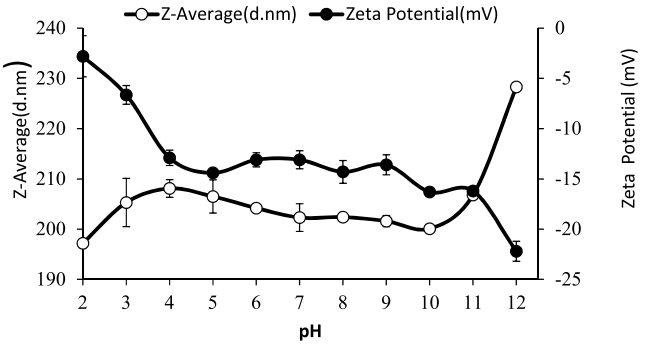

Figure 1. Effect of different pHs on the ζ-potential and Z-average of levan aqueous solution (1 mg/mL).

The ζ-potential of 1% (w/v) levan solution displayed a value ranging from −3 to −23 mV between pH 2.0 and 12.0, respectively. In particular, the value became progressively more negative (from −3 to −15 mV) with the increase of the pH value from 2.0 to 4.0. These findings are clearly indicative of an improvement in the solubility of levan owing to the negative charges increasing on the surface. In fact, in the same range of pH, an increase was observed in the hydrodynamic radius of levan particles from 197 to 208 nm at pH 2.0 and pH 4.0. No changes in the ζ-potential and size were noticed from pH 4.0 to pH 11.0. However, at pH 12.0, a decrease in the ζ-potential (−23 mv) and an increase in particle size to 228 nm were observed, which is suggestive of an improvement in levan solubility. Abid et al.[30] reported that during EPS titration there was a decrease in the overall net charge. These results indicate that levan particles with relatively small diameters (<500 d.nm) can be used to form films.[31]

Moreover, FFSs containing different ratios between BVPC and levan and which were prepared in the presence and absence of GLY at pH 12.0 were found to exhibit ζ-potential values lower than −38 mV (Table 1). Conversely, the same panel shows that the Z-average values increased in parallel with the enhancement of levan concentration. Yet, GLY containing BVPC−levan FFS samples always displayed remarkably higher Z-average values (>420 d.nm), which decrease with the increase in the percentage of GLY, referring probably to their higher conductivity.[20] In fact, GLY is a small molecule able to

Table 1. Effect of Different Ratios between BVPC/Levan on Z-Average and ζ-Potential of the Film-Forming Solutions

FFSs (BVPC/levan)	Z-average (d.nm)	ζ-potential (mV)
(100:00)	285.3 ± 14.4^{b}	−39.5 ± 2.1^{b}
(00:100)	2140.0 ± 49.6^{a}	−51.8 ± 6.0^{a}
(100:00) + 50% GLY	335.0 ± 5.5^{a,b}	−37.3 ± 3.3^{b}
(95:5.0)	337.1 ± 2.9^{a,b}	−38.1 ± 3.6^{b}
(87.5:13.5)	315.5 ± 3.7^{a,b}	−38.1 ± 3.2^{b}
(13.5:87.5)	581.3 ± 8.1^{a,b}	−43.2 ± 5.0
(75:25)	321.6 ± 4.9^{a,b}	−38.3 ± 3.1^{bb}
(50:50)	334.2 ± 3.8^{a,b}	−48.0 ± 8.2
(50:50) + 5% GLY	1192.0 ± 73.0^{a,b}	−48.0 ± 5.4^{a}
(50:50) + 10% GLY	802.0 ± 37.2^{a,b}	−54.0 ± 6.0
(50:50) + 20% GLY	761.0 ± 49.0^{a,b}	−48.0 ± 6.6
(50:50) + 30% GLY	448.0 ± 6.0^{a,b}	−38.0 ± 6.0^{b}

^{a}The values were significantly different from those of the film prepared at 100% BVPC. ^{b}The values of the films prepared at 100% levan.

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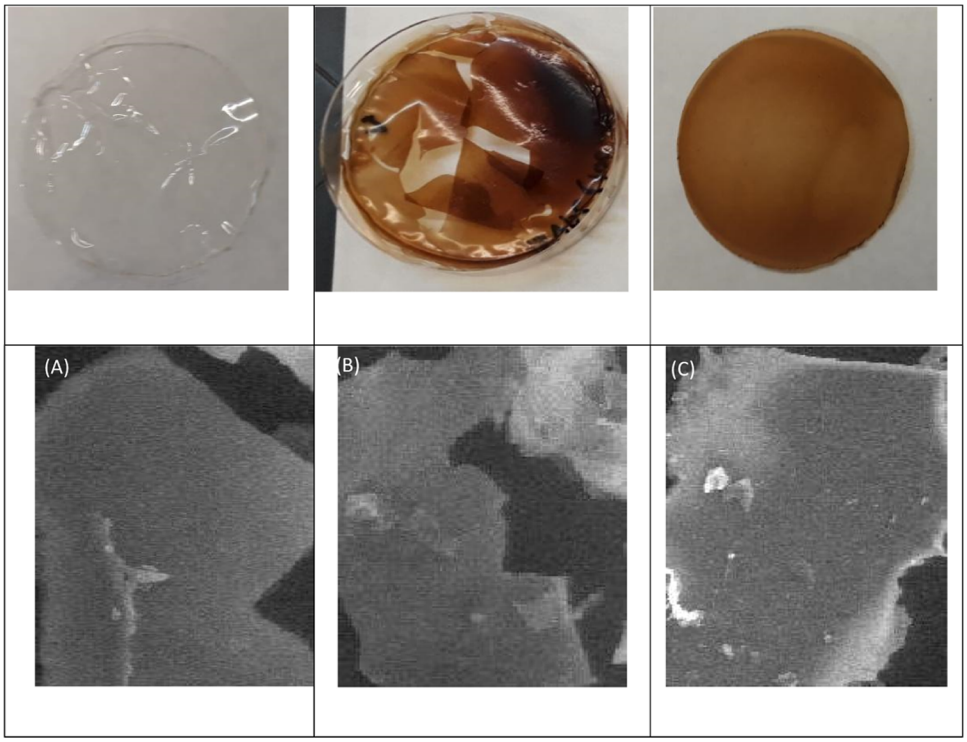

Figure 2. Films obtained by the casting method and SEM micrographs at 500 magnification of the levan (A) BVPC film and (B) levan−BVPC with 50% glycerol. The images shown were chosen as the most representative from each sample.

interact only with protein proton acceptors by intermolecular hydrogen bonds.[20]

As far as FFS is concerned, Table 1 also shows that the levan concentration influences the *Z*-average and it has a more important effect on ζ-potential values for all of the analyzed samples with the aim to determine the best conditions for obtaining manageable (i.e., not brittle or sticky and, thus, easy to manipulate) and characterizable films. These results can be accounted for in terms of the occurrence of aggregation or clustering in polysaccharide solutions. The self-assembly of polysaccharide solutions can be stimulated by the hydrophobic interactions of the hydrophobic constituents, resulting in a concentration-dependent increase of the size of aggregates.[32] Table 1 shows that the ζ-potential is significantly influenced by the levan concentration in this research. Levan solutions present negative values of ζ-potential (Table 1, BVPC: levan −51.8 mV), which may be attributed to their anionic structure. However, increasing the polysaccharide concentrations in the film-forming solution leads to a rise in the negative ζ-potential values.[33] Actually, polysaccharides are reputed to have a high tendency to associate owing to the accumulation in hydroxyl or amino groups present in the macromolecules that facilitate hydrogen bonding. However, at high concentrations of polysaccharides, a reversible association occurs and disappears again by association when the solution is diluted.[34] These effects can be observed in polysaccharide solutions by changing their concentration.

Conversely, polydispersity indexes increase with the increase in the percentage of GLY (5 to 30%). However, films with less than 50% levan and 50% BVPC were too brittle to be handled and tested. Finally, manageable films were formed departing from BVPC/levan, with a ratio of 50:50, with or without GLY.

It is worth noting that their brownish color became more intense by increasing BVPC concentrations (Figure 2 upper part).

Opacity and Thickness. The optical property traits of edible films correspond to an essential sensory aspect for edible films and coatings to be appreciated by clients. In general, it is expected to be colorless or similar to petroleum-based packaging materials.[26,35] Levan films were more or less visually transparent. Furthermore, the films were glossier on the support side compared to the air side, which were more dull and rough. The opacity values decreased with the increase of both levan and GLY concentrations. The opacity of levan−BVPC films was strongly connected with the arrangement of levan chains in the films. The effect of levan and glycerol concentration on the opacities of biodegradable films was investigated (Table3). Glycerol concentration has a significant effect ($p > 0.05$) on film transparency. Films made from levan−BVPC with 5% glycerol produced had the lowest opacities (15.1 nm^{-1}) regardless of the glycerol content. In addition, the opacity was observed to be ranging from 1.8 to 16.1 mm^{-1}, with significant differences among samples ($p > 0.05$).

By casting the BVPC/EPS formulations containing a higher amount of proteins (100:00; 95:05; 87.5:13.5; and 75:25), the obtained films cannot be characterized as they are difficult to peel from the support. As reported in the literature, the presence of BVPC has been shown to increase the opaqueness, indicating the formation of insoluble protein−polysaccharide aggregates during drying as well as the presence of immiscible dispersed phases in the final composite films.[26] Similar observations were made by Kokoszka et al.[36] in their experiment on whey protein isolate films. Unlike the present study, all films exhibit special and outstanding thicknesses. As revealed inTable2, high thickness values (>170 μm) imply

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beneficial mechanical and barrier properties of levan films. This result can be explained by the improvement of the film microstructure.[37]

Table 2. Effect of Different Ratios between BVPC/Levan on Film Thickness and Opacity Properties[a,b]

film (BVPC/levan)	thickness (μm)	opacity (mm^{-1})
(100:00)	nd	nd
(00:100)	90.8 ± 0.3[c]	1.8 ± 0.6[c]
(100:00) + 50% GLY	108.2 ± 9.0[c,d]	5.3 ± 0.5[c,d]
(00:100) + 50% GLY	nd	nd
(95:05)	nd	nd
(87.5:13.5)	nd	nd
(13.5:87.5)	69.8 ± 12.0[c,d]	3.7 ± 0.3[c,d]
(75:25)	nd	nd
(50:50)	85.3 ± 5.0[c]	14.1 ± 0.4[c,d]
(50:50) + 5% GLY	91.0 ± 8.0[c]	15.1 ± 0.2[c,d]
(50:50) + 10% GLY	94.0 ± 6.0[c]	15.4 ± 0.4[c,d]
(50:50) + 20% GLY	99.0 ± 10.0[c]	15.9 ± 0.6[c,d]
(50:50) + 30% GLY	101.0 ± 8.0[c,d]	16.1 ± 0.4[c,d]

[a]The results are expressed as mean ± standard deviation. Further experimental details are given in the text. [b]Non-detectable values because of the film brittleness or stickiness (nd). [c]The values were significantly different from those of the film prepared at 100% BVPC. [d]Values of the films prepared at 100% levan.

The entangled branches of levan contribute to its cohesive strength, and the large number of hydroxyl groups helps form adhesive bonds with a variety of substrates. Basically, levan is referred to as a "green" adhesive, and dried levan adhesives can be easily removed with water. Levan's tensile strength on aluminum and excellent shear strength on plastics make levan superior to many petrochemical-based adhesives.

SEM. SEM images of BVPC/levan films are presented in Figure 4 (lower part). There is a regular roughness and homogeneity in the different film samples. The images of different films clearly indicate a more compact microstructure.

FTIR. FTIR spectra of different films are shown in Figure 3. According to the FTIR spectrum, the strong bands at 3260, 3268, and 3270 cm^{-1} were related to the tensile vibrations of O−H. The absorption band at 2962 cm^{-1} was attributed to symmetrical or asymmetrical forms of methylene (CH_2) and methyl (CH_3) groups of aliphatic chains. The bands at 2870, 2912, and 2871 cm−1 represent asymmetric tensile bands of $=CH_2$. The absorption band at 1565 cm^{-1} represents amide I and the presence of an α helix structure in the secondary structure of the protein. The bands observed at 1001 and 1012 cm^{-1} were related to glycosidic bonds.[38]

Mechanical Properties. The mechanical strength of hydrocolloid films can be identified through the measurement of several parameters, including tensile strength (TS), elongation at break (EB), and Young's modulus (YM) of elasticity. Such information is useful in determining the effectiveness of the polymer for industrial purposes. Research papers have presented various values of the mechanical properties of hydrocolloid films.

This difference may be attributed to the composition and structure of these biopolymers and suppliers, as well as film preparation techniques.[39,40] The properties of levan-based films were studied using TS and EB evaluation. The data fromTable3 suggest that levan films with moderate TS and good EB values have excellent resistance and extensibility. According to the literature, levan films that are plasticized with 20% (W/V) demonstrate greater TS and EB values than κ-carrageenan films that are plasticized with 30% GLY (8.09%).

At this level, we realized that TS values were lower than those reported in carboxymethyl cellulose films plasticized with GLY 4.11 MPa.[41] The variation in the resistance and extensibility of films may be due to multiple factors, including the origin of the material, the type and concentration of plasticizer used, the preparation method of the films, and any postprocessing treatments.[18,42]

It can also be statistically emphasized that the plasticizer type has a significant ($p < 0.05$) effect on mechanical properties of films.Table3 indicates that levan-based films

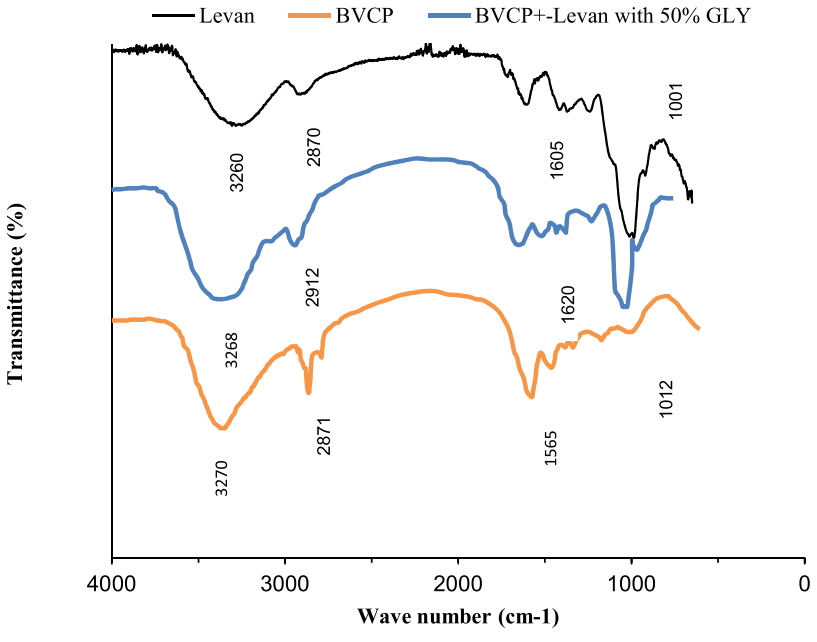

Figure 3. FTIR spectra of levan, (BVPC) protein, and levan−BVPC with glycerol.

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Table 3. Effect of Different Ratios between BVPC and Levan on Film Mechanical Properties[a]

film (BVPC/levan)	TS (MPa)	EB (%)	YM (MPa)
(00:100)	0.5 ± 0.1[b]	812.0 ± 21.0[b]	4.2 ± 1.1[b]
(100:00) + 50% GLY	1.9 ± 0.5[b,c]	147.3 ± 35.0[b,c]	17.1 ± 9.0[b,c]
(13.5:87.5)	2.0 ± 0.1[b,c]	54.3 ± 15[b,c]	80.3 ± 0.8[b,c]
(50:50)	1.5 ± 0.3[b,c]	4.1 ± 0.1[b,c]	168.4 ± 20[b,c]
(50:50) + 5% GLY	0.4 ± 0.1[b]	13.3 ± 0.5[b,c]	34.4 ± 6.3[b,c]
(50:50) + 10% GLY	0.5 ± 0.1[b]	16.3 ± 1.5[b,c]	15.3 ± 3.2[b,c]
(50:50) + 20% GLY	0.6 ± 0.2[b]	34.3 ± 4.0[b,c]	24.0 ± 3.2[b,c]
(50:50) + 30% GLY	0.37 ± 0.1[b]	64.1 ± 12.0[b,c]	5.6 ± 1.2[b]

[a]The results are expressed as mean ± standard deviation. Further experimental details are given in the text. [b]The values were significantly different from the value of the film prepared at 100% BVPC. [c]The value of the films prepared at 100% levan.

with GLY incorporated into their composition tend to demonstrate higher TS and EB.

Similar observations were reported by Zhang et al.[43] and Aguirre et al.[44] The researchers proposed that due to its small size, numerous hydroxyl groups, and hydrophilic properties, GLY may be able to penetrate between the levan polymer matrices, thus weakening the interchain interaction force and changing the mechanical properties of levan films.

The results ofTable3 demonstrate a noteworthy improvement in the mechanical properties of FFS films with different particle size distributions. These findings have offered us invaluable insights into the remarkable effect ($p < 0.05$) on the extensibility of levan films.

Some authors have reported that the incorporation of extra polymeric (such as plasticizer) and the use of polymers with various molecular weights influence the structural rearrangement distribution, thus affecting the mechanical properties of the produced films.[19] Qin et al.[42] revealed that TS and EB are mainly related to the film network, the intermolecular force, and the microstructure materials.

Our research aimed to demonstrate that the successful mechanical properties of levan-based films can be attributed to the size of the plasticizer and BVPC. It was essential to add levan along with a high amount of GLY and BVPC for the formation of complex structures in the films, thus providing good resistance and extensibility, which are essential for the successful production of films.

Barrier Properties of Levan-BV Films. Water vapor permeability (WVP) represents one of the most important properties for food packaging applications when considering food spoilage reactions. WVP works to avoid or lower the transmission of moisture from the food to the environment, as moisture is a major factor in food spoilage.[45] Thus, WVP should be as minimal as possible. For this reason, determining this parameter is crucial. The results of WVP of levan films are illustrated inTable4. The results clearly indicate that the films present a low WVP (3.40×10 cm^3 mm m^{-2} day^{-1} kPa^{-1}). Indeed, the lowest WVP value was obtained in BVPC/EPS with a ratio of 50:50 without GLY. Basically, these observed WVP values proved to be lower than those of films based on semirefined κ-carrageenan, plasticized with different concentrations of GLY (3.26−4.25 cm^3 mm m^{-2} day^{-1} kPa^{-1}).[45] According to WVP values, as the plasticizer concentration increased, the WVP of films increased as well (50:50) + 30% GLY 5.21 cm^3 mm m^{-2} day^{-1} kPa^{-1}. Indeed, it can be seen that the incorporation of low plasticizer concentration in films helps reduce the WVP of films. Thus, it represents another promising step toward the improvement of this material. This effect was evaluated compared to that reported in previous

Table 4. Effect of Different Glycerol (GLY) Concentrations on Water Vapor Permeability (WVP) and Oxygen Permeability (OP)

	permeability (cm^3 mm m^{-2} day^{-1} kPa^{-1})	
film (BVPC/levan)	WVP	OP
0% GLY	3.4 ± 0.2[a]	0.9 ± 0.03[a]
+5% GLY	3.5 ± 0.5[a]	1.11 ± 0.2[a]
+10% GLY	3.6 ± 0.1[a]	1.65 ± 0.1[a]
+20% GLY	4.5 ± 0.4[b]	2.43 ± 0.01[b]
+30% GLY	5.2 ± 1.2[c]	3.56 ± 0.02[c]

[a]The values were significantly different from the value of the film prepared without GLY. (WVP) of BVPC levan films prepared at the ratio of 50/50%.

works such as Tong et al.[47] in pullulan−alginate−carboxymethyl cellulose blend films and in carrageenan films.[46] The results of the study indicated that plasticizers can penetrate the polymer chains and reduce intermolecular forces, which leads to less dense polymer matrices and improved mobility of polymer chains. This rearrangement increases the free volume in the film system. In addition, the type of plasticizer had a statistically significant effect on the WVP of films ($p < 0.05$). It was observed that combining low and high-molecular-weight levan and BVPC in the film composition can improve its water vapor protection properties. Therefore, the selection of the right plasticizer, its concentration, and its type is important for creating food packaging films with good barrier properties. Given the significance of oxygen in the oxidation of food, the oxygen permeability of food packaging materials is very important. The biopolymer chain structure and plasticizer distribution within the biopolymer matrix are important in the permeability of films. Increasing the glycerol levels from 5 to 30% significantly increased the OP values;Table4. This finding could be attributed to an increase in chain mobility of the biopolymer and the creation of void spaces in the film matrix.[48]

Thermal Analysis. In the DSC analysis of levan−BVPC films, the glass-transition temperature (T_g) played an essential role in the thermophysical transition. Therefore, the glass-transition temperature of the control, as well as of levan−BVPC plasticized films, were obtained from DSC analysis. The results obtained from this study showed that the T_g values of levan−BVPC films were 116.27, 114.13, 113.9, 112.3, and 110.5 °C for 0, 5, 10, 20, and 30% glycerol, respectively. The incorporation of glycerol into control films caused a decrease in the T_g. These results were in line with those of previous results that reported the reduction of T_g with the incorporation of plasticizers into starch-based biopolymers. Consequently, the movement of polymer chains increased as the polymer

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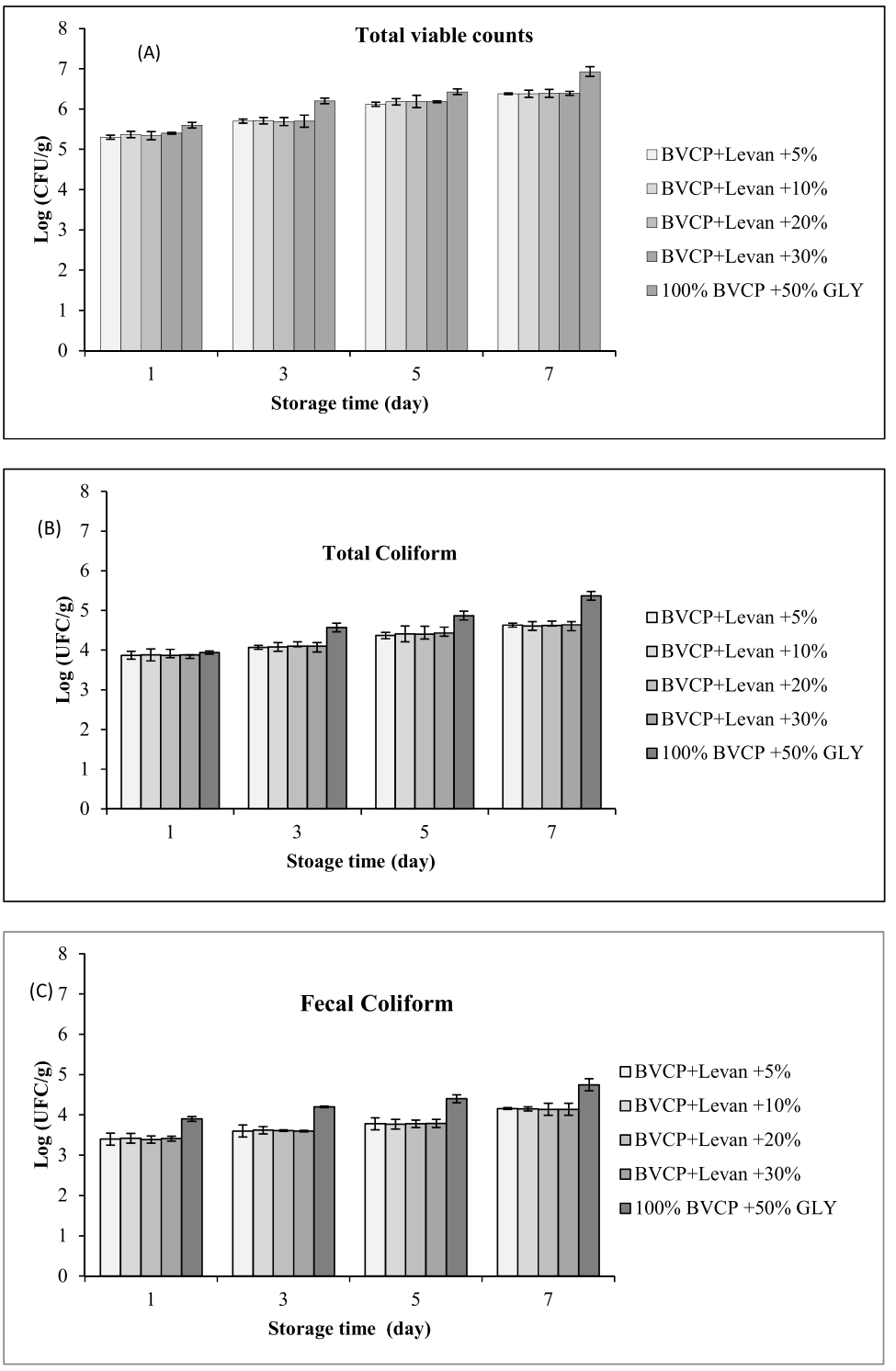

Figure 4. Mean microbial count (log CFU g^{-1}) (A) total viable counts; (B) total coliform; (C) fecal coliform of ground meat covered by BVPC−levan composite films stored at 4 °C for 7 days.

matrix became less compact.[49] However, in the current analysis, the T_g values of levan−BVPC films were significantly reduced as the percent of glycerol incorporated increased from 5 to 30%. The introduction of glycerol led to the production of more hydroxyl groups as active sites, which might be conquered by water molecules. In the same context, Sanyang et al.[49] reported that starch-based films showed a higher moisture content with a higher glycerol content that led to lower glass-transition temperature (T_g) values.

Utilization of Levan−BVPC Films for Meat Packaging. The majority of microbial contaminants found on meat products are on the surface, making antimicrobial packaging a more effective means of protecting food than introducing antimicrobial agents into the food itself since the packaging interacts with the food and its environment.[50] Recent technological developments in nanotechnology have led to the emergence of novel antimicrobial packaging materials that can help prolong the shelf life and enhance the safety of meat

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and meat products.[51] Figure 4 shows the changes of total viable counts, psychrophilic bacteria, total and fecal coliforms, and salmonella of minced beef meat packaged with the BVPC film and BVPC−levan films blended with different concentrations of glycerol during refrigerated storage.

BVPC films incorporated with levan at different ratios of glycerol exhibited significant inhibitory effects ($p < 0.05$) against tested bacteria than the control film (BVPC without levan). Comparisons of the bacterial count reductions among were significantly different ($p < 0.05$). It is noteworthy that the number of bacterial strains increased across the board, except for fecal coliforms, which indicated a decrease in fecal contamination over the course of 7 days when the meat sample was wrapped with a film composite. Coliforms are ubiquitous in nature and are now widely used as indicators of sanitation and food safety. It is noteworthy that the highest bacterial growth was observed in meat wrapped with PVCP, surpassing the BVPC blended films with levan in all treatments. The antimicrobial activity of levan is likely due to the slow release of nanoparticles and their interaction with the negatively charged biomolecules, causing cell wall damage, structural changes in protein and its biological function, and ultimately cell death.[52]

CONCLUSIONS

A series of levan−BVPC blend films in different proportions were prepared. First, levan and BVPC solutions were blended in different volume ratios in the presence and absence of GLY. The ζ-potential was measured to investigate the stability of film-forming solutions. The mean values of the ζ-potential ranged from −48 to −38 mV, which indicated the excellent stability of the film-forming solutions. Edible films of BVPC were observed to have significant effects on the fluid-forming solution as well as the optical and mechanical properties of the formed films. At this stage of analysis, we would assert that our study is a step that may be taken further, extended, and built upon as it lays the groundwork and paves the way for future research to investigate more outstanding features of films. Considering the synergistic effects of levan and BVPC will offer new perspectives for a more comprehensive elaboration of film blends with improved barrier and mechanical properties. Furthermore, the BVPC−levan films showed promising inhibition of bacterial growth for packaging of beef meat, which can help extend the shelf life of food. These results suggest that these films could be a potential natural packaging material for improving the shelf life of different foods.

ASSOCIATED CONTENT

Data Availability Statement

The data sets used during the current study are available from the corresponding author on reasonable request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05627.

Composition of the film-forming solutions such as glycerol with different amounts (PDF)

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Investigation, A.B., M.S.; writing—original draft preparation, A.B., M.S.; writing—review and supervision, A.M.S., M.B., D.P.P., S.E.C. All authors have read and agreed on the published version of the manuscript.

Notes

The authors declare no competing financial interest.

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