Effect of Bee Pollen on the Mechanical and Thermal Properties of Starch Films

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Summary: The use of casein, starch and bee pollen as biodegradable materials has been promise. The objective of this work was the development and characterization of films containing casein, pollen and starch. The films were obtained by casting process and the solvent evaporation was performed at 40 °C/24 h. The films characterization was carried out by microscopy, thermal analysis, opacity test, mechanical properties and barrier methods. The starch films presented heterogeneous on microscopy analysis. The thermal behaviors of pollen films were similar. The formulation containing only pollen 3% was unable to form film. The introduction of pollen in starch film formulation improved the mechanical characteristic and thermal stability of films.

Keywords: bee pollen; casein; films; mechanical profile; starch

Introduction

Biodegradable films have been studied in order to find environmentally friendly packaging, to improve the quality of food products, and to find new markets for existing materials. Film is a thin, continuous layer of polymer that may control mass transfer, provide mechanical protection. The films can also extend the shelf-life of different products by limiting moisture migration. Mechanical characteristics must be sufficient to maintain film integrity throughout production and handling practices.[1,2]

These films have been used in pharmaceutical and food technology, in order to improve the stability of drugs and food. They are used as coating dosage forms and food packaging. Various natural biodegradable macromolecules such as proteins and polysaccharides–based films can serve as coating materials due to their functional properties as barrier. The multicomponent systems, where protein and/or polysaccharides form a continuous, cohesive network have been studied.[1–3]

The characteristics of edible films include the renewable nature of their ingredients.[4] The use of proteins as casein, polysaccharides as starch or product protein/carbohydrate-rich as bee pollen has been promise due to their biodegradable characteristics. Proteins and polysaccharides generally form films with good mechanical properties.[3]

Films are produced using milk proteins. These films may retard moisture loss, show good tensile strength and moderate elongation.[1,5] Starch is one of the most studied biopolymers and starch based blends are an alternative to the most common biodegradable polymers applied in the biomedical field.[6] Bee pollen is a hive derived product of great commercial interest owing to its high nutritional quality and can be considered a potential source of proteins.[7] It contains about 20% of protein, 2.5% ash, 6% lipids.[8,9] The aim of this work was the

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development and characterization of films containing pollen, casein and starch.

Experimental Part

Films Preparation

Casein or pollen filmogenic dispersions were obtained in water at adjusted pH 11.0 and 4.5 respectively. The filmogenic dispersion contained starch was mixed under heating at 75 °C during 5min. Formulations were also obtained using polymers blends. It were used the filmogenic dispersion of casein 3% (CF), of starch 3% (SF), of pollen 3% (PF), and the blends casein:starch (CSF), casein:pollen (CPF) and starch:pollen (SPF). The blends dispersions were obtained using the proportion 1:1 for all mixtures. The blend SPF was obtained also in 1:4 proportion. Plasticizer (glycerol) was added in ratio of 20% (w/w) of polymer. The film was obtained by casting process and the solvent evaporation was performed at 40 °C/24 h. Films were stored in desiccators at ambient temperature for 12 hours before use.

Light Microscopy

Films were inspected using an Olympus C506-ADU optical microscope equipped with a camera (Olympus C-7070).

Fourier Transform Infrared Attenuated Total Reflectance Spectroscopy Analysis (FTIR-ATR)

FTIR spectra of the films were recorded in attenuated total reflection (ATR) mode using a FTIR spectrometer (ILLUMAT IR II with microprobe IR to optic microscopy SMITHS, Microscope Olympus BX51). The films were applied directly onto the KRS-5 crystal (Detector MCT Objective 36X-ATR) and scanned from 650 to 4000 cm^{-1} at 4 cm^{-1} resolution. Each spectrum represented an average of 64 consecutive scans.

Opacity

The films were cut into rectangular shapes (2 mm × 40 mm) and placed on the internal side of a spectrophotometer cell. Opacity of films was measured using a spectrophotometer (Q-108D, Quimis Instruments) between 300 and 600 nm.[10,11] The opacity was calculated as follows:

$$\mathbf{O} = \boldsymbol{I}/\boldsymbol{t}$$

where $\boldsymbol{I}$ is the absorbance with the specimen in the beam and $\boldsymbol{t}$ is the film thickness.

Thermal Analysis

The thermal properties of the free films were determined using a Differential Scanning Calorimeter (TA-50WSI Shimadzu). Film samples of 3.5 mg were accurately weighed into aluminum pans and then sealed. The samples were tested under a nitrogen atmosphere at a heating rate of 10 °C.min^{-1}, at a temperature range of 25 °C to 350 °C (DSC) and 500 °C (TGA).

Mechanical Evaluation

The mechanical properties of films were determined using a texture analyzing instrument (Texture Analyser, TA-XT2; Stable Microsystems, Surrey, UK). Film samples were cut into 2 cm wide and 5 cm length strips using a sharp razor blade. The acquisition parameters were: 1 mm/s prespeed; 1 mm/s test-speed; 1 mm/s postspeed with an acquisition rate of 100 points/s and 5 kg load cell. Film was secured with tensile grips, and a trigger force of 20 g was applied. The resulting profiles were analyzed using Texture Expert, Version 1.22 (Stable Micro Systems, Surrey, UK). Tensile strength (TS) was calculated by dividing the peak load by the cross-sectional area of the film. Elongation was determined by percentile of the change in the length of specimen to the original distance between the grips.

Water Vapor Transmission Rate (WVTR)

The water vapor transmission rate through the films and the permeability were determined according SPROCKEL et al. (1990). The permeability of films was determined by water vapor loss in a gravimetric cup film sealed method under known relative humidity (RH) given by saturated solutions in contact with non dissolved salt ($CaCl_2$

80%RH) placed in a desiccator containing silica in a dehumidified room.[12]

Each experiment was continued for 48 h and carried out with 8 replicates. The permeability was calculated by:

$$\mathbf{WVT = w/A.h}$$

where: WVT is water vapor transmission in $mg.mm^{-3}$; w is lost weight in mg, A is film area in mm^2, h is film thickness in mm.

Statistical Analyses

Data were reported as mean and standard deviation for at least triplicate measurements. Results were tested for statistical significance by ANOVA/ Tukey or Dunnet by Statistic 8.0 software. Differences were considered statistically significant at the $p < 0.05$ level.

Results and Discussion

Films produced with starch (SF) were brittle and the pollen dispersion at 3% was unable to form films. Starch films produced using glycerol at 15.0% (dry polymer weight) were dry and brittle.[13] The addition of plasticizers produced more flexible films, but increase the hydrophilicity and permeability of polar molecules across the films due to formed porous. Then, the characterization was carried out only with the others formulations.

The main objective of the visual and microscopic observation was to decide which films presented the most homogeneous structure, so they could be produced on a large scale. The Figure 1 presents the microscopic analyses of the films. It can observe that casein films (CF) showed homogenous surface by both microscopy methods. The introduction of pollen into formulation (CPF) changed the film color with small alterations in film homogeneity. The blends of casein-starch (CSF) and starch-pollen (SPF) become the film surface more heterogeneous, but on SPF that characteristic was more evident.

The infrared spectroscopy, considering interactions at a local range order, has already been used to describe the organization and structure of starch at various water contents. Amorphous starch was characterized by an absorbance band around $1022\,cm^{-1}$ (C–OH bending), the crystalline state could be identified by the development of a band at $1047\,cm^{-1}$, which one is composed of two overlapping bands at 1040 and $1053\,cm^{-1}$.[14] These bands can be observed at Figure 2 in SPF and CSF spectra.

The spectral region between 1065–$870\,cm^{-1}$ was assigned to C–O, C–C and C–H stretching modes in alcoholic COH moieties. Infrared absorbances generated from deformations of the various C–O–H valence angles are very sensitive to the degree of hydration. Therefore, the high intensity in this spectral region can suggest that SPF presents a higher hydrophilicity than CSF, with a higher degree of hydration. The hydrogen bond of the OH group at C6 are located between the double helices, it could allow modification in helices associations, favoring the increasing water content.[15]

Proteins molecules exhibit many vibrational frequencies. Nine characteristics group frequencies arise from the peptide linkages.[16] Amide I band (1,620 to $1{,}700\,cm^{-1}$) may inform about protein secondary structure. Therefore this band is very complex and its components are not obtained individually, obtaining a broad band.[17] At this region, the casein films (CF, CSF and CPF) presented a double signal, characteristics of casein spectra. At SPF spectrum, the signal is unique, probably due to the pollen protein presence. At 3050–$3600\,cm^{-1}$ (O–H stretching) and 2920, $2880\,cm^{-1}$ (C–H stretching) regions, all samples presented similar profiles.

The opacity of films was higher in CF and CSF. These formulations presented higher content of macromolecules, while the pollen films (CPF and SPF) showed between 380 and 480 higher light permeability.

Figure 4 showed a differential scanning calorimeter (DSC) curves of starch:pollen films at different proportion (1:0, 1:1 and 1:4). At the dehydratation event (until

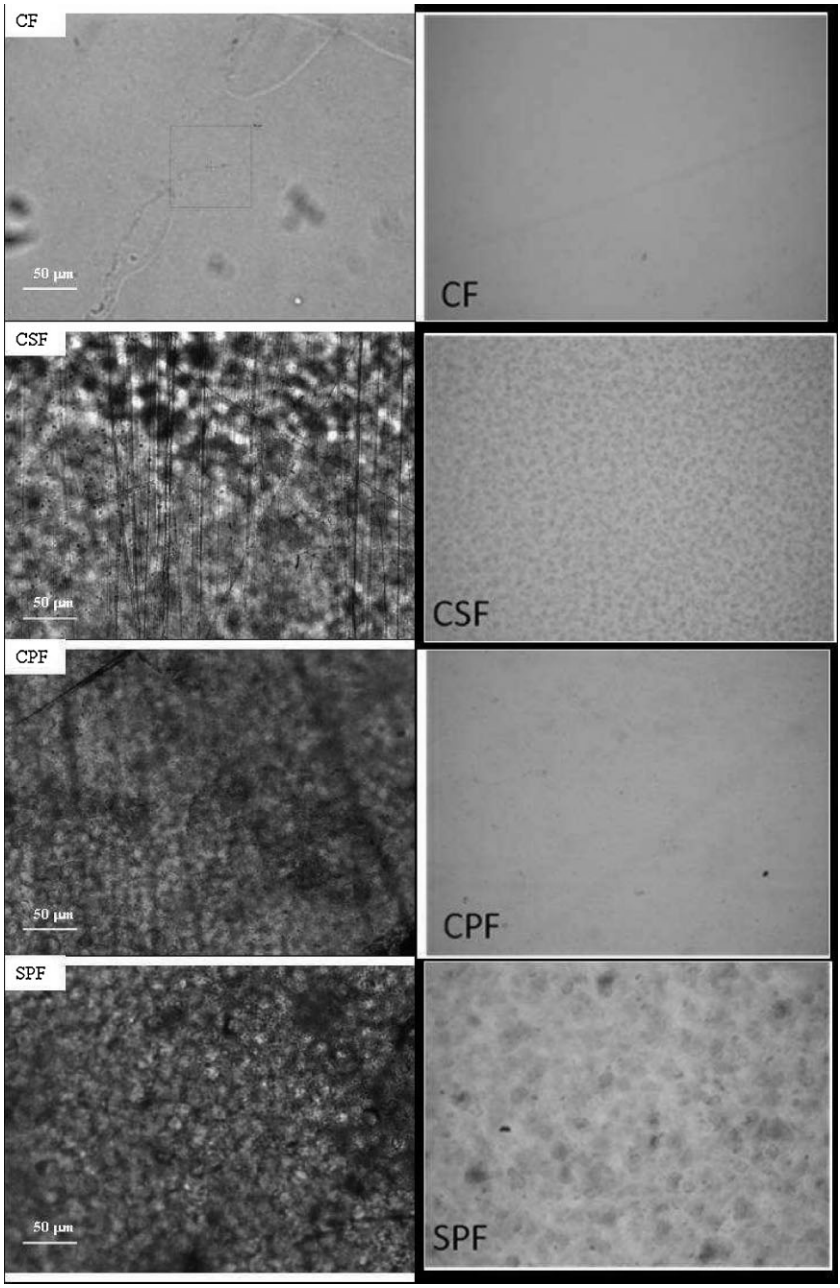

Figure 1.
Reflectance photomicrography (A – magnification 36×) and Light microscopy (B – magnification 100×) of casein film (CF), casein-starch film (CSF), casein-pollen film (CPF) and starch-pollen film.

100 °C) the thermal profiles of films were similar, except to SPF 1:4. This film, due to pollen proportion, showed a higher interaction with water molecules, difficult the elimination of these molecules. This event can be observed in TGA curve, where this film presented the lowest weight lost until 100 °C (Figure 5).

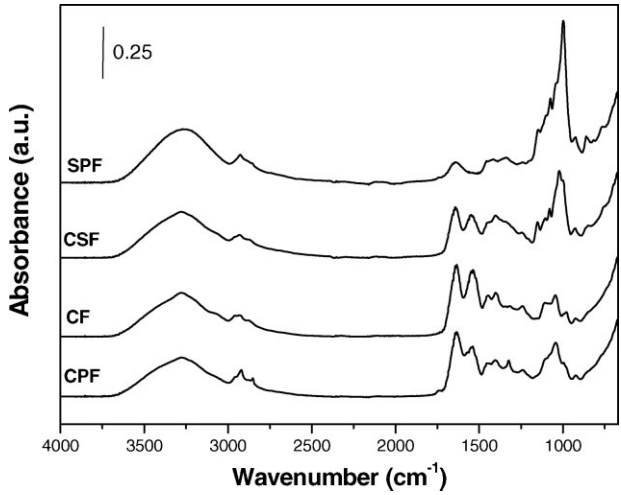

Figure 2.
FTIR-ATR spectra of starch:pollen 1:1 film (SPF), casein:pollen film (CSF), casein film (CF) and casein:pollen film (CPF).

After 100 °C, the degradation step begins and we can observe that the films with pollen presented higher thermal stability when compared with SF. The pollen content improves the thermal characteristic probably due to the protein presence. Generally, protein-based films have more interesting mechanical and barriers characteristics than polysaccharides.[3]

The tensile strength results of films showed that the casein films presented higher maximal tensile. The introduction of pollen and starch into casein formulations decrease significantly the tensile strength ($p = 0.0002$ and $p = 0.0497$ for CSF and CPF respectively). The SPF showed the lowest tensile strength value.

The inclusion of plasticizers, like glycerol, in film formulation can decrease the brittleness and ensure the formation of free-standing films.[18] Plasticizers are used to improve the processibility, flexibility, and elasticity of polymers. These compounds alter the thermal properties of the polymer by disrupting the intermolecular interactions of the polymer chains.[2]

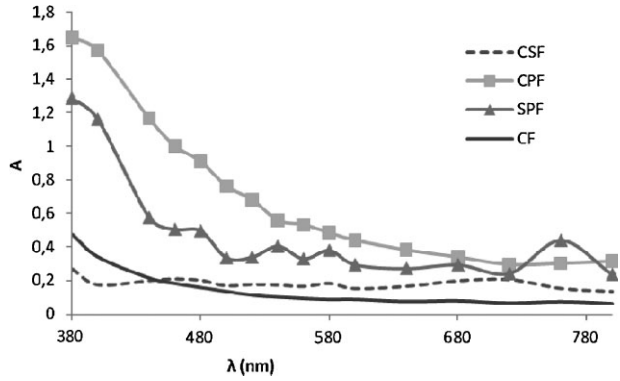

Figure 3.
Opacity of films.

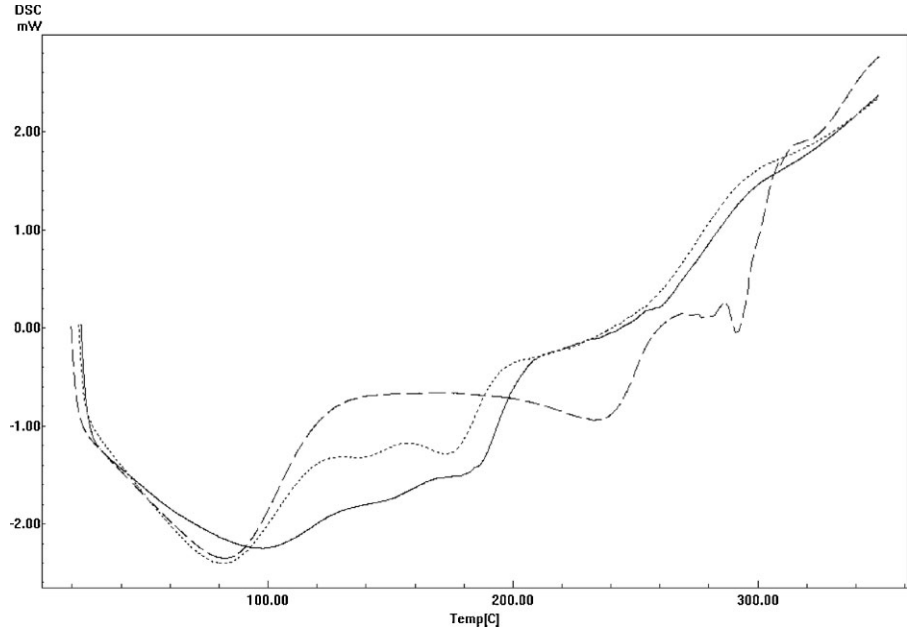

Figure 4.
DSC curves of starch:pollen films (__SPF 1:4), (. . .SPF 1:1) and starch (- - - SF).

Generally, less stiff and rigid, more extensible films may be obtained by increasing of plasticizer concentration in the film formulation, due to the reduction in interactions between the biopolymer chains. Low molecular weight hydrophilic molecules, such as carbohydrates presents in pollen, can easily fit into proteins networks and establish hydrogen bonds with reactive groups of amino acid residues, reducing the protein-protein interactions.[19] The reduction of biopolymer chain-to-chain interaction results in the improvement of film flexibility and stretch ability, however also increase the film permeability.[2]

Elongation results demonstrated that there were no significative difference between CF and CPF ($p = 1{,}00$). On the other hand, the introduction of starch into casein filmogenic dispersion became the film brittle. The interaction between pollen and starch improved significantly the elongation capacity of films.

The composition of pollen is about 20% of proteins and 40% of carbohydrates.[20] The film containing only pollen in filmogenic dispersion do not viable due to the low concentration of macromolecules. However, the introduction of pollen into a polysaccharide films, like starch film showed a suitable mechanical response ($p = 0{,}001$). The same concentration of pollen into protein films, like casein, seems do not alter de mechanical results ($p = 1{,}0$).

Banana and mango starch films with 50% of glycerol presented 40 and 30% of elongation respectively.[21] These results demonstrated that 20% of glycerol is not sufficient to improve mechanical properties of starch films. The interaction of casein:starch showed no positive results, decreasing the elongation of films when compared to casein films ($p = 0.0002$).

The films with pollen (CPF and SPF) presented similar profile of WVT when compared with casein film ($p > 0.05$). This result indicated that the hydrophilicity of these films are similar. The CSF showed higher permeability than all films studied ($p < 0.05$), suggesting a different interaction between casein and starch. The three-dimensional arrangement of these macromolecules seems to form a dense and

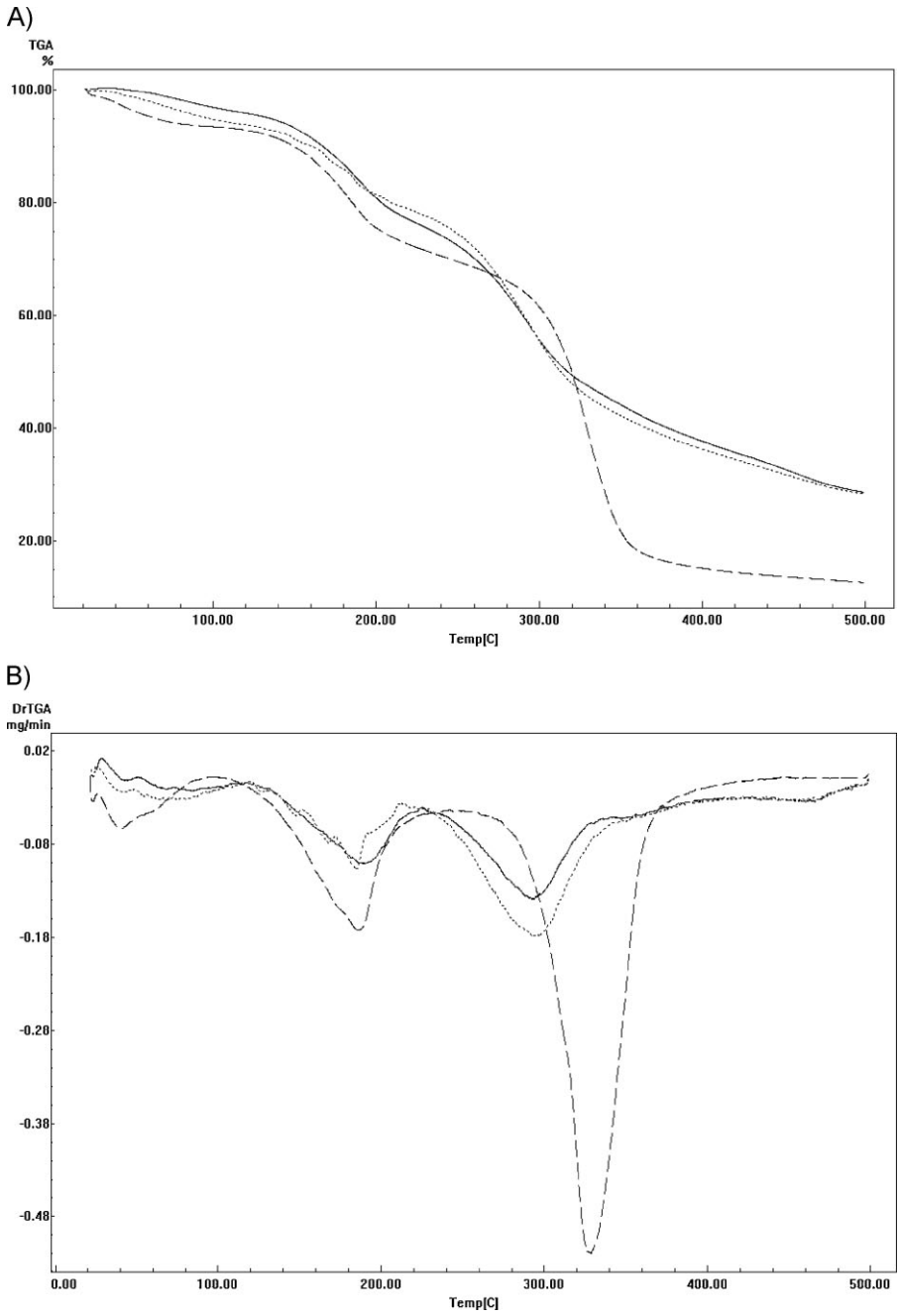

Figure 5.
TGA (A) e DrTGA (B) curves of starch:pollen films (__SPF 1:4), (. . .SPF 1:1) and starch (- - - SF).

cohesive network, where the plasticizer interaction can be difficult. The both polymer presents hydrophilic characteristic, improving the polarity of system.

Conclusion

The blends casein-pollen and starch-pollen films can be formed with suitable charac-

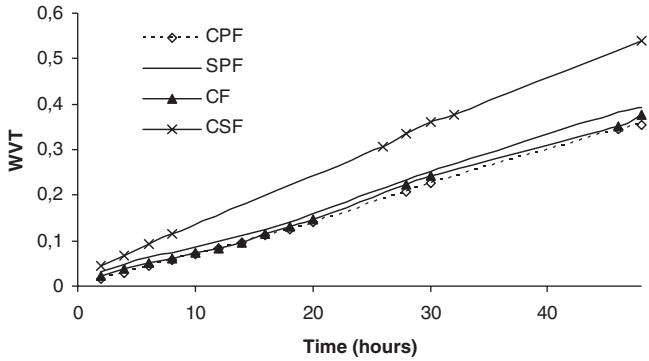

Figure 6.
WVT of casein (CF), starch:pollen (SPF), casein:pollen (CPF) and casein:starch (CSF) films.

Table 1.
Mechanical properties of films.

Films	Tensile strength (MPa)	Elongation (%)	Young's modulus (MPa)
CF	16.87 ± 1.35^{a}	26.7 ± 4.9^{a}	32.6 ± 8.4^{a}
CSF	$2.64 \pm 0{,}59^{b}$	1.7 ± 0.5^{b}	854.6 ± 298.5^{b}
CPF	5.72 ± 1.81^{c}	26.7 ± 3.7^{a}	10.9 ± 4.2^{c}
SPF	1.28 ± 0.29^{b}	97.3 ± 27.3^{c}	6.1 ± 1.5^{c}

Columns with different superscript letters are significantly different ($P < 0.05$).

teristics. The introduction of pollen into formulations of casein and starch films improved mechanical and barrier characteristic.

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