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RESEARCH

Hibiscus Sabdariffa L. Extract as a Natural Additive in Food Packaging Biodegradable Films to Improve Antioxidant, Antimicrobial, and Physicochemical Properties

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Abstract
In this study, biodegradable active films were prepared from potato starch and polyvinyl alcohol at different proportions, mixed with acetone extract of *Hibiscus sabdariffa* L. (HS) and using glycerol as a plasticizer. Functional properties, antimicrobial, and antioxidant capacity were evaluated. Potato starch films with a proportion of polyvinyl alcohol up to 50% and HS extract had significant antioxidant capacity and antibacterial effect against most of the analyzed strains. Adding polyvinyl alcohol (PVOH) and HS extract improved the mechanical performance and reduced water vapor permeability of the materials. The active biobased films with HS extract presented good physicochemical, antimicrobial, and antioxidant properties. These materials are considered as suitable for food packaging, and the active compounds in the roselle extract are a natural antibacterial option for the food area. The materials based entirely on biodegradable products are an excellent alternative when developing and marketing biobased materials, minimizing the environmental impact of food packaging.

Keywords Edible plant extracts · *Hibiscus sabdariffa* · Active packaging · Bio-based polymers · Antimicrobial activity · Antioxidant capacity

Introduction

The pandemic caused by Covid-19 significantly increased the utilization of single-use plastics in the food industry, particularly for packaging and take-out food. This increase has aggravated the environmental pollution by plastic packaging materials which are manufactured using polymers derived from fossil fuels with low or null biodegradability. Notably, in some industrialized countries, the use of plastic increased from 40 to 240 metric ton per day, before and during the pandemic, respectively [1]. Packaging is fundamental to preserve and commercialize food. Biodegradable films are

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a promising alternative to replace plastic materials made from synthetic polymers. Biobased materials are mainly composed of natural polymers such as gelatin, pectin, gums, chitosan, carrageenan, or starch. Many research works have studied starch due to its availability from various sources, making it renewable and readily accessible [2, 3]. Starch is a polymer composed of amylose (linear structure) and amylopectin (branched structure), both macromolecules formed by glucose units [4]. Due to its rapid degradation, abundance, and low cost, it can be used to manufacture biodegradable packaging materials. However, films made from pristine starch exhibit poor mechanical properties, so the addition of reinforcing materials or compounds that improve their mechanical performance is essential to use them as a packaging material [5, 6]. Polyvinyl alcohol (PVOH) is a biodegradable and water-soluble synthetic polymer that has been used to improve the mechanical performance and gas barrier properties of biobased materials intended for several applications in food or pharmaceutical industries [7, 8].

Biodegradable films can serve as a carrier for active compounds including vitamins, minerals, antioxidants, and antimicrobial agents [4, 9]. *Hibiscus sabdariffa* Linne is a herbaceous plant typical in dry subtropical, mountainous, and thorny scrub climates. This edible hibiscus is part of the Malvaceae family, also known as jamaica, karkade or roselle, which can be found in Africa, Central America, South America, and Southeast Asia [10–12]. This plant, rich in anthocyanin and phenolic compounds is widely used by various industries such as pigments, food additives, and medicine [10, 13, 14]. Extracts obtained with various solvents have shown that *H. sabdariffa* has non-toxic chemical compounds and they can be used as antimicrobial, antifungal, antioxidant agents for food preservation [9, 15, 16], or as adjuvants to maintain food safety. These natural additives are non-toxic when ingested [16] and are environmentally friendly making them a viable option to develop clean-label and active packaging materials.

For all of the above, we hypothesize that adding PVOH allows obtaining a starch-based film with improved functional characteristics that can be used as a vehicle for the incorporation of an active extract of HS. The main goal is to develop active films with antioxidant and antibacterial capacity based on potato starch/polyvinyl alcohol with an acetone extract of *Hibiscus sabdariffa* intended for food packaging applications.

Table 1 Antimicrobial effect of 30% acetonic extract (w/w of total polymers) of *Hibiscus sabdariffa* L. (HS) present in biodegradable films based on several ratios of potato starch-PVOH

Potato starch-PVOH ratio	Pathogenic microorganism			
	L. monocytogenes	S. aureus	Salmonella spp.	E. coli
S100P0-HS	18.5 ± 0.2 [d]	12.0 ± 0.0 [b]	15.0 ± 0.0 [c]	12.0 ± 0.0 [c]
S75P25-HS	15.5 ± 0.1 [c]		12.0 ± 0.0 [b]	11.0 ± 0.0 [b]
S50P50-HS	12.5 ± 0.1 [a]	9.0 ± 0.0 [a]	9.5 ± 0.1[a]	10.0 ± 0.0 [a]
S25P75-HS	15.5 ± 0.1 [c]			
S0P100-HS	14.0 ± 0.0 [b]			

Mean ± standard deviation of three replicas of zone of inhibition diameter (mm). (–) No inhibition halo was observed during the test. Values with different letter in a same column denote significant difference (Tukey test; $p < 0.05$)

Results and Discussions

Antimicrobial Capacity

The antimicrobial capacity of potato starch-PVOH biodegradable films containing acetonic extract of *Hibiscus sabdariffa* L. (HS) was assessed against four pathogenic microorganisms, *Salmonella* spp., *L. monocytogenes*, *E. coli*, and *S. aureus*. Table 1 shows the antimicrobial activity of the starch-PVOH with and without HS extract. Inhibition halos (areas with no microbial growth around the film sample disc) were observed in all microorganisms with three of the evaluated films. Starch films with PVOH proportions from 0 to 50% and HS acetone extract (S100P0-HS, S75P25-HS, and S50P50-HS), exhibited antimicrobial activity against most of the tested pathogenic microorganisms. Films containing high proportions of PVOH (S25P75-HS, S0P100-HS) only presented antimicrobial effect against *L. monocytogenes*. The films with no HS extract did not show inhibition halos, therefore they are not reported in Table 1. Films made from pristine starch (with HS extract) presented the highest inhibition halo against all the evaluated microorganisms. Likewise, increasing the amount of HS extract added to the filmogenic solutions increases the antimicrobial capacity of the film [17].

Hibiscus sabdariffa leaves of a wide number of varieties contain high levels of polyphenolic compounds [11, 13, 15, 18]. The antimicrobial mechanism of these active compounds comprises the adhesion to the cell wall, promoting the rupture of the cytoplasmic membrane, and releasing the cell's internal components; another antimicrobial mechanism is enzymatic inactivation [4]. The antimicrobial capacity of *Hibiscus sabdariffa* extract is very promising making it a viable option as a natural preservative that can potentially replace chemical-based additives commonly used in the food industry.

Antioxidant Capacity

To evaluate the antioxidant capacity of the biodegradable starch-PVOH films with HS extract, the materials were immersed in water for 48 h and the releasing of the active compounds was assessed at 3, 24, and 48 h. The results revealed a substantial release of total phenolic compounds (TPC) over the immersion period. The TPC were expressed in milligrams equivalent of gallic acid (mg eq GA), for each gram of biodegradable film. The highest concentrations obtained were 2.89 ± 0.61 and 2.75 ± 0.28 mg eq GA/g of film containing PVOH ratios of 0 and 75, respectively (Fig. 1a). The highest concentration of phenolic compounds in plants has been reported in flowers, probably because large amounts of these compounds are needed to protect this part of the plant from the damage caused by phytopathogens [19]. *Hibiscus sabdariffa* has a rich content of polyphenolic compounds, anthocyanins, and flavonoids [18]. The primary phenolic acids reported are chlorogenic acid, kaempferol glycosides, and quercetin, contributing to its antioxidant and anti-inflammatory capacity [12, 13, 20]. The possible reason why the highest total phenol values occur after 48 h of immersion in water and the lowest ones after 3 h, is because as time elapses, more OH groups are

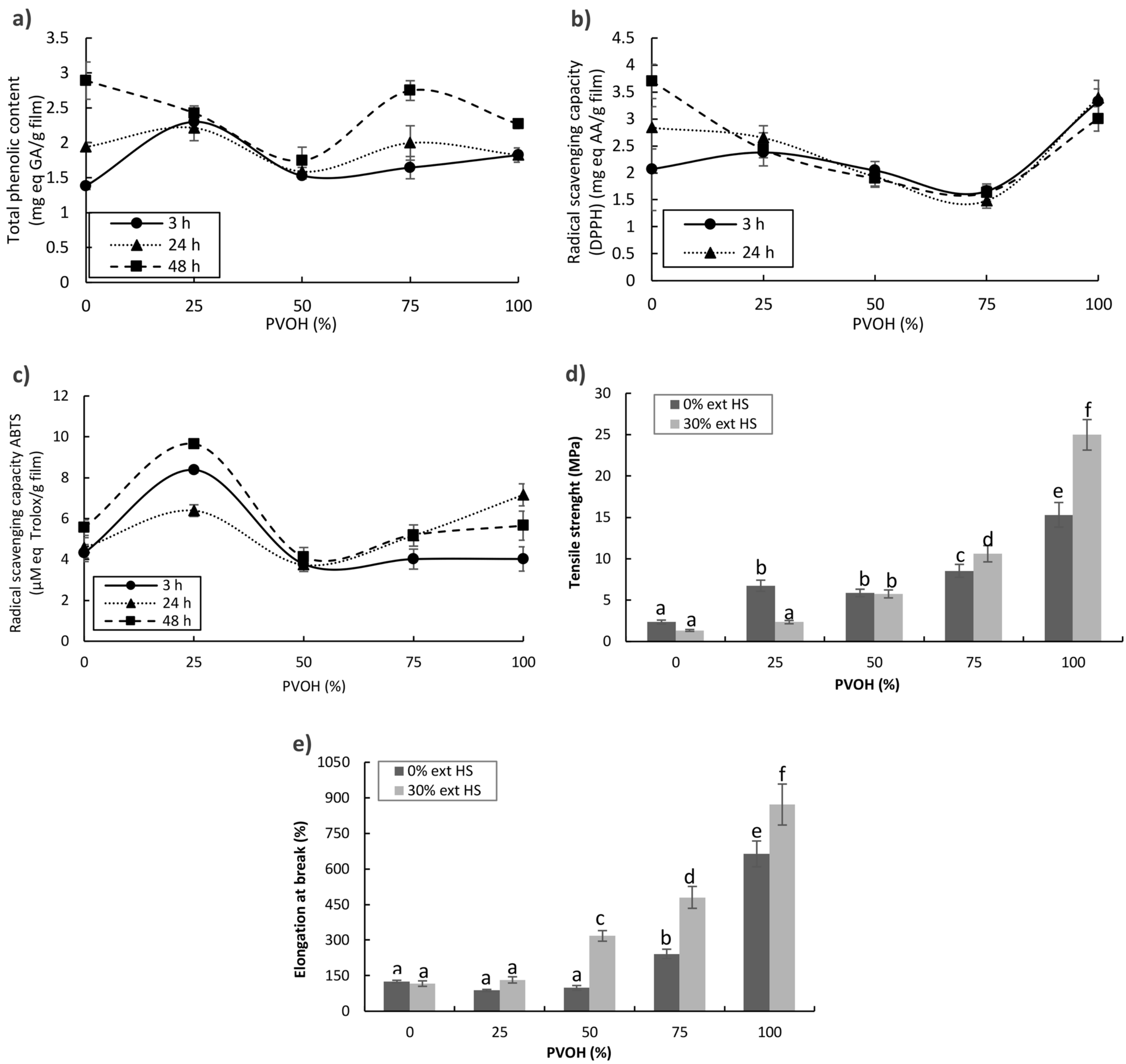

Fig. 1 **a**), **b**), **c**) Antioxidant capacity, **d**) Tensile strength (TS) and **e**) elongation at break (%E) of potato starch-PVOH films with 0% and 30% acetonic *Hibiscus sabdariffa* L. (HS) extract (w/w of total polymers)

available in the polymeric matrix, facilitating the flow of water molecules and releasing the HS extract that is dispersed within the polymer molecules.

Antioxidant activity depends on the concentration [10, 21]. In this study, the antioxidant capacity was assessed by the DPPH, ABTS, and radical scavenging methodologies [10]. Films were elaborate adding 30% by weight of acetone extract relative to the total polymer content. The highest concentration was obtained after 48 h since time allowed the acetonic extract to dissolve in the liquid suspension (Fig. 1c). All biodegradable films exhibited good free radical scavenging properties against the radical cation ABTS (Fig. 1b) with no significant difference among most extract film formulations, except for potato starch with no PVOH (S100P0). These findings are similar to those reported for phenolic compounds obtained from mixtures of natural extracts of cocoa, HS, ginger, betel, and gedabu [9, 22, 23]. This antioxidant capacity, along with the antimicrobial effect, makes these films a suitable material for food preservation offering clean-label and active solutions derived from natural source, with simple processing methods, and with no toxic chemicals for the consumer or the environment.

Physicochemical Characterization

Film Appearance and Morphology

All starch-PVOH films, with and without 30% (w/w) acetonic extract of HS, exhibited a good appearance. Films containing HS extract and pristine polymers showed a more homogeneous appearance than those from the mixtures of both polymers with the antimicrobial extract (Fig. 2A), even so, they were very easy to manipulate. The surface morphology (both top and cross-section) of the starch-PVOH biodegradable films is shown in Fig. 2B.

Some ungelatinized starch granules were observed on the upper surface of films with a high starch ratio (without HS extract). This effect was reduced when the proportion of PVOH increased in the formulation. However, adding HS

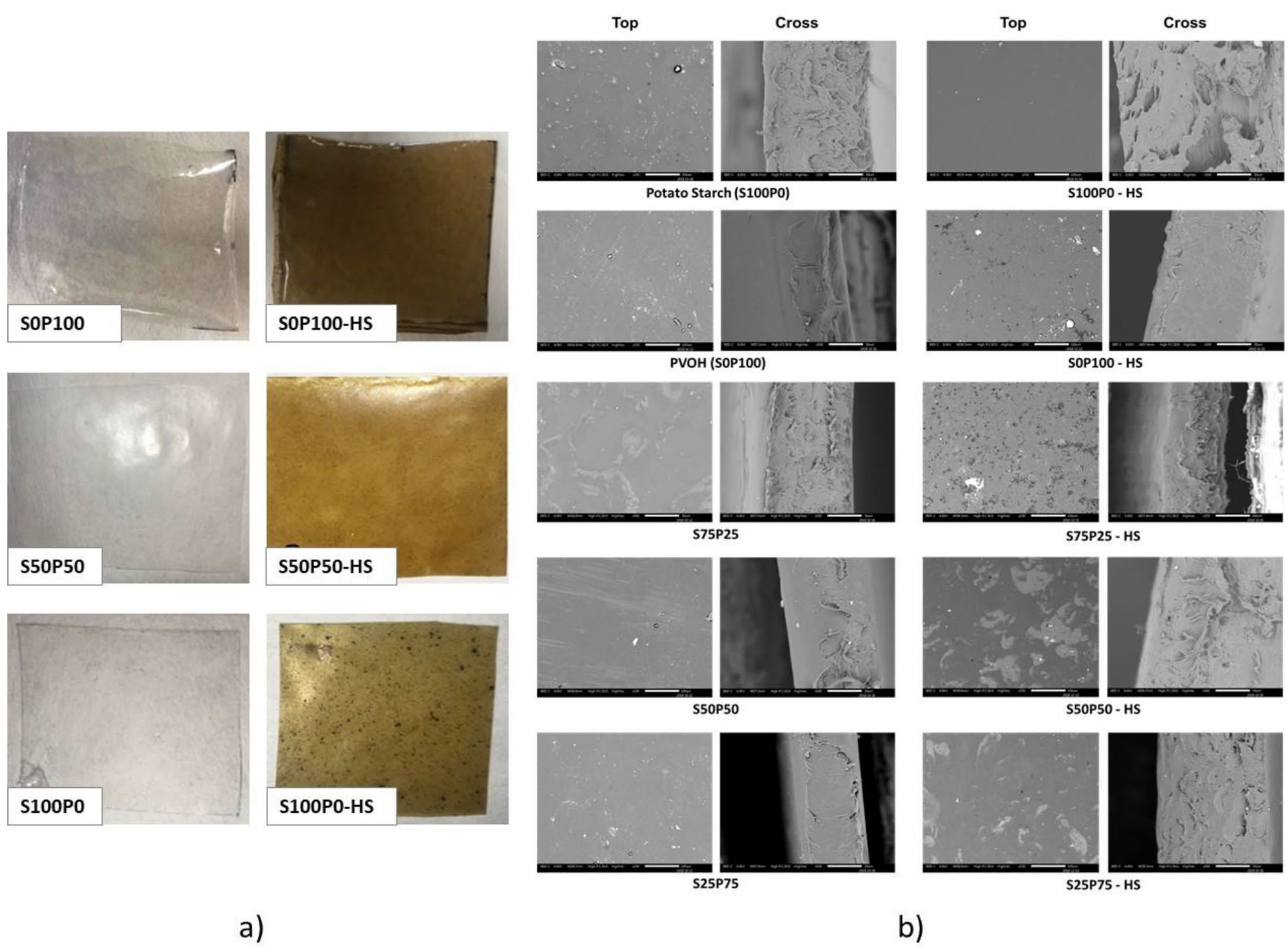

Fig. 2 (**a**) Appearance, and (**b**) Micro-photographs of the surface morphology of the top (250x) and cross-section (500x) of starch (S)-PVOH (P) films and with HS extract

extract to starch-PVOH films resulted in a heterogeneous morphology with no ungelatinized starch granules. This irregular morphology of the films with HS extract could be due to an incomplete solubilization of the extract during preparation of the filmogenic solution, although macroscopically, they show a homogeneous appearance. Cross-sectional analysis of the films with the highest starch content showed heterogeneous and fibrous appearances. However, the cross-section became more homogeneous with the increase of PVOH content in the films.

Thickness and Percentage of Water Solubility

The thickness of the starch-PVOH composite films, with and without HS acetone extract, ranged between 109 and 218 µm (Table 2), decreasing as the proportion of PVOH increased up to 75% in the polymer blends, an effect observed both in the films with and without HS extract. The solubility of polymeric materials composed of potato starch and PVOH with no HS extract, did not present significant difference (Table 2) when the proportion of PVOH increased from 25 to 75%; meanwhile, the control starch film (S100P0) had the highest solubility in water. The incorporation of the acetone HS extract increased the solubility of the materials compared to the films with no extract. Notably, when comparing the different films added with HS extract, the susceptibility to water increased significantly when the proportion of PVOH increased to 75% (S25P75 – HS).

Mechanical Properties

Films of pristine starch with no PVOH showed high brittleness and poor strength and elongation. To improve the mechanical properties, the potato starch was blended with PVOH seeking to improve dimensional stability and strength [7]. Results showed a significant change of the mechanical properties when the ratio of PVOH in the films increased (Fig. 1). Also, the incorporation of the acetone HS extract had a significant effect on the mechanical performance of the films. The percentage of elongation at break (Fig. 1e) increased with the addition of the HS extract which could have improved the compatibility between starch and PVOH, resulting in a robust matrix with better mechanical capacity. This plasticizing capacity has been reported in some other compounds extracted from natural sources [21, 24, 25].

Water Vapor Permeability (WVP)

The water vapor barrier capacity is an essential property in packaging materials intended for food use. A correlation was observed among water vapor permeability (WVP) (Table 2), proportion of PVOH, and the addition of HS extract. In films with no extract, WVP significantly decreased when the starch: PVOH ratio was 50:50, much lower than in pristine starch film (S100P0). In starch-PVOH films with HS extract, WVP decreased when the natural extract was added or when the PVOH ratio increased. Adding PVOH improves the gas barrier properties due to the hygroscopicity of the synthetic polymer, which enhances the plasticizer's ability to modify both the mechanical performance and the water vapor barrier capacity of the films. The significant decrease in WVP observed when adding the acetone HS extract is probably associated to a reduction in the free volume of the starch-PVOH matrix, resulting in a more compact structure due to the cross-linking. A more compact matrix could have prevented the starch from swelling, restricting molecular movement, leading to a decrease in WVP [26].

Thermal Properties

An increase in the glass transition temperature (Tg) of the potato starch-based films was observed as the ratio of PVOH in the mixture increased (Table 2). However, films with HS extract, exhibited higher Tg values compared to the same films with no extract, excepting films from pristine PVOH (S0P100-HS). Increasing the proportion of PVOH in

Table 2 Thickness, water solubility, Tg, WVP of potato starch (S)-PVOH (P) films and with the incorporation of *Hibiscus sabdariffa* L. (HS) extract

Potato starch-PVOH ratio	Thickness (µm)	Solubility (%)	T_g (°C)	WVP x10^{-10} (g/m.s.Pa)
S100P0	165.45 ± 28.99^{bc}	36.14 ± 2.86^{bc}	27.08	6.27 ± 0.06^{d}
S75P25	164.67 ± 14.33^{bc}	33.05 ± 2.03^{ab}	32.44	5.48 ± 0.20^{bcd}
S50P50	134.10 ± 19.14^{ab}	28.57 ± 1.92^{a}	34.68	4.88 ± 0.24^{abc}
S25P75	109.67 ± 8.80^{a}	29.09 ± 1.88^{a}	38.94	4.61 ± 0.13^{abc}
S0P100	180.69 ± 8.73^{cde}	33.54 ± 1.57^{ab}	47.42	5.48 ± 0.35^{cd}
S100P0 – HS	216.30 ± 42.75^{ef}	47.23 ± 1.78^{d}	40.97	4.75 ± 0.28^{abc}
S75P25 – HS	218.12 ± 9.08^{f}	41.28 ± 0.53^{c}	47.00	4.56 ± 0.42^{cb}
S50P50 – HS	209.33 ± 6.29^{def}	38.06 ± 1.68^{bc}	48.00	4.25 ± 0.11^{a}
S25P75 – HS	203.91 ± 24.87^{def}	63.07 ± 0.80^{e}	43.87	4.16 ± 0.21^{a}
S0P100 – HS	180.28 ± 8.18^{cd}	34.97 ± 3.48^{b}	39.64	4.91 ± 0.11^{abc}

*Mean values ± standard deviation ($n = 3$). Values with different letter in a same column denote significant difference (Tukey test; $p < 0.05$)

films with HS, Tg values increased from 40.97 °C for films of pristine starch to 48 °C in the sample with 50% PVOH (S50P50 - HS). However, when the proportion of PVOH exceeded 50%, Tg decreased to 39.64 °C in the sample with no starch (S0P100 - HS).

Conclusions

Biodegradable films were prepared from different mixtures of potato starch and PVOH adding *Hibiscus sabdariffa* L. (HS) extract. Films with HS were colored and non-transparent materials with antimicrobial effect against four pathogens (*Salmonella* spp., *E. coli*, *L. monocytogenes*, and *S. aureus*), as well as a good antioxidant capacity, due to the presence of phenolic compounds. The morphological, mechanical, and water vapor permeability properties of the films indicate that the acetone extract of HS can act as a plasticizer, improving the mechanical performance of the material. In future studies, these materials will be tested to assess their antimicrobial and antioxidant capacity for food preservation, either as a preformed film or as a direct coating on the product.

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Author Contributions F.A.H.-H. Investigation, Formal analysis, C.A.G.-A. Conceptualization, J.C.-R. Investigation, E.A.V.-L. Investigation, Methodology, M.C.G. Methodology, G.V. Investigation, E.J.J.-R. Investigation, R.Y.A.-L. Conceptualization, Visualization. All authors reviewed the manuscript.

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Data Availability The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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