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Influence of grape and acerola residues on the antioxidant, physicochemical and mechanical properties of cassava starch biocomposites

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ABSTRACT

The aim of this work was to develop cassava starch biocomposites with varius concentrations of grape skin (Gr) and acerola (Ac) residues (0.1, 1.0, 5.0 and 10.0 wt%) using extrusion and injection molding processes. Grape residue had the highest concentration of total monomeric anthocyanin (ANC) and Ac had the highest concentration of total phenolic content (TPC) and total sugars. The starch and fruit residues had thermal degradation onset temperatures above the processing temperature profile of polymer biocomposites. The results showed that the antioxidant, physicochemical, mechanical, and morphological properties of these biocomposites were influenced by the type and concentration of the fruit residues. The addition of grape skins and acerola residues to the cassava thermoplastic starch resulted in better antioxidant characteristics, indicating the potential of these formulations for the development of new bioactive packaging obtained by large-scale processes.

1. Introduction

In recent years, the production and use of biodegradable polymers obtained from natural sources has become a viable alternative to replace the synthetic polymers used in short-life applications. Due to their technical and economic viability, these materials have great potential for expansion in their production. Polymers obtained from natural sources may come into contact with food and have the ability to degrade more quickly, reducing the environmental impact, which justifies their use in packaging, disposable products and other applications [1–4].

The use of starch as a raw material has been of great interest in the study of biodegradable materials obtained from renewable sources. Starch is a polysaccharide that is abundant in nature, low cost, and can be transformed into polymer biocomposites. The materials produced from starch are known as thermoplastic starch (TPS) and can be obtained by conventional processes already used for synthetic polymers, such as extrusion, injection, compression, and blow molding [4–7]. The biopolymer obtained from starch is easy to produce and, has a very short degradation time, but it has limitations regarding its mechanical properties and water absorption when compared to synthetic polymers [8,9].

Recent research [10–16] has introduced fillers of by-products and fruit and vegetable residues in synthetic polymer and starch matrices. The fibers and bioactive compounds of the natural fillers obtained from fruits and vegetables, when incorporated into the TPS, contribute several functions, including pigment, mechanical reinforcement, and antioxidant action, in addition to sensory and microbiological properties [17–20].

Use of by-products from the grape skin and acerola industries have been incorporated into starch and other polymers obtained from natural sources, due to the phenolic compounds and anthocyanins present in these residues [21–25]. Phenolic compounds are natural substances present in fruits, which are responsible for several functions, mainly antioxidant properties. Anthocyanins are phenolic substances also present in red and purple fruits such as acerola and grape, which have antioxidant, antimicrobial, pigmentary and pH indicator functions, beneficial for bioactive packaging made of TPS with these residues [26–28].

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Starch biocomposites and other polymeric matrices from natural sources with fillers obtained from fruit were processed largely by casting or compression molding [19–21,23–26,29], processes used on a laboratory scale, but not suitable for development on a commercial scale. Processing of TPS in a twin screw extruder has shown potential for starch modification, owing to its high shear capabilities [30].The objective of this work was to develop biocomposites of cassava starch with grape skin (Gr) and acerola (Ac) residues obtained by extrusion and injection molding processes as well as to evaluate the influence of the addition and varying concentration of these residues on the antioxidant, physicochemical, mechanical, and morphological properties of polymer biocomposites.

2. Materials and methods

2.1. Materials

Cassava starch from Yoki (with 25.6% amylose) and Gr, both commercial products in powder form, were purchased from supermarkets in the city of Natal-RN in Brazil. Ac was purchased from Delícia da Fruta in the city of Macaíba-RN in Brazil. The glycerol p.a. from Dinâmica was used as a plasticizer. The Folin-Ciocalteu reagent, gallic acid standard, DPPH (2,2-diphenyl-1-picryl-hydrazyil), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich. All the plant-based materials used in this work are already registered in the Brazilian Government Genetic Patrimony System and Associated Traditional Knowledge (SisGEn), the Access Register No. A7EB1D1.

2.2. Obtaining acerola residue powder

The acerola residues consisted of mostly fruit peels, but also contained residual pulp and seeds, and were obtained after mechanical depulping of the acerola fruits. Acerola powder was obtained by freeze-drying for 24 h at 0.26 mBar and −55 °C in Terroni Enterprise I freeze dryer. Freeze-dried samples were milled in at Tecnal TE-621 mechanical agitator and sieved in a 24-mesh sieve.

2.3. Preliminary characterization of raw materials

2.3.1. Moisture content

The moisture content of starch, grape skin, and acerola powders was assessed by the gravimetric method using a BEL Engineering M5-Thermo 163L infrared moisture analyzer at 105 °C.

2.3.2. Characterization of the bioactive components

In order to extract the bioactive components, 0.3 g of sample was added to 50 mL of 1% acetic acid (v/v) and stirred for 2 h at 25 °C. The extracts were filtered using Whatman Grade 1 filter paper and centrifuged for 10 min at 4000 rpm and 4 °C.

The total phenolic content (TPC) was evaluated using a microplate-adapted Folin-Ciocalteu method, according to Singleton et al. [31]. A gallic acid standard was used to construct an external calibration curve between 10 and 500 mg/L. The absorbance of the samples was measured at 765 nm in a Biochrom UVM340 microplate reader, with the results expressed in milligrams of gallic acid equivalent per gram of sample on a dry basis (mg GAE/g DW).

Total monomeric anthocyanin (ANC) content was assessed by the pH differential method using two buffer solutions (0.025 M potassium chloride, with a pH of 1 and 0.4 M sodium acetate, with a pH of 4.5), according to Giusti and Wrolstad [32]. The absorbance of the samples was obtained at 510 and 700 nm for both pH solutions using a Thermo Fisher Scientific Genesys 10S UV–Visible spectrophotometer, with the results being expressed in milligrams of cyanidin 3-glycoside equivalent per 100 g dry weight of the sample (mg YA/100g DW).

2.3.3. Total reducing sugars

The reducing sugar content of the raw materials was assessed by the 3,5-dinitrosalicylic acid (DNS) method, based on Miller [33], with modifications. Briefly, 0.5 g of the powdered sample was added to 20 mL of distilled water with 1 mL of HCl. The mixture was heated to 70 °C for 10 min and then the content was neutralized with 4 M NaOH, and the final volume was adjusted to 100 mL with distilled water. Aliquots of 0.5 mL were mixed with 2.5 mL of DNS solution 1% (v/v) and heated in a hot water at 100 °C for 10 min. The samples were cooled to room temperature and 3 mL of distilled water was added. The absorbance was measured at 540 nm using a Thermo Fisher Scientific Genesys 10S UV–Visible spectrophotometer. The results were expressed in grams per 100 g of dry sample (g/100g DW) using an external calibration curve of glucose and fructose.

2.3.4. Thermal characterization

Thermogravimetric analysis (TGA) was performed with a PerkinElmer STA 6000 simultaneous thermal analyzer from ambient temperature of up to 500 °C at a rate of 10 °C $\cdot$min^{-1} under a nitrogen atmosphere with a flow rate of 50 mL min^{-1}.

2.3.5. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy was performed with a Shimadzu IRTracer-100 spectrophotometer using the attenuated total reflection (ATR) mode in the region from 4000 to 400 cm^{-1}.

2.3.6. Morphological characterization

The preliminary morphological analysis of the raw materials used in the development of polymer biocomposites was performed using a Hitachi TM 3000 scanning electron microscope (SEM). The samples were coated with a thin conductive layer of gold, operated at a voltage of 5–15 kV using a tungsten filament.

2.4. Polymer biocomposite processing

TPS formulations were developed with cassava starch, using 45 wt% glycerol and 15 wt% distilled water in relation to the starch wt.%, and will be expressed in parts per hundred of resin (phr). Grape skin and acerola residues were added to the starch formulations at concentrations of 0.1, 1.0, 5.0, and 10.0 wt% in relation to the starch wt.%, according to Table 1.

A co-rotational twin screw extruder from AX Plásticos Máquinas Técnicas, with a diameter of 16 mm and aspect ratio of 40, was used to prepare pure TPS and polymer biocomposites under the same conditions, that is, with a temperature profile of 50, 60, 110, 110, 120, 125, 130, and 135 °C from feed to die, a screw speed of 250 rpm, using a die for pellet production.

After the extrusion mixing of the polymer biocomposites, injection molding (test specimens, ASTM D638 type V) was performed using a Thermo Scientific HAAKE MiniJet II injection molding system with a gun temperature of 130 °C, pressure of 400 bar, and mold temperature

Table 1
Formulations of pure TPS and polymer biocomposites with grape skin or acerola residues.

Formulation Starch/Glycerol/Water/Filler	Composition (phr)
TPS	100/45/15
0.1%Gr	100/45/15/0.1
1.0%Gr	100/45/15/1.0
5.0%Gr	100/45/15/5.0
10.0%Gr	100/45/15/10.0
0.1%Ac	100/45/15/0.1
1.0%Ac	100/45/15/1.0
5.0%Ac	100/45/15/5.0
10.0%Ac	100/45/15/10.0

of 36 °C.

2.5. Characterizations of polymer biocomposites

2.5.1. Color parameters and visual appearance

The analysis of the L*, a*, and b* parameters of the injection-molded specimens were performed using a Konica Minolta CM-5 colorimeter. The color change with the introduction of fillers in the polymer biocomposites in comparison with TPS was measured according to Eq. (1):

$$\Delta E^{*} = \sqrt{(\Delta l^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2}} \tag{1}$$

where ΔE^{*} is the color difference, $\Delta l^{*} = L^{*}_{control} - L^{*}_{specimen}$, $\Delta a^{*} = a^{*}_{control} - a^{*}_{specimen}$; $\Delta b = b^{*}_{control} - b^{*}_{specimen}$. The control parameters are related to the pure TPS sample and the sample parameters are related to each formulation of polymer biocomposites with grape skin and acerola residues.

2.5.2. Quantification of bioactive compounds

The presence of bioactive compounds was evaluated in the samples after the mixing processes by extrusion and injection molding. For bioactive compound extraction, 2.5 g of sample was added to 50 mL of 1% acetic acid (v/v) and stirred for 2 h at 25 °C. The extracts were filtered using Whatman Grade 1 filter paper and centrifuged for 10 min at 4000 rpm at 4 °C. The quantification of TPC and ANC was performed as described in section 2.3.2.

2.5.3. Antioxidant activity

The antioxidant activity of the injection-molded samples was assessed using the DPPH method, described by Crouvisier-Urion et al. [34]. The radical scavenging activity (RSA) kinetics of the TPS formulations was performed at 25 °C for 150 min. The samples were added to 30 mL of a 50 mg/L DPPH methanolic solution and kept under constant magnetic stirring, and protected from light. The absorbance of the solutions was measured at 517 nm and the reduction in RSA was expressed as a percentage, according to Eq. (2):

$$RSA(\%) = 100 - [(\frac{A_{blank}(t) - A_{sample}(t)}{A_{blank}(t)})*100] \tag{2}$$

where A_{blank} (t) is the absorbance of the DPPH solution at time t, and A_{sample} (t) is the absorbance of the DPPH solution with each sample at the same time.

2.5.4. Migration test

The migration test evaluated the capacity of the antioxidant bioactive compounds to migrate from polymeric biocomposites to food simulators, as described by Sarantópoulos et al. [35]. In this analysis, three food simulators (distilled water, 3% aqueous acetic acid, and olive oil) were evaluated in duplicate for 10 days at 40 °C. After the incubation period, the test specimens were removed from the solutions prior to centrifugation (4000 rpm, 10 min, 4 °C). The supernatant was assessed for antioxidant capacity using the DPPH scavenging activity method. Briefly, aliquots of 200 μL of a methanolic DPPH solution (40 mg/mL, absorbance at 517 nm) were mixed with 40 μL of sample supernatants. After 25 min at 25 °C, the absorbance was measured using a Biochrom UVM340 microplate reader. A standard curve (10–200 μM) was constructed using a Trolox standard. The results were expressed as μmol Trolox equivalent per gram of sample (μmol TE/g).

2.5.5. Mechanical characterization

Tensile tests were performed using an EMIC DL-3000. The displacement rate was 1 mm min^{-1} and the load cell was 30 kN by according to ASTM D638 type V.

2.5.6. Morphological characterization

Pure TPS and polymer biocomposites with grape skin and acerola residues were cryo-fractured, and coated with a thin layer of gold. The morphological characterization was performed using a SEM with a field-emission gun in a Philips XL-30FEG with a tungsten filament.

3. Results and discussion

3.1. Preliminary characterization of raw materials

3.1.1. Characterization of bioactive components

The results of the characterization of bioactive compounds and the moisture content of cassava starch, grape skin, and acerola residues are shown in Table 2. Cassava starch did not contain bioactive compounds and had no sugars, since starch commercial products are purified and are sugar free, corroborating the results observed by Teixeira et al. [36].

The acerola residue had a higher amount of TPC (78.6 ± 7.8 mg GAE/g DW) than the grape residue (11.5 ± 0.8 mg GAE/g DW). Similar results for grape residue were observed by Carmona-Jiménez et al. [37]. The concentration of anthocyanins in the grape residue (429.5 ± 14.5 mg of CYA/100 g DW) was higher than that of acerola residue (227.3 ± 9.8 mg of CYA/100 g DW). These results were expected due to the intense purple coloration of the grape skin residue.

The difference in the amount of TPC and ANC of the grape skin residue, when compared to the acerola residue, may have been influenced by several factors, such as the type of fruit, extraction method, chemical composition, and origin of the residue, among others. As verified by da Cruz et al. [38] and Moraes et al. [39], acerola is a fruit rich in phenolic compounds, and the grape skin have significant amounts of anthocyanins, whose natural pigments are related to the red, blue, and purple colors present in some fruits and vegetables.

The amount of reducing sugars found in the acerola residue powder was higher than the values presented by the grape skin residue powder (Table 2), which may be related to the different composition of these residues.

The lyophilized acerola residue powder consisted of husks, seeds, and residual pulp. The presence of the residual pulp was responsible for the greater amount of reducing sugars present in the acerola residue powder because fruit pulp has a higher concentration of total reducing sugars.

Grape skin residue powder is a commercial product that contains grape skins and is a suitable replacement for flour in the diet. The content of reducing sugars in the grape skin powder was lower than that of the acerola residue powder because the amount of reducing sugars present in the fruit skin was lower than that found in the fruit pulp.

3.1.2. Thermal characterization

Fig. 1 shows the thermal stability of the raw materials used in the development of polymer biocomposites. Starch, grape skin, and acerola residues had a water loss of 12.3, 4.7, 6.0 wt%, respectively (Fig. 1a). These results, obtained from the TGA curves, are close to the results of the moisture content presented in Table 2.

Table 2
Physicochemical characterization of starch (St), grape (Gr), and acerola (Ac) powders.

	Cassava starch	Grape powder	Acerola powder
Moisture content (%)	12.8 ± 0.5	3.5 ± 0.1	6.7 ± 0.1
TPC (mg GAE/g DW)	–	11.5 ± 0.8	78.6 ± 7.8
ANC (mg CYA/100 g DW)	–	429.5 ± 14.5	227.3 ± 9.8
Reducing sugars (g/100 g DW)	–	26.7 ± 2.6	43.1 ± 1.4

Legend: TPC – Total phenolic compounds; ANC – Total monomeric anthocyanins; GAE – Gallic acid equivalent; CYA - cyanidin 3-glycoside equivalent; DW – dry weight.

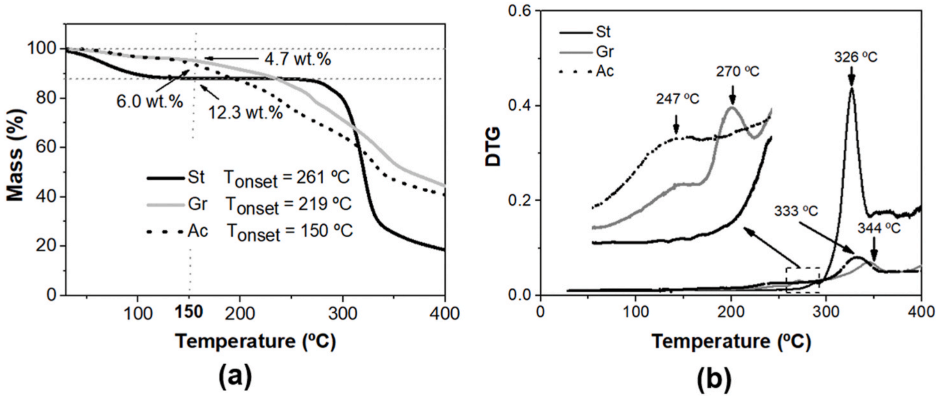


Fig. 1. Thermogravimetric curves of starch, grape skin, and acerola residues. a) TGA. b) DTG.

The thermal degradation onset temperature (T_{onset}), shown in Fig. 1a, for cassava starch, grape skin, and acerola residues are 261, 219, and 150 °C, respectively. Cassava starch was the most thermally stable material, followed by grape skin residue. The acerola residue was the material that had the lowest thermal stability among the materials. All thermal degradation onset temperatures were above the processing temperatures used in the mixture by extrusion and injection molding. The temperature profile minimized the thermal degradation of the materials, showing that conventional thermal processing already used for synthetic polymers is viable for obtaining TPS biocomposites with fruit residues.

Cassava starch showed a single peak of mass loss at 326 °C (Fig. 1b), associated with the decomposition temperature of its main components, that is, thermal degradation of amylose and amylopectin. These results corroborate the results observed by Gutiérrez et al. [62] for commercial starch because of its high purity. Grape skin residue showed two mass loss peaks, the first occurring at 270 °C and the second at 344 °C (Fig. 1b). These can be associated with the decomposition of hemicellulose and cellulose, respectively, as observed by Gutiérrez et al. [40], and Ferri et al. [41]. The acerola residue showed two mass losses at 247 and 333 °C, which are also related to the decomposition of hemicellulose and cellulose, whose temperatures were close to those found by Silva et al. [42].

3.1.3. FTIR spectroscopy

Fig. 2 shows the FTIR spectrum obtained from starch, grapes skin, and acerola residues, where the chemical composition of these raw materials can be observed. The broad peak observed around 3351 to 3376 cm^{-1} in the three spectra can be associated with the hydroxyl (OH) groups of alcohols, phenols, and carboxylic acids present in the grape skin and acerola residues, as well as the presence of acetal (COC) and hydroxyl groups of the constituent molecules of cassava starch [24,29,43,44]. The bands observed between 2914 and 2926 cm^{-1} can be attributed to C–H stretching [44] and were observed in the three spectra, with greater intensity for the grape skin residue.

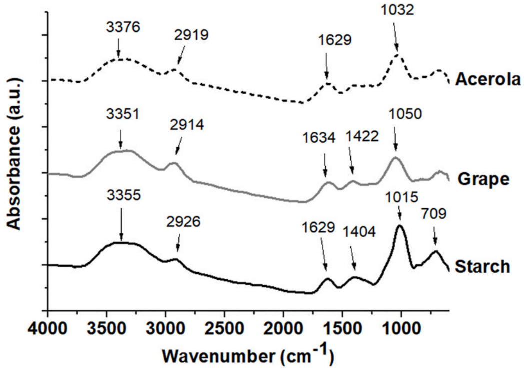


Fig. 2. FTIR spectrum of starch and grape skin and acerola residues.

The peaks observed between 1634 and 1415 cm^{-1} for the grape skin residue (related to the stretching of the aromatic ring, C=C, and –OH) the peak observed at 1404 cm^{-1} for the acerola residue (regarding to bending of the COH bond) are related to the functional groups present in the phenolic compounds of these residues [24,29,44].

Starch and acerola residue have a peak at 1629 cm^{-1} that can be attributed to the bending mode of the absorbed water; that is, they are associated with the angular curvature of the –OH bonds in water molecules [43]. The presence of this peak corroborates the results of moisture and water loss, where higher values were found for these properties for starch and acerola residue, showing a greater interaction of these materials with water. The peaks at 1050 and 1032 cm^{-1} observed in the grape skin and acerola residues, respectively, may be related to the COC stretching of the ester bonds and, the COH and COC bending which could from the esters present in carbohydrates and vitamin C [29].The peaks around 1386 and 1015 cm^{-1} can be attributed to the COH and OH bonds present in polysaccharides, such as starch [43].

3.1.4. Morphological characterization

The size distribution of cassava starch, and the grape skin, and acerola residues are shown in Fig. 3. The photomicrographs obtained by SEM show a wide variation in the size of the raw materials that were used in the development of polymer biocomposites. The large size distribution of cassava starch may favor the plastification stage to obtain TPS compared to the different types of starch obtained from other plant sources.

The grape skin and acerola residues have vastly different sizes and formats, as the production processes of these residues were not controlled. This research aimed to evaluate the use of these residues without adding additional processing costs and/or use materials that were produced for other applications.

3.2. Characterization of polymer biocomposites

3.2.1. Color parameters and visual appearance

The color parameters L*, a*, b*, the color variation (ΔE*), and the visual aspect are shown in Table 3. Pure TPS had a higher value in the luminance parameter (L*) of 32.02 and a value of 0.03 and 0.32 for the parameters a* and b*, respectively. The results showed that there was a change in the luminosity values and in the parameters a* and b* of the biocomposites due to the variation in the concentration of grape skin and acerola residues in the TPS matrix.

In the biocomposites with grape skin residue, it was observed that an increase in the amount of incorporation of grape skin residue caused a decrease in L* and an increase in the values of a*, b*, and ΔE*, with a

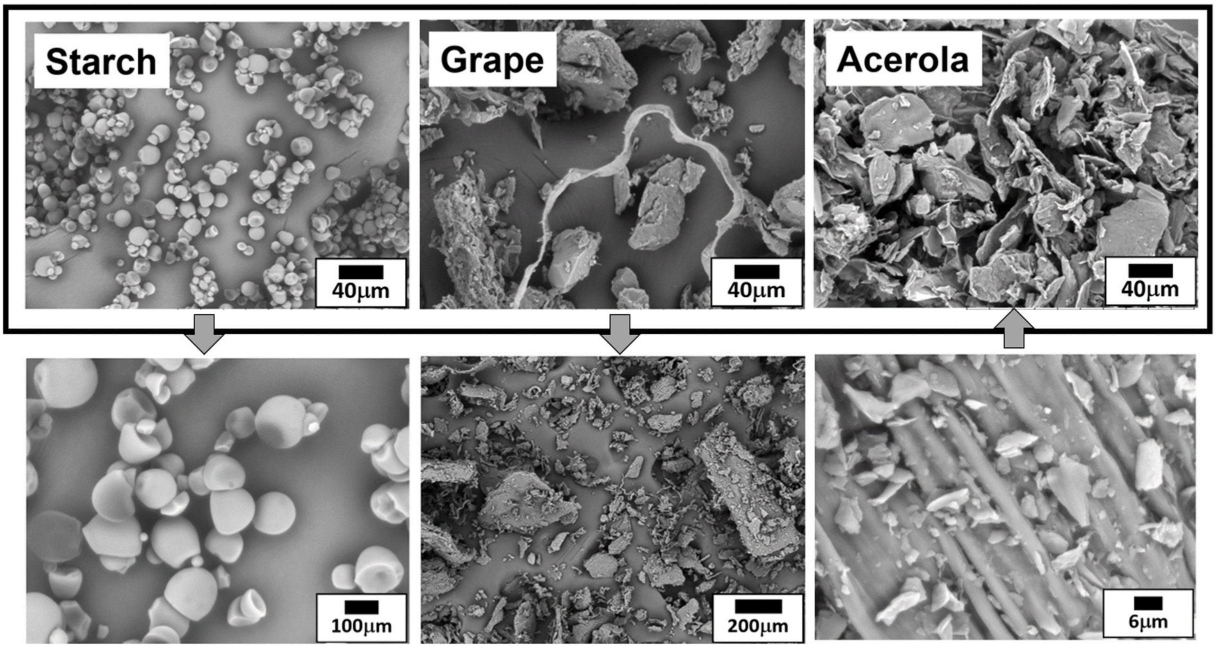


Fig. 3. Size distribution of cassava starch, grape skin, and acerola residues.

Table 3
Color parameters and visual appearance of pure TPS and biocomposites with grape skin and acerola residues.

Colour parameters and visual aspect											
Grape						Acerola					
	L*	a*	b*	ΔE*	Visual Aspect		L*	a*	b*	ΔE*	Visual Aspect
TPS	32.07	0.03	0.32	–		**TPS**	32.07	0.03	0.32	–	
0.1%Gr	30.86	0.55	0.44	1.32		**0.1%Ac**	31.12	0.24	0.70	1.04	
1.0%Gr	26.16	0.43	−0.65	6.00		**1.0%Ac**	25.47	0.66	1.19	6.68	
5.0%Gr	23.8	0.15	−0.26	8.3		**5.0%Ac**	26.40	0.99	2.86	6.28	
10.0%Gr	25.11	0.57	−0.03	7.00		**10.0%Ac**	26.24	1.21	1.3	6.03	

more pronounced visual effect and a dark reddish purple tint, like the color of pure grape skin.

The biocomposites with acerola residue had a decrease in luminosity; however, it had an increase in the values of a* and b*. A variation in color was visually observed, the biocomposites had a light or dark brown color, due to the increase in parameter b*. These characteristics and changes presented in the coloring of cassava starch biocomposites with grape skin and acerola residues can be attributed to the Maillard and caramelization browning reactions [45], which can occur due to the temperatures of the extrusion and injection molding processes, as well as the presence of sugars and other compounds present in each fruit residue.

3.2.2. *Bioactive characterizations*

Fig. 4a and b shows the content of bioactive compounds present in TPS biocomposites with grape skin and acerola residues after being subjected to mixing processes through extrusion and injection molding. The TPC content in the granules obtained after extrusion processing (Fig. 4a) was observed for all compositions with Gr and Ac, mainly for formulations with a higher fillers content (5.0 and 10.0 wt%), with a greater retention of TPC for compositions with Ac, due to the higher TPC content in the acerola residue (Table 2). In the injection-molded specimens (Fig. 4b) that were subjected to two thermal processes, that is, extrusion and injection molding, no TPC content was found for the compositions with 0.1 and 1.0 wt%, with less TPC retention for the injection-molded biocomposites with grape residue. However, injection-molded specimens with compositions of 5.0 and 10.0 wt% retained the TPC content equal to the extruded materials of the same composition.

In the results of the total anthocyanin concentrations shown in Fig. 4c and d, it can be seen that the biocomposite with grape skin residue has higher ANC values in the granules (Fig. 4c) and in the specimens (Fig. 4d) than the biocomposite with acerola residue, due to the grape skin residue having a higher concentration of this bioactive compound than the acerola residue. The biocomposites with the two fillers had a greater amount of ANC for the extruded materials, but there was a reduction in the presence of this bioactive compound for the injection-molded specimens. However, biocomposites with acerola residues showed greater stability in the ANC retention in the specimens

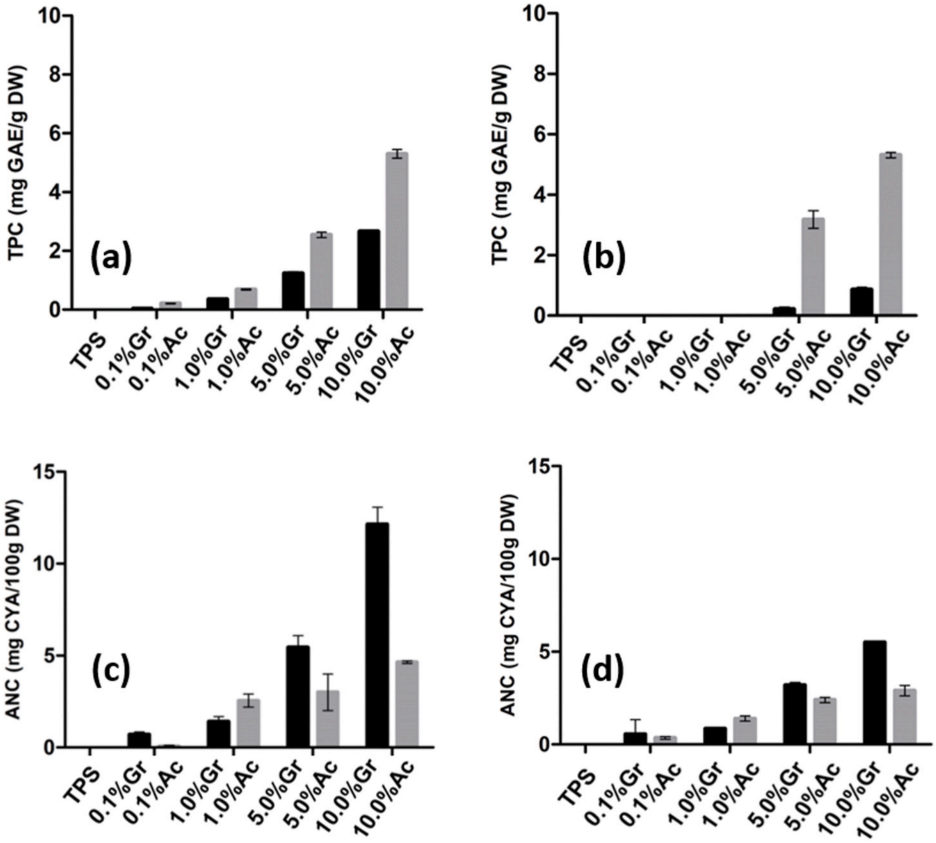


Fig. 4. Total phenolic compounds (TPC), and Total monomeric anthocyanins (ANC) content, respectively. a) and c) Granules obtained after mixing by extrusion. b) and d) Injection molding specimens.

prepared by injection molding, as a smaller loss in the amount of anthocyanin was observed in the biocomposite with acerola residue than in the biocomposite with grape skin residue.

Anthocyanins are known to have a strong chemical affinity for sugars, as verified by Frank et al. [46], so the higher content of reducing sugars in the acerola residue (Table 2) may have helped to retain a greater amount of ANC in the cassava starch biocomposites with acerola residue.

The bioactive properties of biocomposites with grape skin and acerola residues show different behaviors in the retention of bioactive compounds after the processing used to obtain the biocomposites. The retention of TPC and ANC was better for biocomposites containing acerola residue. This can be attributed to the content of reducing sugars present in this residue, and the caramelization effect observed in the visual aspect, and the color change of the specimens presented in Table 3, which may have caused a protective effect that helped to retain

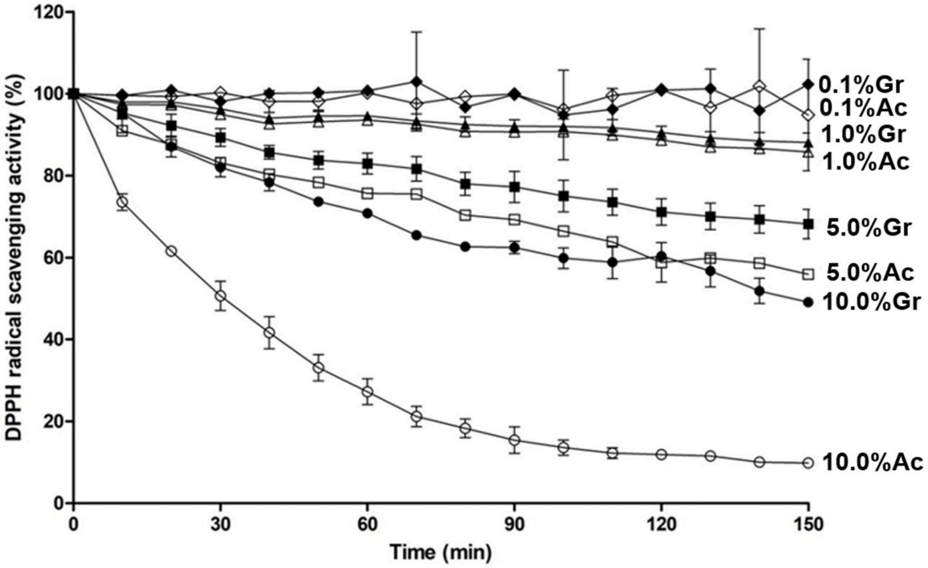


Fig. 5. Kinetic profile of the ability to scavenge DPPH free radicals in biocomposites with grape skin and acerola residues.

the bioactive compounds after the biocomposites were subjected to two types of thermal processing.

Some authors [47–49] have also observed the presence of bioactive compounds in functional foods obtained by extrusion. The retention of bioactive compounds, such as anthocyanins, in extruded foods was possible with an adequate adjustment in the processing conditions (screw temperature profile, shear rate and residence time). These results demonstrated that the composition of the residues and the adjustment in the processing conditions used may have contributed to obtaining cassava starch biocomposites with bioactive functional properties.

3.2.3. Antioxidant activity

The efficiency of migration of the antioxidant compounds is shown in Fig. 5, displaying a kinetic profile for the reduction of DPPH elimination activity in the polymer biocomposites. The biocomposite with 10.0 wt% of acerola residue was the most efficient formulation to eliminate DPPH free radicals, achieving a balance of approximately 10% of residual RSA after a reaction time of 150 min, which may be associated with a higher concentration of phenolic compounds (Fig. 4a and b). The biocomposite with 10.0 wt% of grape skin reached about 50% of residual RSA in the same period. The release of antioxidant components in the biocomposites with 0.1 wt% grape skin and 0.1 wt% acerola was low, which corroborates with the migration tests (Fig. 6). The biocomposites with 1.0 and 5.0 wt% of fruit residues presented a continuous reduction in RSA, as can be observed in Fig. 5.

These release profiles can be used to adjust for future applications in packaging using cassava starch biocomposites with fruit residues. For example, formulations with a faster release profile, such as 10.0 wt% of Ac, can be used to contain products ready for consumption, that is, food with a shorter shelf life that will be in contact with the packaging for less time. Formulations with lower release profiles, such as 1.0 wt% of Ac or 5.0 wt% of Gr, can be used for products that will be stored for longer periods.

3.2.4. Migration test

The final antioxidant activity (expressed as DPPH elimination activity) was significantly higher in food simulators A (water) and B (3% acetic acid solution) for biocomposites with grape skin residue (Fig. 6a) and with acerola residue (Fig. 6b), with a higher final antioxidant activity of acerola in simulator B, as phenolics are released more quickly in acetic acid than water. Acidic solvents can cause the hydrolysis of glycoside groups linked to flavonoids and phenolic acids, thus increasing their migration to acidic solvents.

Both groups related to the antioxidant activities present in fruit residues (phenolic compounds and anthocyanins) are mainly hydrophilic [50], which explains the greater transfer of components to the water-based simulators. In addition, starch is also soluble in water [51], which can facilitate the migration of antioxidant-promoting components through a solubilizing TPS matrix. Carotenoids are a class of hydrophobic components that are associated with antioxidant properties [52]. It has been reported that both acerola [53] and grapes [54] have carotenoids, which may also be associated with the antioxidant capacity quantified in the environment of a fatty food simulator.

The results of the migration test of biocomposites with higher concentrations (5.0 and 10.0 wt%) of the Gr and Ac residues show higher values of antioxidant activity in vitro have the potential to protect and increase the useful life of food. They may also be suitable for applications in packaging for fatty food products, such as mayonnaise, sauces that contain oils, butters, margarines, peanut butter, and pates.

3.2.5. Mechanical characterization

Fig. 7a shows the stress *versus* strain curves obtained in the tensile test and the fracture surface morphologies obtained by SEM of pure TPS and biocomposites with grape and acerola residues. The pure TPS had a deformation of 201%; this value can be attributed to the amount of glycerol used to plasticize cassava starch, which may have led to a decrease in the interaction forces between the starch chains and, consequently, greater mobility, resulting in a decrease in the values of maximum strength, modulus of elasticity, and a significant increase in elongation at break (Fig. 7b). The homogeneous smooth surface visualized in the photomicrograph indicates a high degree of plasticization of cassava starch for the extrusion processing conditions. A similar result [55] was previously obtained with other types of TPS.

Biocomposites with grape skin and acerola residues had different tensile mechanical behaviors than TPS, since the mechanical properties depend on the physicochemical properties and morphology (Fig. 3) presented by each fruit residue used as fillers in the starch matrix.

The addition of grape residue in TPS resulted in a decrease in elongation at break when compared with pure TPS, which was more significant for compositions with higher concentrations of grape residue (5.0 and 10.0 wt% of Gr), these results may indicate that the grape skin residue acted as a crosslinking agent (Fig. 7a).

Gutierez et al. [40] and Deng and Zhao [56], and also reported similar mechanical behavior in biocomposite with grape skin residue. In these studies, the authors observed that the grape skin residue contained naturally occurring crosslinkers, including minerals (such as Ca^{2+}, Mg^{2+}, and Fe^{3+}); proteins; and organic, amino, and phenolic acids that act as crosslinking agents and are responsible for increasing the mechanical properties of maximum strength, modulus of elasticity and reduction in elongation at break.

The increase in maximum strength and elasticity modulus of biocomposites with grape skin residue may be related to the good interfacial adhesion between the starch matrix and the grape skin residue as seen in the SEM photomicrograph (Fig. 7a). This may have been caused by crosslinking and it may also be responsible for the fragile fracture present in starch biocomposites with Gr.

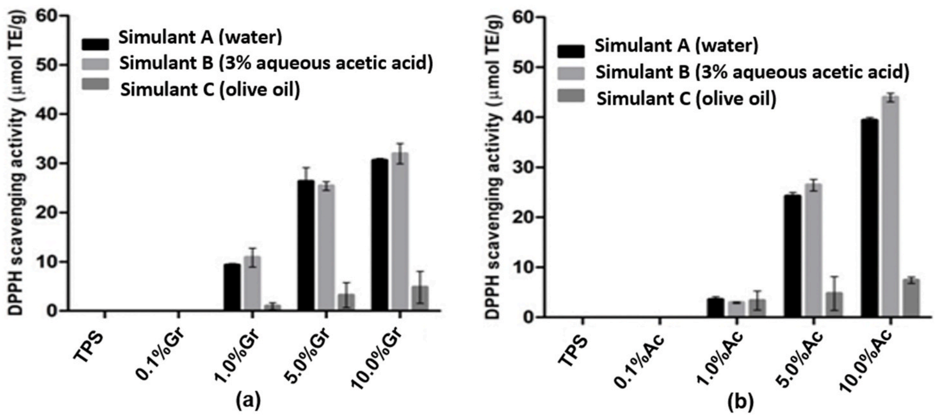
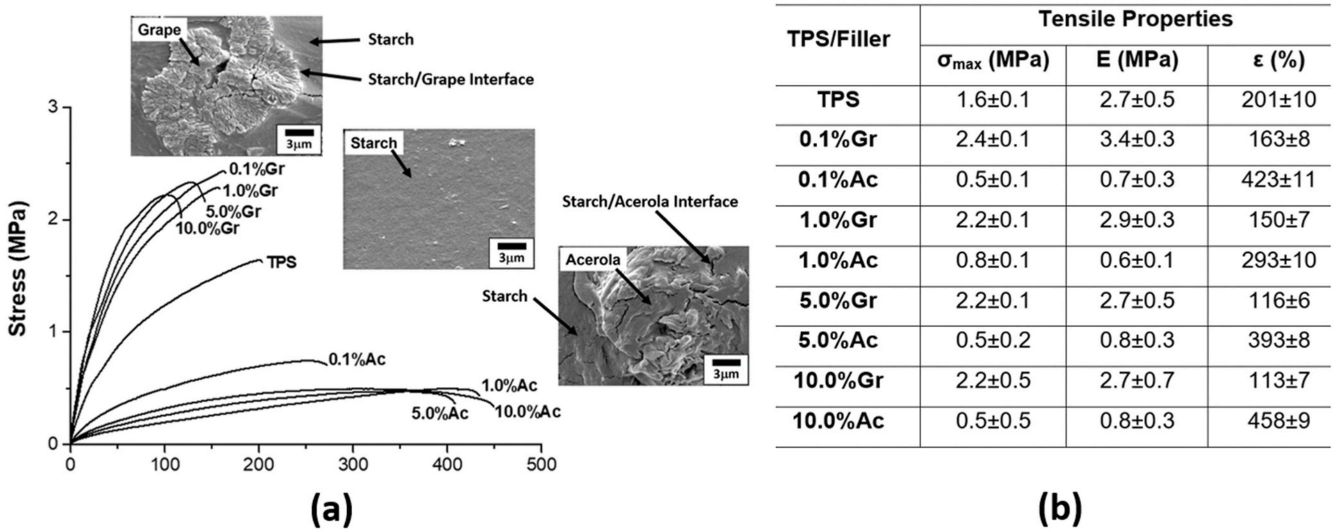


Fig. 6. Migration test on polymer biocomposites in contact with three different food simulators (water, 3% acetic acid solution and olive oil). a) Gr. b) Ac.



TPS/Filler	Tensile Properties		
	σ_{max} (MPa)	E (MPa)	ε (%)
TPS	1.6±0.1	2.7±0.5	201±10
0.1%Gr	2.4±0.1	3.4±0.3	163±8
0.1%Ac	0.5±0.1	0.7±0.3	423±11
1.0%Gr	2.2±0.1	2.9±0.3	150±7
1.0%Ac	0.8±0.1	0.6±0.1	293±10
5.0%Gr	2.2±0.1	2.7±0.5	116±6
5.0%Ac	0.5±0.2	0.8±0.3	393±8
10.0%Gr	2.2±0.5	2.7±0.7	113±7
10.0%Ac	0.5±0.5	0.8±0.3	458±9

Fig. 7. Mechanical and fracture behavior. a) Stress versus strain curve and morphology for pure TPS and for biocomposites with grape skin and acerola residues. b) Tensile properties.

The mechanical properties from the tensile tests and the fracture surface morphology of biocomposites with acerola residue showed different mechanical behavior and fracture mechanisms than biocomposites with grape skin residue. The stress *versus* strain curves (Fig. 7a) of the biocomposite with acerola residue showed a significant deformation and a reduction in the properties of maximum strength and elasticity modulus. As seen in Table 2, the acerola residue had a higher total sugar content, which may have influenced the mechanical properties and fracture mechanisms of these biocomposites. The presence of sugars in fruit residues acts as a natural plasticizer in TPS matrices [20], reducing their glass transition temperature to values below room temperature, which may promote an increase in the elongation at break and a decrease in the modulus of elasticity and maximum resistance due to the plasticization effect.

In the photomicrograph of biocomposites containing with acerola residue (Fig. 7a), it was verified that the plasticization effect due to the presence of sugars in these biocomposites promoted a ductile fracture and provided significant elongation at break values in these biocomposites, even with a smaller interfacial adhesion between the starch matrix and the acerola residue.

4. Conclusions

Biocomposites with grape skin and acerola residues were obtained by conventional extrusion mixing and injection molding processes. The antioxidant, physicochemical, mechanical, and morphological properties of the biocomposites with grape skin and acerola residues strongly depend on the composition and origin of these residues, leading to significant changes in the properties of the polymer biocomposites. The biocomposite with acerola residue had more desirable final properties with a greater application potential for flexible bioactive packaging, whereas the biocomposite with commercial grape skin residue was suited for applications in rigid bioactive packaging. This study provided useful information for the technological development of cassava starch biocomposites containing residues with antioxidant properties for applications in biodegradable packaging or disposable products obtained by large-scale processes.

CRediT authorship contribution statement

Juciklécia S. Reinaldo: Investigation. **Carlos H.R. Milfont:** Investigation. **Felipe P.C. Gomes:** Investigation. **Adriano L.A. Mattos:** Investigation. **Fábio G.M. Medeiros:** Investigation, Formal analysis. **Paula F.N. Lopes:** Investigation. **Men de sá M. Souza Filho:** Conceptualization. **Kátia N. Matsui:** Methodology, Conceptualization. **Edson N. Ito:** Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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