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Additional Information

1 **Bio-based plastic based on ozonated cassava starch produced by extrusion**[★]

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Abstract

Green methods of modification, such as ozone, can bring new functionalities to starch. In this study, starch-based plastics were produced by extrusion, using cassava starch ozonated with a gas flow of 1 L·min^{-1} at a concentration of 41 mg O$_3$·L^{-1} resulting in doses of 78.9 and 145.3 mg O$_3$/g starch. Pre-mixes (starch, glycerol, and water) were processed in a co-rotating twin-screw extruder. The main results showed that ozonated sheet produced with the treatment of 78.9 mg O$_3$/g starch resulted in an increase in Young Modulus by 43 % and a decrease in hydrophilicity by 37 % in comparison to the native one. Conversely, treatment with 145.3 mg O$_3$/g starch did not increase the bio-based plastic mechanical properties or surface characteristics, showing a specific behavior tendency between ozonation and extrusion processes. The ozonation of starch showed to be an alternative for producing by extrusion bio-based plastics with enhanced properties, by selecting adequate processing conditions.

Keywords: biodegradable plastics; ozonation, green technologies, starch modification

42   **Nomenclature**

  A   area (m^2)

  E   Young's Modulus (MPa)

  p   pressure (kPa)

  RC   Relative Crystallinity

  t   time (h)

  TS   Tensile Strength (MPa)

  WVP   Water Vapor Permeability (g·mm/m^2·day·kPa)

  x   thickness (mm)

  Y   opacity (%)

43

44 **Subscripts**

  b   black standard

  w   white standard

45

46 **Greek**

  ε   elongation at break (%)

47

1. Introduction

Starch-based plastics have been developed as an alternative to petroleum-based ones, being cassava starch an interesting raw material for this application [1]. However, starch-based plastics can show lower performance when compared with traditional plastic, such as worse mechanical properties, low transparency, and high-water absorption [2,3] mainly due to the hydrophilic character of this polymer[4].

Different modification techniques can be used to overcome those issues, being the so-called "green technologies" interesting approaches to enhance performance for attaining sustainability. The ozonation process (i.e., processing using the ozone gas) is considered an environmentally friendly technology that promotes three main modifications in starch molecules, achieving different properties: partial depolymerization, oxidation of hydroxyl groups into carbonyl and carboxyl groups [5]. In fact, ozonation was able to enhance the performance of the bio-based plastics of cassava [6,7] and potato[8] bio-based plastics produced by the casting technique.

However, although casting is an interesting technology to understand the polymeric interactions with some other compounds or process conditions, as a first approach, it is a laboratory-scale technique, difficult to apply on an industrial scale. Extrusion technology is widely used in the polymer industry [2], but studies on starch-based plastics are still scarce in the literature. The extrusion technique has many advantages, being a continuous and low time-consuming process, and efficient to produce viscous materials [9,10].

When both processes are compared (casting *vs*. extrusion), drying conditions are quite different. Casting plastics are slowly dried (~12 h) at low temperatures and rest (i.e., at negligible stress), while extruded plastics are quickly dried at high temperatures and high shear stress, resulting in a different matrix conformation and recrystallization ability

[11,12]. These differences affect the mechanical, barrier, and physical properties of the material, highlighting the importance of this research.

Therefore, in this study, ozonation, a "green emerging technology", was used to modify cassava starch before bio-plastic production. Ozonated starch-based plastics were produced by extrusion, reported for the first time in the literature. Those new plastics were characterized by correlating their mechanical, barrier, physical and thermal characteristics with the starch modification process.

2. Material and Methods

2.1.Materials and ozonation process

Native cassava starch (Amilogill 1500; 28.94 g/100 g of amylose) provided by Cargill Agrícola – Brazil, glycerol P.A. grade (Sigma-Aldrich, Brazil), and Milli-Q water were used to produce the ozonated starch-based plastics.

The ozonation process and the full characterization of cassava starch were described in a previous study [13]. Briefly, a rich stream was produced with a gas flow of 1 L·min^{-1} with an ozone concentration of 41 mg O_3·L^{-1} going through the samples for 15 min or 30 min at 25 ± 1 °C, which resulted in the ozone doses (consumption during the reaction) of 78.9 and 145.3 mg O_3/g starch, respectively (and hereafter called O78.9 and O145.3, respectively). This was followed by decantation, centrifugation, and air-drying in a circulation oven (Marconi, MA 035, Brazil) for ~ 12 h at 35 °C, until reaching a moisture content of ~12 g/100 g w.b. The O78.9 and O 145.3 samples resulted in carbonyl, carboxyl, and amylose content of (0.080; 0.020) groups/100 glucose units, 25.85 g/100 g and (0.12; 0.040) groups/100 glucose units, 23.79 g/100 g, respectively [13].

2.2. Bio-based *plastic production*

Premixes of starch, glycerol (43.1 g/100 g of starch), and water (25.9 g/100 g of starch)[14] were homogenized in a mixer (Kitchen Aid, 525, Brazil) and then extruded in

98   a corotating twin-screw extruder (Thermo Fisher Scientific, Process 11, Germany) L/D

99   40, and 11-mm screw diameter to produce pellets. The premixes were fed through a

100   double screw feeder (Brabender, Volumetric Minitwin Process 11, Germany) at 0.05 kg/h

101   and a thread speed of 80 rpm. The temperature profile to obtain pellets from the feed zone

102   (1) to the extrudate outlet (zone 8) was based on Vedove et al. [14], being: 60, 75, 90,

103   100, 110, 110, 105, and 100 °C. The cylindrical material obtained was then cut to 2 mm

104   to obtain the pellets.

105   Those pellets were fed from a conical type feeder (Brabender, Kulturstrasse 55-73,

106   Germany), with a feed flow of 0.2 kg/h to the same co-rotating twin-screw extruder. The

107   sheets were obtained by the extrusion process using the same temperature profile, the die

108   (3 cm x 1 mm) with the mold to form the sheets at 105 °C and the thread speed at 80 rpm.

109   For each level of ozone applied to cassava starch (78.9 and 145.3 mg O_3/g starch),

110   the pellets and their respective sheets were produced by extrusion in duplicate. The pellets

111   and sheets were also produced from native cassava starch (no ozonated) for comparison.

112   *2.3. Bio-based plastic characterization*

113   The materials were conditioned for 7 days in desiccators (75 % RH) at room

114   temperature (~ 25 °C) and then evaluated according to their morphological, structural,

115   mechanical, barrier, physical, and thermal characteristics.

116   *2.3.1. Morphology*

117   The morphology of the bio-based plastics was visualized by a scanning electron

118   microscope (SEM). The specimens were coated with 10-nm platinum and observed using

119   the microscope (FEI, model Quanta 600FEG, Netherlands) at 15 kV and the

120   magnification ranged from 1500× (surface) to 150× (cross-section) images.

121

122   *2.3.2. Relative Crystallinity*

6

The crystallinity of the materials was analyzed by an X-ray diffractometer (PANalytical, model X'Pert PRO, Netherlands) at 45 mA and, 40 kV. The scanning rate was 0.02°/s with 2θ angles from 2° to 40°. The Origin 2020 software was used for data analysis (OriginLab Corporation, Massachusetts, USA).

2.3.3. Fourier-transform infrared (FTIR) analysis

Sheet spectra were investigated by Fourier transform infrared spectroscopy (Pekin Elmer Frontier IR-4700, USA) in attenuated total reflectance (ATR) mode using a diamond ATR crystal in a wavenumber range from 540 to 4000 cm^{-1}; 32 scans were recorded per sample. The software Origin 2020 was employed for data obtained from the spectra (OriginLab Corporation, Massachusetts, USA).

2.3.4. Mechanical Properties

Plastic thickness was determined at five random positions using a digital micrometer (precision 0.001 mm) (MITUTOYO, Japan). Their mechanical properties were evaluated using a uniaxial tensile assay according to the ASTM D882-12 standard method [15] on a texture analyzer (Stable Micro Systems, TAXT2i, UK). Tensile strength (*TS*) and elongation at break (ε) were evaluated for ten strips (100 mm x 25 mm) using the A/TGT self-tightening roller grips at a speed of 1 $mm \cdot s^{-1}$ and grip separation of 50 mm. Young's modulus (*E*) was calculated as the slope of the initial linear portion of the stress *versus* strain curve.

2.3.5. Barrier Properties

Water vapor permeability (*WVP*) was determined through the gravimetrical method according to the ASTM E96/E96M-16 Standard Method [16], in triplicate. The specimens were placed in circular permeation cells containing silica gel and then in a desiccator (75 % RH) in a climate chamber (TIRACLIMA, model TCC 7034, Germany) at a temperature

147   of 25 °C. The weight gain was recorded at intervals of 1 h. The *WVP* was calculated

148   according to Eq. (1).

149    $$WVP = \frac{\Delta m \cdot x \cdot 24}{\Delta t \cdot A \cdot \Delta p}$$ (1)

150   wherein: $\Delta m / \Delta t$ is the moisture gain per unit of time (g·h^{-1}); A is the film area exposed to

151   permeation ($4.9 \cdot 10^{-4}$ m^2); x is the film thickness (mm); Δp is the difference in vapor

152   pressure (kPa) of the atmosphere over silica gel and pure water.

153   *2.3.6.   Wettability*

154   The wettability of the bio-based plastic surface was determined by the contact angle

155   with Milli -Q water, according to ASTM D7334 08 standard methods [17] through an

156   optical system (Dataphysiscs, OCA 15, Germany). The water contact angle was

157   determined in six small specimens per sample. Angle measurements were quickly carried

158   out to avoid water vaporization.

159   *2.3.7.   Solubility and moisture content*

160   The bio-based plastic solubility in water was evaluated in triplicate with discs

161   (diameter of 20 mm) immersed in water at 25 °C, being agitated at 120 rpm for 24 h,

162   according to Gontard et al. [18]. The solubility was calculated according to Eq. (2).

163    $$S = \frac{m_f - m_o}{m_o} \cdot 100$$ (2)

164   wherein: m_o and m_f are the initial and the final mass of the discs (g), respectively.

165   Moisture of bio-based plastics was determined in triplicate, according to Gontard

166   et al. [18] in an oven (Marconi, M030/2057, Brazil) at 105 °C, until constant mass.

167   *2.3.8.   Opacity and Light Transmittance*

168   The transmission of UV to visible light of the materials was measured with a

169   spectrophotometer from 200 nm to 800 nm (FEMTO, model 700.Plus, Brazil). The bio-

170 based plastics opacity was determined, in triplicate, using a colorimeter (HUNTERLAB,

171 model ColorQuest XE, USA) using Eq. (3).

172 $$Y = \frac{Y_b}{Y_w}$$ (3)

173 wherein: Y_b is the opacity on the black standard and, Y_w is the opacity on the white

174 standard.

175 *2.3.9. Thermogravimetric analysis (TGA)*

176 The thermogravimetric analysis was performed in duplicate using a thermal

177 analyzer (TA Instrument, model TGA-Q500 V20.13, EUA), using ~ 10 mg of samples,

178 which were heated from 25 °C to 700 °C, at a rate of 10 °C/min under nitrogen flow. The

179 sample mass was continuously measured to describe its reduction over temperature.

180 *2.4. Statistical analyses*

181 All data were evaluated by the Analysis of Variance (ANOVA), applying Tukey`s

182 test ($p<0.05$) using the Statgraphics Centurion XV software (StatPoint, Inc., USA).

183 **3. Results and Discussion**

184 Figure 1 presents the visual aspect of the bio-based plastics produced by extrusion,

185 which were classified as "sheets" due to their thickness (> 0.250 mm) according to the

186 standard terminology of ASTM D882-18 [15].

187 Different performances were achieved in each treatment, while the ozonation

188 conditions resulted in a hormetic pattern (increasing performance at low doses and

189 decreasing it at high doses) for some properties. This indicates there is an interesting range

190 of starch molecular modification levels from which the ozonation process becomes

191 deleterious to the bio-based plastic – therefore highlighting the importance of the present

192 evaluation.

193   For instance, while the sheet produced with cassava starch ozonated at 78.9 mg

194   O₃/ g starch seems more homogenous and thinner, with adequate transparency to be used

195   as packaging material, the plastics produced with the native and 145.3 mg O_3/g ozonated

196   starches were rougher and heterogeneous - the latter (145.3 mg O_3/g starch) presented

197   higher opacity. More details are discussed in the following.

198   *3.1.Morphology*

199   Figure 2 presents the morphology of the sheets obtained. In general, the 15-min

200   ozonated sheets present a compact and smooth structure on the surfaces and cross section,

201   while for the O145.3, some irregularities are visualized. Higher heterogeneity and

202   roughness are observed in the sheet produced from native cassava starch, which could be

203   attributed to the formation of bubbles/drops in the extruder exit section [19]. A smoother

204   surface for ozonated cassava films in comparison to native films produced by casting was

205   reported by La Fuente et al. [6]. According to Biduski et al.[20], the oxidation of hydroxyl

206   groups allows a higher penetration of the plasticizer in the starch chains, improving the

207   interactions between molecules. However, in this study, it is important to highlight the

208   difference in the drying process, which can affect the final conformation of the matrix.

209   Actually, extrusion drying favors the organization of the molecules in the flow direction

210   [21], while in casting, molecules can be entangled due to slow drying under steady

211   conditions, resulting in morphology with different characteristics.

212   The presence of micro-voids and cracks in extruded bio-based plastics is

213   associated with the accelerated drying process [22,23], as well as the formation of

214   bubbles/droplets at the exit of the die, due to the pressure and temperature drop [19].

215   *3.2.Crystallinity and Fourier-Transform Infrared (FTIR) spectra*

10

Figure 3A shows the X-ray diffractograms for the native and ozonated cassava sheets, in which peaks were observed at 5.1 °, 17.0 °, 20.0 °, 22.0 °, 31.0 °, and 33.9 ° (2θ), indicating a type-B crystalline structure. The peaks at 5.1 °, 17.0 °, 20.0 °, and 22.0 ° were also observed by La Fuente [6] in ozonated cassava starch films produced by casting, while Valencia [24] also observed peaks near 31.0 ° in native cassava films produced by casting. Vedove [14] observed peaks near 20.0 ° and 22.3 ° in native cassava starch sheets produced by extrusion, under conditions similar to those applied in this study.

Ozonated sheets presented higher values of relative crystallinity (Table 1) in comparison with the native starch, the same behavior was observed in ozonated potato [8] and cassava [6] starch films produced by casting. This suggests that depolymerization occurred during ozonation and the partial replacement of the hydroxyl groups by carbonyl and carboxyl groups [5,13], resulting in molecules with better organization, increasing the crystalline regions.

Figure 3B presents the FTIR spectra of the sheets obtained. In general, the spectra showed a typical behavior for cassava starch films plasticized with glycerol [25–27]. The absorbance at 3300 cm^{-1} is attributed to O–H stretching, while C–H stretching appeared at 2922 cm^{-1}. The band near 1650 cm^{-1} is assigned to the water adsorbed by starch molecules [5,26], near 1455 cm^{-1} to C–H bending, near 1242 cm^{-1} to C–O stretching, or the C–OH stretching [25]. Then, at 1000 cm^{-1} to the C–O stretching, while near 925 cm^{-1} designated to the C–O and the C–H stretching [25,26].

Satmalawati et al. [28] observed a difference in the peak sharpness at 1780–1680 cm^{-1} between native and ozonated cassava starch, which they attributed to the carbonyl groups due to the ozone oxidation process. However, no difference in this region among samples was visualized in Figure 3. Vicentini et al. [27] discussed that different molecular

241 changes could occur in other regions, which probably explains why carboxyl and
242 carbonyl groups were not visualized.

243   According to the literature [5,26,27], starch shows a fingerprint region within
244 1200–900 cm^{-1} bands, which is attributed to changes in starch structure. However, once
245 again, no differences were visualized in this region among samples. At this point, it is
246 important to note that, in ATR spectra (mode used in this study), the penetration depth of
247 infrared light in the sample is about 15 µm and, probably, the higher thickness of the
248 sheets (Table 1) could interfere with visualization of this functional groups in all ranges
249 studied herein.

250 *3.3.Mechanical properties*

251   Table 1 presents the mechanical properties of the materials. Similar values have
252 been reported for native cassava sheets [14]. As shown, there was no significant variation
253 ($p>0.05$) in tensile strength between the native and the O78.9 cassava sheet, while for the
254 O145.3 sheet, this property was ~ 20 % lower. Similarly, the elongation at break was ~25
255 % lower for ozonated starch sheets compared to the plastic produced with the native
256 starch. Table 1 allows verifying that the Young Modulus follows the same behavior as
257 the tensile strength, as expected. It increased by 43 % for O78.9 in comparison to the
258 O145.3 and native cassava sheets, indicating that O78.9 increased the mechanical
259 properties, while for O145.3 this property is similar to that of native starch.

Table 1.

Relative crystallinity, thickness, tensile strength, elongation at break, Young modulus's, moisture content, and opacity of native and ozonated cassava starch sheets.

Ozone dose (mg O_3/g starch)	RC (%)	Thickness (mm)	Tensile Strength (MPa)	Elongation at break (%)	Young Modulus (MPa)	Moisture (g/100 g)	Opacity (-)
0	3.81 ± 0.59 [a]	1.19 ± 0.00[b]	1.13 ± 0.11[b]	38.82 ± 1.32[b]	5.23 ± 0.70[a]	20.47 ± 0.35[a]	49.33 ± 1.15[a]
78.9	5.68 ± 0.17 [a]	0.96 ± 0.10[a]	1.29 ± 0.09[b]	29.84 ± 4.39[a]	7.50 ± 0.46[b]	19.65 ± 0.29[a]	79.33 ± 1.53[b]
145.3	5.69 ± 0.81 [a]	1.17 ± 0.01[b]	0.88 ± 0.08[a]	29.41 ± 2.36[a]	4.90 ± 0.61[a]	19.23 ± 1.22[a]	93.33 ± 0.58[c]
Tukey HSD*	2.46	0.15	0.26	7.46	1.50	0.02	2.90

The means with different letters in the column are significantly different ($p<0.05$).

* Tukey's honestly significant difference (HSD).

266   The increase in stiffness and the decrease in the elongation of cassava sheets,

267 depending on the process conditions, can be interesting since we could produce materials

268 with unique characteristics according to the market demand. This behavior can be

269 attributed both to the new matrix conformation obtained after determining the ozonation

270 process and to the drying conditions in the extruder. Probably, the higher degree of

271 depolymerization of this material (O145.3 cassava starch), in combination with higher

272 temperatures and shear stress during extrusion, was drastic enough to weaken the

273 polymeric matrix, thus resulting in the lower values of Young Modulus, while for the

274 O78.9 material, the degree of depolymerization reached, in combination with the

275 extrusion process (temperature and shear stress) was good enough to increase the

276 toughness of the polymeric matrix.

277   *3.4.Barrier and physical properties*

278   As shown in Figure 4, there is no statistical difference in water vapor permeability

279 between native and the ozonated cassava sheets. In addition, an increase of 13 % in the

280 solubility of the O78.9 sheet can be observed compared to the other materials. The values

281 are similar to those reported by Pérez-Vergara et al. [29] for native cassava starch films.

282   Although a decrease in water vapor permeability was expected due to the decrease

283 in hydroxyl groups, leading to a decrease in hydrophilic properties [30], this did not occur.

284 A possible explanation may be the carboxyl groups formed as a result of ozonation, which

285 are electronegatively charged, change the polarity of the polymeric matrix, increasing the

286 repulsive forces, favoring the mobility of water, as Biduski et al. [20] exposed. In fact, an

287 increase in the water vapor permeability was observed in cassava [6] and potato [8]

288 ozonated starch films produced by casting. These electrostatic repulsion forces could also

289 be responsible for the increase in the solubility of the O78.9 sheets. However, for O145.3

290 sheets, the greater ozonation might cause the opposite behavior. Additionally, it is also

291 possible that the intense shear stress and high temperature during the extrusion process

292 resulted in a molecular conformation that reduces the effect of these repulsive forces,

293 without affecting the water vapor permeability or the solubility.

294   Figure 5 presents the water contact angle formed with the first drop of water

295 released onto the surface of the material, indicating the hydrophobicity or hydrophilicity

296 of the surface of the material. According to Chimonyo et al. [31], hydrophobic

297 interactions occur when non-polar starch groups escape from the water to interact with a

298 hydrophobic surface. In this sense, the ozonated cassava sheet for O78.9 could be

299 considered a hydrophobic material with an increase of 37 % in contact angle in

300 comparison to the native cassava starch sheet. On the contrary, we observed a decrease

301 in the contact angle for the O145.3 sheet, which could be attributed to the strong

302 polarization and resonance stabilization of the carboxyl-OH bonds formed (higher for the

303 O145.3 starch), which enhances the level of polarity on the polymeric matrix [31],

304 resulting in the decrease in the water contact angle.

305   There was no statistical difference in the moisture content of the bio-based plastics

306 (Table 1), showing that ozonation did not interfere with this parameter, being the the

307 values similar to those reported for native cassava sheets [14].

308   *3.5.Opacity and transmittance*

309   As shown in Table 1, the native cassava starch sheet showed lower values of

310 opacity in comparison to the O78.9 sheet, similar to those reported by La Fuente et al.

311 [6,7] for ozonated cassava films produced by casting. However, for the O145.3 material,

312 while the films obtained by casting were less opaque [6] the sheets were more opaque.

313 This could be attributed to several factors, such as the thickness of the material (Table 1),

314 different molecules' conformation due to the extrusion process, and also the

315   bubbles/droplets formation, which could interfere with the measurement of this property.

316   Figure 6 presents the spectra of transmittance of the sheets, confirming that the ozonated

317   sheets presented less transmission from 200 to 800 nm. Specifically, in the UV region

318   (200 to 400 nm), considerably lower transmittance values can be observed, which means

319   the starch ozonation treatment on starch improves the ability of films to protect against

320   UV light. Note that this is important for different applications, such as for food packaging.

321   Other studies reported this achievement but after the addition of other compounds, such

322   as nano-encapsulated lycopene, β-carotene, or blueberry and pomace extracts [32,33]. It

323   is worth highlighting that the ozonated starch sheets do not need the addition of any

324   components to achieve this good behavior. This result can be a consequence of the new

325   polymeric matrix formed by ozonation, which, according to Fakhouri et al. [22], can also

326   be related to the high relative crystallinity of these sheets (Table 1).

327   *3.6.Thermogravimetric analysis (TGA)*

328   The thermogravimetric analyzes and their respective differential

329   thermogravimetric (DTG) profiles are presented in Figure 7, where different profile

330   slopes can be observed. According to Medina-Jaramillo et al. [34], the first stage (100–

331   150 °C) is related to the evaporation of water and/or volatiles. The second stage (180–

332   260) °C is correlated with the decomposition of the glycerol-rich phase, which also holds

333   starch; and the third stage (250–350 °C) is related to the degradation of the components

334   of the starch. As observed in Figure 7 (A to C), no differences are observed between

335   sheets of (350-500 ℃), which implies that ozonation did not interfere with starch

336   degradation. Finally, the region from (350-500) ℃ corresponds to carbon burning [35].

337   From the weight loss profile (Figure 7D), faster degradation of native and O78.9

338   cassava starch sheets can be observed within the step from (100–150) °C and (250–350)

339   °C. According to Medina-Jaramillo et al. [34], the degradation at lower temperatures

16

suggests a reduction in the molecular interactions between starch-starch chains and strengthening of hydrogen bonding interactions between hydroxyl groups and other molecules. Therefore, ozonation could reduce the molecular interactions between the starch–starch chains and favor the interactions between the hydroxyl groups of the starch and carbonyl and carboxyl groups, thus resulting in a decrease in the degradation temperature for the O78.9 sheet. This was not observed for the O145.3 sheet, probably due to the higher degree of ozonation, which can promote other interactions between the hydroxyl, carbonyl, and carboxyl groups, as well as the different molecular size distribution (molecular depolymerization).

4. Conclusions

The starch ozonation process resulted in the formation of the carbonyl and carboxyl groups, as well as partial molecular depolymerization of the amylose and amylopectin molecules. Consequently, a polymeric matrix with different molecular size distribution, charge distribution, and thus, packing properties, was achieved.

In this work, first reported in the literature, it was possible to produce cassava sheets with unique characteristics, from ozonated starch processed by the extrusion process. The level of ozone processing was shown to be a key factor in selecting the desired properties.

The sheet produced with ozonated starch for O78.9 resulted in a homogeneous structure, with improved mechanical behavior (increased Young Modulus of 43 % and decreased elongation at break of 23 %), increase hydrophobicity (37 %), without interfering with the water vapor permeability. Furthermore, a lower transmittance was achieved in the UV-region (200 to 400 nm) was achieved.

Conversely, for the O145.3 starch sheet, the higher degree of ozonation in combination with the conditions of the extrusion process did not improve the mechanical properties, the water vapor permeability, or the wettability characteristics. This behavior

could be attributed to the combination of the molecular modification during the pre-treatment with ozone, and the network formation under the shear-temperature conditions during extrusion. Therefore, an adequate combination of ozonation and extrusion processes yields materials with specific characteristics, with a wide range of applications for starch-based bioplastic production.

We suggest future studies including other starch sources, processing conditions, formulations, and a combination of processes to obtain bio-based plastics with better performance.

Author's Contribution / CRediT author statement

Carla I. A. La Fuente: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Project administration.

Larissa do Val Siqueira: Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing.

Pedro E.D. Augusto: Conceptualization, Methodology, Formal analysis, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

390   **Carmen C. Tadini:** Conceptualization, Methodology, Formal analysis, Resources,

391   Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

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Figure 1. Images of bio-based plastics produced with native and ozonated cassava starch by extrusion.

Figure 2. Morphology of native and ozonated cassava starch plastics observed by scanning electron microscopy (SEM).

Figure 3. **(A)** X-ray diffraction patterns and **(B)** FTIR spectra of native and ozonated cassava starch sheets.

Figure 4. Water vapor permeability and solubility of native and ozonated cassava starch sheets produced by extrusion.

Figure 5. The water contact angle of native and ozonated cassava starch sheets produced by extrusion.

Figure 6. Transmittance of native and ozonated cassava starch sheets produced by extrusion.

Figure 7. Thermo gravimetrical analysis of native **(A)** and ozonated **(B)** and **(C)** cassava starch sheets produced by extrusion. **(D)** Comparatives profiles.