Biodegradability of bio-based materials produced with DHT-modified cassava starch

Carla I.A. La Fuente[a], Gabriel A. Atanazio- Silva[a] Carlos A. R. Anjos[b], and Carmen C. Tadini[a,c*]

[a] Universidade de São Paulo, Escola Politécnica, Department of Chemical Engineering, Main *Campus*, São Paulo, SP, 05508-010, Brazil

[b] University of Campinas, School of Food Engineering, Department of Food Engineering and Technology, Campinas, SP, Brazil

[c] Universidade de São Paulo, Food Research Center (FoRC/NAPAN), São Paulo, SP, Brazil

***Corresponding author:** C. C. Tadini; Universidade de São Paulo, Main *Campus*, São Paulo, SP, 05508-010, Brazil. (catadini@usp.br).

Abstract

Among the different types of starch modification that increase their performance, dry heating treatment (DHT) is a promising green technique that results in plastic materials with enhanced characteristics, mainly in their mechanical and barrier properties. The effect of this type of modification on its biodegradability and disintegration behavior has yet to be described in the literature. Therefore, this work aims to produce bio-based plastic with cassava starch previously modified by dry heating treatment (DHT) and study its disintegration and biodegradation in compost media. The main results showed that all materials showed up to 80 % and 90 % biodegradation and disintegration, respectively, for all materials in 45 days. Moreover, higher rates of disintegration were visualized until day 7, as well as microbial growth and disappearance of functional groups, which is attributed to the highly hydrophilic nature of starch that facilitates the hydrolysis of the polymer in the early stages. In particular, the sheet submitted to DHT for 4 h showed higher rates.

Keywords: extrusion, biodegradable plastics, compostable materials.

1. Introduction

The amount of materials used for packaging is constantly growing. If current consumption models and waste management practices do not improve by 2050, there will be more than 12 billion tons of plastic litter [1]. The inappropriate disposal of plastic materials has led to their dispersion and accumulation in the marine environment in smaller pieces called “microplastics” [2]. The finding of plastic degrading into microparticles in oceanic environments is no longer shocking news [3]. In fact, during the current COVID-19 pandemic, the worldwide production and disposal of face masks, plastic laboratories and medical materials have increased drastically, adding microplastic waste to the environment [3].

Therefore, the development of biopolymer-based packaging is one such alternative to reduce the use of conventional plastics made from petrochemicals. Regarding starch, in 2017, the global production of starch-blend materials corresponded to 43.8 % of the total biodegradable plastic production [2]. However, it is well-known that native starch performance is reduced, and the properties of their plastics are not competitive against conventional plastics. Among the numerous varieties of starch modifications to improve the performance of native starch, dry heating treatment (DHT) is an interesting alternative with potential use in different industries, resulting in materials with increased mechanical and barrier properties [8,9]. According to the literature, DHT results in partial replacement of hydroxyl groups by carbonyl groups, resulting in new intermolecular associations. Moreover, DHT led to a reduction of molecular size, in particular the molecules of intermediate size, forming two well-defined groups with larger and smaller molecules, similar molecule sizes are more suitable for reassociation favoring short- and long-term retrogradation [12]. Literature also reported that polymer

53 structure, i.e., polymer chain length, and functional groups influence the biodegradation
54 process [10].
55 Biodegradation studies of polymers in compost media started to be studied for some
56 bio-based packaging, i.e., wheat gluten-based [4,5]; Polylactic acid (PLA) - Poly(3-
57 hydroxybutyrate-co-3-hydroxyvalerate), PHBV [6] and starch [7], among others.
58 However, the effect of the DHT modification process on the biodegradation and
59 disintegration of plastic materials is not yet described in the literature. Obtaining
60 materials with increased performance is a good achievement; however, these materials
61 must also result in proper rates of biodegradation and disintegration, otherwise, the
62 grave problem of plastic waste contamination in the environment will not be solved at
63 all.
64 Therefore, this work aims to produce bio-based plastic with cassava starch previously
65 modified by dry heating treatment (DHT) and study its disintegration and
66 biodegradation in compost media.

67 ## 2. Material and Methods

68 ### *2.1.Materials and dry heating process (DHT)*

69 Native cassava starch (Amilogill 1500) was supplied by Cargill Agrícola - Brazil.
70 Subsequently, it was divided into 50 g portions, then allocated into aluminum envelopes
71 (30 x 30) cm^2 and submitted to dry heating treatment (DHT) process in a convective
72 oven (FANEM, model 515, Brazil), for 2 h (DH2T) and 4 h (DH4T) at 130 °C,
73 following the modification process described in La Fuente [14]. The modified starch
74 was then cooled, grounded, sieved (250 mm), packaged, and stored until further use.
75 Glycerol grade P.A. (Sigma-Aldrich, Brazil) and Milli Q water were used as
76 plasticizers.

77 ### *2.2.Extrusion process*

78 The mixes of starch, glycerol (43.1 g/100 g of starch) and water (25.9 g/100 g of starch)
79 were mixed in a blender (Kitchen Aid, 525, Brazil). These mixes were fed to the twin-
80 screw extruder (Thermo Fisher Scientific, Process 11, Germany) L/D 40, and 11-mm
81 screw diameter from a double screw feeder (Brabender, Volumetric Minitwin Process
82 11, Germany) at 0.05 kg/h and 80 rpm to produce pellets (2 mm). The temperature
83 profile from the feed zone (1) to the extrudate outlet (zone 8) was 60, 75, 90, 100, 110,
84 110, 105, and 95 °C. Subsequently, from a conical-type feeder (Brabender,
85 Kulturstrasse 55-73, Germany), those pellets were supplied to the same corotating twin-
86 screw extruder with a feed flow of 0.3 kg/h. The plastic materials were produced using
87 the same temperature profile of the pellets at 95 °C and 80 rpm with a die of (3 x 0.1)
88 cm^2, as previously described in La Fuente et al. [14].
89 Those materials were conditioned for at least 7 days in aquariums (75 % RH) at ~25 °C
90 before the disintegration/biodegradation experiments.
91 *2.3.Water Content and Solubility*
92 The water content of the biobased materials was determined in triplicate in an oven
93 (Marconi, M030/2057, Brazil) at 105 ºC, according to the methodology described by
94 Gontard et al. [15].
95 The solubility was evaluated in quintuplicate with disks (diameter = 20 mm) in Milli Q
96 water at 25 °C and 58 °C, stirred at 120 rpm in a shaker (SOLAB, mod. SL-223, Brazil)
97 for 24 h. After that, water was filtered and the retained material was weighed and then
98 dried to constant mass at 105 ºC according to Gontard et al. [15]. Solubility (*S*) and
99 water uptake capacity (*WUC*) were calculated according to Eq. (1) and Eq. (2),
100 respectively.

101 $$S(\%) = \frac{m_f - m_0}{m_0} \cdot 100 \quad (1)$$

102 $$WUC(\%) = \frac{m_w - m_f}{m_f} \cdot 100 \quad (2)$$

103 wherein: m_o, m_w, and m_f are the initial, the wet, and the final mass of the discs (g),
104 respectively.

105 *2.4.Synthetic Solid Residue (SSR)*

106 A synthetic solid residue (SSR) was prepared according to the EN ISO 20200 standard
107 [12]. The water content was corrected at 55 g/100 g at the beginning of the composting
108 period. At time zero and the end of the assay, the SSR was characterized by the dry
109 (*DS*) and volatile solids (*VS*), in triplicate, according to Eq. (3) and Eq. (4).

110 $$DS(\%) = \frac{m_d^{105}}{m_w^0} \cdot 100 \quad (3)$$

111 $$VS(\%) = \frac{m_d^{105} - m_d^{550}}{m_d^{105}} \cdot 100 \quad (4)$$

112 wherein: m_w^0 is the initial mass of the sample, m_d^{105} is the mass after drying at 105 °C
113 and m_d^{550} is the mass of the ashes dried at 550 °C.

114 The assay was validated at the end of the composting time according to Eq. (5).

115 $$R = \frac{\left[m_0 \cdot (DS)_0 \cdot (VS)_0\right] - \left[m_f \cdot (DS)_f \cdot (VS)_f\right]}{\left[m_0 \cdot (DS)_0 \cdot (VS)_0\right]} \cdot 100 \quad (5)$$

116 wherein: m_0 is the initial mass, DS_0 is the initial dry solids, and VS_0 is the initial volatile
117 solids, while *mf*, DS_f, and VS_f are their respective values at the end of the composting
118 time.

119 *2.5.Disintegration assay*

120 The film disintegration assay was performed on a laboratory scale according to the EN
121 ISO 20200 [12] methodology, with some modifications, as described below.

122 The amount of 5 g of samples cut into (25 × 25) mm^2 was weighed using an analytical
123 balance (±0.1 mg) and put into mesh bags, later placed into plastic reactors containing 1
124 kg of SSR, prepared as described previously. Before the experimental setting, plastic
125 reactors were perforated with a 5 mm diameter hole on one side of the reactor to allow
126 gas exchange between the inside and the outside. Three reactors per film formulation
127 and then placed in the oven (FABBE-PRIMAR, Brazil) at 58 ± 5 °C to ensure
128 thermophilic conditions. Throughout the testing period, the reactors were weighed and,
129 if necessary, Milli-Q water was added to keep the compost wet and stirred as specified
130 in ISO 20200 [12]. The disintegration percentage (D_t) after 45 days was determined
131 according to Eq. (6).

132 $$D_t(\%) = \frac{m_0 - m_t}{m_0} \cdot 100 \quad (6)$$

133 wherein: m_0 is the initial mass and m_t is the mass after day 45 dried at 40 ºC under
134 vacuum in an oven (Marconi, M030, Brazil), until a constant mass.
135 Additionally, samples of materials were removed from the reactor at different control
136 times on days 2, 7, 10, 14, 21, 28, 35, and 45 to monitor sample weight loss,
137 morphological and mechanical changes, and FTIR spectra. Before these analyses, the
138 mesh bags (holding the pieces of material) were cleaned with a brush to eliminate the
139 adhered residues and then dried at 40 ºC, under a vacuum in an oven (Marconi, M030,
140 Brazil), until constant mass.

141 *2.5.1. Morphology*

142 The morphological changes of the biobased materials throughout the disintegration
143 experiments were photographed with a digital camera and with an image capture system
144 consisting of a light microscope and a digital camera (LEICA, model S6D, Germany) at
145 80x magnification, immediately after being collected from the reactors. Pieces of the
146 materials, previously dried at 40 ºC, were visualized by a scanning electron microscope

147 (SEM) (FEI, model Quanta 600FEG, Netherlands), coated with 10-nm platinum, and
148 observed at 15 kV and 5000x.

149 *2.5.2. Mechanical Properties*

150 Plastic material thickness was determined at five arbitrary positions using a digital
151 micrometer (±0.001 mm) (MITUTOYO, Japan). Tensile strength (*TS*), elongation at
152 break (ε), and Young's modulus (*E*) were estimated throughout the uniaxial tensile assay
153 according to the ASTM D882-12 standard method [13] on a texture analyzer (Stable
154 Micro Systems, TAXT2i, UK), using the probe A/TGT self-tightening grips at a speed
155 of 1 $mm \cdot s^{-1}$ and grip separation of 50 mm; ten strips (100 x 25) mm^2 per formulation
156 were prepared.
157 Strips of materials were carefully taken out of the reactors and tested on day 0, day 2,
158 and day 4. The materials from days 2 and 4 were previously dried under vacuum in an
159 oven (Marconi, M030, Brazil) at 40 ºC until a constant mass was reached to eliminate
160 excess water. Afterward, they were conditioned for at least 2 days in aquariums (75 %
161 RH) at ~25 °C before the mechanical test. However, for both DHT sheets (DH2T and
162 DH4T), it was impossible to remove the material because it was already broken or
163 broken during the collection procedure after 2 days. From day 7, it was impossible to
164 collect materials from any formulation.

165 *2.5.3. Fourier-transform infrared (FTIR) assay*

166 Plastic material spectra were evaluated by Fourier Transform Infrared spectroscopy
167 (Shimadzu, IR Prestige-21, Japan) in the Attenuated Total Reflectance (ATR) mode
168 from 4000 to 500 cm^{-1}, with a resolution of 2 cm^{-1} and 20 scans. The data were then
169 processed using Origin 2020 software (OriginLab Corporation, Massachusetts, USA).

170 *2.6.Biodegradation assay*

171 Biodegradation was evaluated throughout the laboratory setup methodology for 45 days
172 at 58 ± 2 °C simulating aerobic composting following ISO 14855-1 [14] with
173 modifications. Before the test, the C, N, and H contents were determined by an
174 elementary analyzer (PERKIN ELMER, model 2400 Serie II, USA). The reactors (glass
175 containers of 2 L) containing two polypropylene flasks were then prepared, one with 3 g
176 of synthetic solid residue (SSR) with 1 g of vermiculite and approximately 100 mg of
177 material, while the other flask had distilled water to ensure 100 % RH. Reactors were
178 prepared in triplicate per formulation; reactors containing cellulose microcrystalline
179 (CMC) as the reference material and reactors with only compost as blank were also
180 prepared in triplicate.
181 The CO_2 generated inside the reactors throughout the biodegradation process was
182 directly measured using a CO_2 analyzer (MOCON, PAC CHECK model 650, U.S.A), at
183 intervals of 2 days. The theoretical concentration of CO_2 generated from the plastic
184 material (${CO_2}^{Th}{}_S$) was calculated by Eq. (7).

185 $$ {CO_2}^{Th}{}_S = m_S \cdot C_S \cdot \frac{M_{wCO_2}}{M_{wC}} \quad (7) $$

186 wherein: m_s is the dry mass of the sample (g), Cs is the concentration of carbon in the
187 dry material determined by the elementary analysis, M_{wCO2} and M_{wC} are the molecular
188 weight of CO_2 and C, respectively.
189 The percentage of biodegradation was determined by Eq. (8), assuming that all the
190 carbon present in the material was converted to CO_2.

191 $$ B(\%) = \frac{\sum (CO_2)_t - \sum (CO_2)_{bt}}{{CO_2}^{Th}} \cdot 100 \quad (8) $$

192 wherein: $\sum CO_{2t}$ is the cumulative amount of CO_2 produced in the sample reactor and $\sum$
193 CO_{2bt} is the cumulative amount of CO_2 produced in the blank reactor.
194 The kinetics of biodegradation was adjusted to Hill's equation [19, 20].

$$B(\%) = B(\%)_{\max} \cdot \frac{t^n}{k^n + t^n} \quad (9)$$

wherein: $B(\%)_{\max}$ is the percentage of biodegradation at infinite time, t is the time (days), k is the time at which $B(\%) = 0.5\ B(\%)_{max}$ (days), and n is the curve radius of the sigmoid function.

2.7.Statistical analyses

All data were evaluated by the Analysis of Variance Multifactor (ANOVA), applying Tukey`s test (p<0.05) using the Statgraphics Centurion XV software (StatPoint, Inc., USA).

3. Results and Discussion

3.1. Solubility, water uptake capacity, water content, thickness, and carbon content

Solubility and water uptake capacity was determined at 58 °C because the disintegration/biodegradation in compost media was held at this temperature, and these properties could infer or predict the behavior of disintegration/biodegradation since one of the main components of the compost is water (55 g/100 g). Table 1 presents the solubility and water uptake capacity at 25 ºC and 58 ºC of the native and DH2T and DH4T materials. Similar values of solubility have been reported in the literature for cassava starch materials produced by extrusion [21]. At 25 ºC, the solubility of all the materials was statistically similar; however, the effect of the temperature is observed at 58 ºC. As shown in this table, the DHT modification process increased the solubility of biobased materials probably caused by the depolymerization that occurred during the modification. Indeed, Guo et al. [22] showed a decrease from 1.75×10^{20} (native starch) to 1.89×10^{19} and 5.61×10^{16} (g/mol) after 2 h or 4 h at 130 ºC of the DHT process. Lei et al. [23] also showed a dramatic decrease in DHT-modified starch granules size from 100 to 10 nm in the case of maize starch.

219 Moreover, Shah et al. [24] reported that this molecular polymerization increases the
220 solubilization due to the fragmentation of starch into low molecular weight chains and
221 La Fuente et al. [21] showed that the ozone-modified cassava starch sheets produced by
222 extrusion also resulted in a higher solubility than the native cassava starch materials.
223 However, the modification process is different (ozone modified *versus* DHT);
224 depolymerization of molecules occurs in both modification processes, confirming that
225 polymerization facilitates water penetration and dissolves polymeric fractions more
226 easily, thus increasing the solubility of the DHT films.
227 At 25 °C, a decrease in water uptake capacity (WUC) can be observed due to the DHT-
228 time, while at 58 °C, a decrease in this property was observed only for the DH4T
229 cassava starch sheet. According to Edhirej et al. [25] the presence of hydroxyl groups
230 in water molecules facilitates the formation of hydrogen bonds with starch, resulting in
231 the highest tendency of WUC in the plasticized polymer material. As exposed
232 previously, DHT promotes the oxidation of hydroxyl to carbonyl groups; therefore, the
233 lowest hydroxyl groups available to form hydrogen bonds and lower WUC, are in
234 agreement with the authors' hypothesis, which probably describes the results herein. In
235 sum, the total solubility of the DHT materials (both 2 h and 4 h) is greater than the
236 native one, which implies that DHT-modified materials could probably degrade at
237 higher rates than the native ones.
238 Table 1 also presents the values of moisture, thickness, and carbon content of the
239 materials studied. Similar values of moisture and thickness were also reported in the
240 literature for cassava materials produced by extrusion [9,15]. Similar carbon content
241 values were reported for starch films [5,7]. No statistical differences were observed for
242 the thickness and the C content, which is a positive result, as both properties influence
243 the disintegration and biodegradation (CO_2 production) processes. According to ASTM

244 D882-18 [13], the plastics obtained from extrusion are ‘sheets’ since their thickness is
245 greater than 0.250 mm.

246 *3.2.Compost characteristic*

247 Table 2 presents the compost characterization in dried solids (*DS)* and volatile solids
248 (*VS),* as well as the reduction in the volatile content (*R*) of the compost after the
249 composting period. The initial values (time = zero composting) were *DS* of 33.75 ± 0.63
250 g/100 g and *VS* of 92.07 ± 2.00 g/100 g. Table 2 allows observing the decrease in the *VS*
251 as regards the initial value*,* which is an expected behavior already described in the
252 literature [7,18]. After 45 days, no statistical differences were observed in the
253 percentage of disintegration for the native, DH2T, and DH4T sheets; similar values of
254 disintegration were reported in the literature for native pea starch after 73 days [19] and
255 corn starch after 84 days [7]. Since the *R* values were greater than 30 % and the
256 standard deviations of the disintegration were less than 10 %, the test could be validated
257 according to EN ISO 20200 [16].

258 *3.3. Morphological changes*

259 The visual appearance of the sheets at different composting times is presented in Figure
260 1. Initially, at time = zero, all materials presented the same morphological characteristic
261 without difference between native and DHT sheets (2 h and 4 h). On the second day, all
262 the sheets are verified to have absorbed water; however, while the native sheet remained
263 with a joint structure, the DHT materials started to separate into pieces, with the visible
264 presence of cracks. On day 7 the disintegration of all the sheets could be visualized; the
265 zoom images show that DHT materials, especially DH4T sheets, resulted in more and
266 smaller pieces, implying a higher disintegration rate. From day 14 to the end of the
267 assay (day 21 - 28 images are not shown), the disintegration continues to occur. Finally,

on day 45, all the materials are observed to practically completely disintegrate, with few particles bound in the mesh bags.

The morphological changes shown by SEM at different composting times are presented in Figure 2. According to Oliveira et al. [20] the SEM allows the detection of microbial colonization and changes in the physical aspect of the polymer surface, which therefore implies surface degradation. At time zero, a smooth surface could be seen for the native sheet, and in the case of DHT materials (2 h and 4 h), ungelatinized starch granules could be seen, which is probably caused by two reasons. Chandanasree et al. [28] reported that during the modification process starch granules grew agglomerated due to the leaching of amylose as the decreased granule interspacing. Moreover, Lei et al. [29] point out that the low moisture content of DHT-starches (due to the heating) reduces the available water acting as a plasticizer during gelatinization. In fact, ungelatinized granules of DHT-modified starch were also seen for materials produced by extrusion [13] and by melt blending and subsequent compression molding [10].

On day 2, a smoother surface remained for the native sheet, while cracks are visualized in the DHT sheets, more visible in the DH4T one, which agrees with the images shown in Figure 1. Czaja-Jagielska [32] also visualized a porous structure with cracks and cavities in the disintegration of corn and potato starch films on the initial days of degradation. On day 7, it is possible to observe the microorganisms attacking the starch granules, again more markedly for the DH4T sheet. From day 14 to the end of the assay, microbial colonization is clearly visualized, with significant increases day after day. Balaguer et al. [11] observed fungal growth in wheat gluten films with the formation of a mycelium network, similar to Trujillo-Hernández et al. [33] in the degradation of *jicama* starch films, in which the presence of fungal spores and mycelia invading the

surface were visualized after 30 days. In our study, the mycelium network was observed on day 35, markedly in the DH2T sheet.

3.4. Weight loss curve

Figure 3 presents the weight loss of biobased sheets during the composting period, whereby the behavior of the curve is similar to those described in the literature [5,7,18] for biopolymer materials degradation under similar conditions. Higher weight loss rates are observed till day 7 when they start decreasing considerably. In fact, high rates of disintegration were also observed on the initial days of the assay for starch materials, which was related to the highly hydrophilic nature of starch, facilitating the degradation of the polymer backbone and promoting faster surface erosion [19]. On day 45, weight loss reached values from 80 to 90 % for all the materials; moreover, during experimentation time, the DH4T sheet showed a slightly higher weight loss rate during composting time, especially at the end of the assay (day 45). This behavior could probably be attributed to the modification process in which molecular depolymerization occurs. Maniglia et al. [8] reported that DHT promoted the formation of molecules of lower size, while Sheel & Pant [9] said that polymer depolymerization increases the available polymer sections for microorganism attack, which probably explains the results here, similar to those exposed by Chiu & Solarek [34] who reported that starch oxidation (DHT is a type of oxidation) produced more biodegradable materials; however, this is an initial hypothesis and additional analysis may be needed in the future to prove this.

As pointed out before, Sheel & Pant [9] reported that solubility is directly related to biodegradability, which partially explains the results herein, since higher solubility was observed for DHT sheets. The fact that DH2T resulted in higher values of solubility (Table 1) could imply that not only hydrophilic characteristics (available hydroxyl

317 groups) interfere with biodegradability behavior but also does polymer structure, since
318 higher depolymerization was visualized in the DH4T sheet, as described by Maniglia
319 [8].

320 *3.5. Mechanical properties*

321 Figure 4 shows the mechanical properties of the native, DH2T and DH4T sheets during
322 the composting time. At time zero, similar values were reported for tensile strength,
323 elongation, and Young's modulus for cassava sheets produced by extrusion [9,15]. As
324 previously exposed, materials were also collected on day 2 and day 4. However, for the
325 DHT sheet (both 2 h and 4 h), the collection of material was impossible because the
326 materials were already broken into pieces or broken during the collection procedure, as
327 can be seen in Figures 1 and 2. For the native sheet, a decrease in the mechanical
328 properties is directly related to the experimentation time. Tosin [35] also observed a
329 decrease in mechanical properties during the biodegradation period of *Mater-Bi* films (a
330 biodegradable plastic produced by Novamont), which according to Oliveira [20] are
331 indications of biodegradation caused by microbial attack on polymers. Sheel & Pant [9]
332 also attributed this decrease in the mechanical properties to the reduced polymer chain
333 length and their weakness due to chain scissions. Therefore, according to these results,
334 the biodegradation process of all biobased sheets had already started on day 2.

335 *3.6. Fourier-transform infrared (FTIR) spectra*

336 Figure 5 shows the FTIR spectra of the sheets obtained from native cassava starch and
337 modified by DHT (2 h and 4 h), collected during the disintegration assay. FTIR spectra
338 provide information on the polymer chemical functional groups, which, according to
339 Oliveira [20], give information on the chemical changes during biodegradation, either
340 through their consumption or by the production of functional groups due to microbial
341 activity.

342 At time zero, the spectra exhibited a conventional behavior for native cassava starch
343 plastics plasticized with glycerol [26,27] and also for sheets produced with cassava
344 starch previously modified by DHT [9]. No differences were observed among the
345 different materials (native, DH2T, and DH4T) as previously discussed in the literature
346 (7). The band at 3300 cm^{-1} is associated with the free hydroxyl groups (O—H), while at
347 2925 cm^{-1} related to C—H stretch [26,27], near to 1645 cm^{-1} to the bending vibration of
348 H—O—H, water molecules absorbed in the amorphous region and, at 1000 cm^{-1} the
349 band is related to the C–O stretching [28,29].
350 Note that the same profile is maintained for native and DHT- modified materials until
351 day 2, while from day 7 to the end of the assay (day 45), the presence of peaks is not
352 verified, showing that all functional groups disappeared during the disintegration.
353 According to Ostadi et al. [40] the peak declines near 3404 cm^{-1} indicating that glycerol
354 has percolated from the material, which, according to Mittal [41] is due to the breakage
355 of the hydroxyl groups bonds during the degradation. The decrease in the peak near
356 2700-2900 cm^{-1} is due to the breakage of methylene groups [31] and near 1666 cm^{-1} due
357 to the duction of carbonyl groups [32]. Ostadi et al. [40] also reported that the decrease
358 in the peak near 1027 cm^{-1} is related to the breakage of the α (1→4) bond of starch,
359 suggesting the degradation of the polymer by α-amylase produced by the
360 microorganisms. The fact that this change in the FTIR path was observed on day 7, is
361 consistent with the images shown in Figure 2, in which microbial growth can be seen,
362 and also with the higher rate of weight loss observed in Figure 3.
363 *3.7. Biodegradation*
364 Figure 6 presents biodegradation during 45 days of the composting period of native,
365 DHT (2h and 4h) materials and the reference material, in which the symbols are
366 experimental data in comparison with the adjusted Hill's model. First, it can be seen

367 that the biodegradation of the reference material (CMC) reached 70 % after 45 days; the
368 assay could thus be validated according to the ISO 14855-1 guidelines [14]. In general,
369 the model describes the experimental data well, and the biodegradation curves showed a
370 sigmoidal shape, characteristic of bio-based polymer biodegradation [4]. Similar values
371 of biodegradation were reported in the literature for commercial potato starch films [5]
372 and wheat gluten [4].
373 In general, the polymer biodegradation process can be divided into *i)* biodeterioration,
374 *ii)* depolymerization, *iii)* bio assimilation, and *iv)* mineralization [9]. In the first step, the
375 formation of a microbial biofilm leads to superficial degradation in which the polymeric
376 material is fragmented into smaller particles. During depolymerization, the biofilm
377 microorganisms secrete extracellular enzymes, which catalyze the polymer chain into
378 oligomers, dimers, or monomers [10]. During this step, a lag phase can occasionally be
379 detected in the sigmoid curves, corresponding to the time needed for water to penetrate
380 the matrix and enzymes to cleave the polymer chains [5]. Uptake of the small molecules
381 produced in the microbial cell and the subsequent production of primary and secondary
382 metabolites is called bioassimilation. Lastly, these metabolites are mineralized and
383 products such as CO_2, CH_4 and H_2O are formed and released into the environment [10].
384 The materials produced with cassava starch (native or DHT) reached at least 80 % of
385 their biodegradation after 45 days (Figure 6A). A higher market value was reached by
386 DH4T, in comparison to the native and the DH2T material. Analogous to the weight
387 loss curve rate (Figure 3), the microbial growth (Figure 2), and the losses of functional
388 groups (Figure 5), the biodegradation effects were observed mainly on day 7, when the
389 maximum value of the biodegradation rate was observed, again for the DH4T sheet, as
390 shown in Figure 6B.

391 Note that biodegradation values of the DH4T sheets reach values higher than 100 %
392 (considering the standard deviation), which is due to the 'priming' effect, which occurs
393 when the compost inoculum in the reactors produces more CO_2 than the blank sample
394 [7]. This is attributed to the stimulation of organic matter mineralization as a
395 consequence of its interaction with microorganisms [19].
396 Table 3 presents the parameters of Hill's model for the native, DH2T, and DH4T bio-
397 based sheets. It can be observed that the DH4T sheet presented the highest value of
398 $B(\%)_{max}$, followed by the native, the DH2T, and the CMC materials; similar values are
399 found in the literature for starch films [5,7,19]. The time in days to reach 0.5 $B(\%)_{max}$ (k
400 value) was similar for all the materials and also similar to those reported previously
401 [5,7,19].
402 According to the literature, the higher degree of crosslinking in starch resulted in a more
403 resistant matrix (due to the new linkages created); lower swelling capacity (measured as
404 water uptake capacity), thus resulting in lower biodegradation rates due to the new
405 bonds being cleaved by microorganisms and the lower water vapor diffusion coefficient
406 [5]. Moreover, Wang et al. [46] stated that both high crystallinity and low
407 hydrophilicity retard penetration and attack by water, resulting in a slow hydrolysis
408 process. Those statements oppose the results reported herein. The DHT modification
409 process results in new functional groups (more resistant matrix due to the new linkages
410 created) and also reduces the water uptake capacity as observed for DH4T (Table 1).
411 The biodegradation of DHT materials was expected to be slower; however, DH4T
412 resulted in higher disintegration (Figure 3) and biodegradation (Figure 6A and 6B)
413 rates. The 'key' factor could be the depolymerization, as previously exposed, which
414 results in shortening the polymer chain and makes it more susceptible to microorganism
415 attack. Indeed, Wang et al. [46] showed that even with the same structure, the size of the

polymer chain can severely affect the rate of hydrolysis during biodegradation. Moreover, Sheel & Pant [9] reported that the introduction of the carbonyl groups such as C=O increases the number of chromophores, increases the sites for photon absorption, and thus the rate of photodegradation.

The results presented here are a great achievement because the literature reported that DHT modification enhances the starch performance for several applications, i.e., 3D impression [23] and especially in the packaging field [8,9]; from this study, depending on the conditions of modification, the biodegradation process can be assumed to also be improved. For example, compared to the native sheet, with the modification of starch for 2 h (DH2T), the behavior of the disintegration/biodegradation patterns is not altered. However, when the modification is carried out for 4 hours (DH4T), the mechanical performance of the sheets at time zero (Figure 4), as well as the disintegration/biodegradation rates are improved (Figure 3 and Figure 6A and 6B).

4. Final Remarks and Conclusions

Disintegration and biodegradation of native and DHT sheets were determined by the ISO 20200 and ISO 15855-1 methodology, respectively, and both assays were validated according to the criteria described in both standards. In general, the disintegration and biodegradation of all the materials resulted in up to 90 % and 80 %, respectively, after 45 days. In both assays, maximum rates were achieved on day 7, which is attributed to the highly hydrophilic nature of starch that facilitates the hydrolysis of the polymer in the early stages.

Complementary assays, morphological and mechanical changes, and FTIR spectra helped to describe the disintegration/biodegradation process, in a specific way. The microbial colonization was visualized on day 7 by SEM images, concurrently with the disappearance of functional groups in the FTIR spectra. On day 2, a decrease in

441 mechanical performance was observed; in fact, it was not possible to measure it in DHT
442 sheets due to the higher hydrolyzed structure, as a result of the DHT modification
443 process, which results in the shortened polymer chain, and probably this fact facilitates
444 the microorganism attack.
445 The solubility characteristic partially explains the disintegration/biodegradation
446 behavior. According to the literature, higher values of molecular depolymerization
447 were reported in the literature for DH4T (in comparison to native and DH2T starch),
448 which increases solubilization in water. In this study, higher solubility was observed in
449 the DH2T sheets; conversely, higher disintegration/biodegradation was observed for the
450 DH4T sheets. This could imply that the greater polymeric depolymerization of this
451 material and the presence of the microorganism population in the compost are crucial
452 elements promoting increases in the rates of disintegration/biodegradation.
453 These results are an advance from the knowledge related in the literature today because
454 DHT modification showed to enhance starch performance resulting in improved plastic
455 material performance and, from the results reported herein, it is possible to assume that
456 depending on the conditions of modification, the biodegradation process could also be
457 improved, which helps to reduce the world's waste accumulation problem. Therefore,
458 impacting positively the packaging sector.

459 **Acknowledgments**

460 The authors acknowledge the São Paulo Research Foundation (FAPESP) for the
461 postdoctoral fellowship of CIA La Fuente (2017/05307-8), the project EMU
462 2016/12385-2; and the National Council for Scientific and Technological Development
463 (CNPq, Brazil) for the grant to CC Tadini (309548/2021). The authors are also grateful
464 to Bianca Maniglia *Ph.D*. from the Department of Chemistry, Ribeirão Preto School of

465 Philosophy, Science and Literature, University of São Paulo for the assistance with the
466 FTIR assay.

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.

Gabriel A. Atanazio- Silva: Methodology, Validation, Formal analysis, Investigation.

Carlos A. R. Anjos: Conceptualization, Methodology, Resources, Writing - Review & Editing.

Carmen C. Tadini: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

References

1. United Nations Development Programme. PLASTICS AND CIRCULAR ECONOMY COMMUNITY SOLUTIONS [Internet]. 2020. Available from: file:///C:/Users/carla/Downloads/SGP_Plastics_and_Circular_Economy--Community_Solutions.pdf
2. Haider TP, Völker C, Kramm J, Landfester K, Wurm FR. Plastics of the Future? The Impact of Biodegradable Polymers on the Environment and on Society. Angew Chemie Int Ed [Internet]. 2019 Jan 2;58(1):50–62. Available from: https://onlinelibrary.wiley.com/doi/10.1002/anie.201805766
3. Alfaro-Núñez A, Astorga D, Cáceres-Farías L, Bastidas L, Soto Villegas C, Macay K, et al. Microplastic pollution in seawater and marine organisms across the Tropical Eastern Pacific and Galápagos. Sci Rep [Internet]. 2021 Dec 19;11(1):6424. Available from: http://www.nature.com/articles/s41598-021-85939-3
4. Alfaro-Núñez A, Astorga D, Cáceres-Farías L, Bastidas L, Soto Villegas C, Macay K, et al. Microplastic pollution in seawater and marine organisms across

the Tropical Eastern Pacific and Galápagos. Sci Rep. 2021 Dec;11(1):6424.

5. Haider TP, Völker C, Kramm J, Landfester K, Wurm FR. Plastics of the Future? The Impact of Biodegradable Polymers on the Environment and on Society. Angew Chemie Int Ed. 2019 Jan;58(1):50–62.

6. Freitas PA V., Arias CILF, Torres-Giner S, González-Martínez C, Chiralt A. Valorization of Rice Straw into Cellulose Microfibers for the Reinforcement of Thermoplastic Corn Starch Films. Appl Sci [Internet]. 2021 Sep 11;11(18):8433. Available from: https://www.mdpi.com/2076-3417/11/18/8433

7. La Fuente CIA, do Val Siqueira L, Augusto PED, Tadini CC. Casting and extrusion processes to produce bio-based plastics using cassava starch modified by the dry heat treatment (DHT). Innov Food Sci Emerg Technol. 2021 Dec;102906.

8. Maniglia BC, Lima DC, Matta Junior MD, Le-Bail P, Le-Bail A, Augusto PED. Preparation of cassava starch hydrogels for application in 3D printing using dry heating treatment (DHT): A prospective study on the effects of DHT and gelatinization conditions. Food Res Int. 2020;128(November 2019).

9. Sheel A, Pant D. Microbial Depolymerization. In 2018. p. 61–103. Available from: http://link.springer.com/10.1007/978-981-10-7413-4_4

10. Chevillard A, Angellier-Coussy H, Cuq B, Guillard V, César G, Gontard N, et al. How the biodegradability of wheat gluten-based agromaterial can be modulated by adding nanoclays. Polym Degrad Stab [Internet]. 2011 Dec;96(12):2088–97. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141391011003272

11. Balaguer MP, Villanova J, Cesar G, Gavara R, Hernandez-Munoz P. Compostable properties of antimicrobial bioplastics based on cinnamaldehyde cross-linked gliadins. Chem Eng J [Internet]. 2015 Feb;262:447–55. Available

517 from: https://linkinghub.elsevier.com/retrieve/pii/S1385894714012959
518 12. Hernández-García E, Vargas M, González-Martínez C, Chiralt A. Biodegradable
519 Antimicrobial Films for Food Packaging: Effect of Antimicrobials on
520 Degradation. Foods [Internet]. 2021 Jun 1;10(6):1256. Available from:
521 https://www.mdpi.com/2304-8158/10/6/1256
522 13. Tampau A, González-Martínez C, Chiralt A. Biodegradability and disintegration
523 of multilayer starch films with electrospun PCL fibres encapsulating carvacrol.
524 Polym Degrad Stab [Internet]. 2020 Mar;173:109100. Available from:
525 https://linkinghub.elsevier.com/retrieve/pii/S014139102030032X
526 14. La Fuente CIA, do Val Siqueira L, Augusto PED, Tadini CC. Casting and
527 extrusion processes to produce bio-based plastics using cassava starch modified
528 by the dry heat treatment (DHT). Innov Food Sci Emerg Technol [Internet]. 2021
529 Dec;102906. Available from:
530 https://linkinghub.elsevier.com/retrieve/pii/S1466856421003076
531 15. Gontard N, Guilbert S, Cuq J -L. Water and Glycerol as Plasticizers Affect
532 Mechanical and Water Vapor Barrier Properties of an Edible Wheat Gluten Film.
533 J Food Sci. 1993;58(1):206–11.
534 16. EN ISO 20200. Determination of the degree of disintegration of plastic materials
535 under simulated composting conditions in a laboratory-scale test. 2004;
536 17. ASTM. Standard Test Method for Tensile Properties of Thin Plastic Sheeting
537 [Internet]. 2018. Available from: https://www.astm.org/Standards/D882.htm
538 18. ISO 14855-1. Determination of the ultimate aerobic biodegradability of plastic
539 materials under controlled composting conditions. Method by analysis of evolved
540 carbon dioxide. 2012;
541 19. Balaguer MP, Villanova J, Cesar G, Gavara R, Hernandez-Munoz P.

Compostable properties of antimicrobial bioplastics based on cinnamaldehyde cross-linked gliadins. Chem Eng J. 2015 Feb;262:447–55.

20. Hernández-García E, Vargas M, González-Martínez C, Chiralt A. Biodegradable Antimicrobial Films for Food Packaging: Effect of Antimicrobials on Degradation. Foods. 2021 Jun;10(6):1256.

21. La Fuente CIA, do Val Siqueira L, Augusto PED, Tadini CC. Bio-Based Plastic Based on Ozonated Cassava Starch Produced by Extrusion. J Polym Environ [Internet]. 2022 Jun 9; Available from: https://link.springer.com/10.1007/s10924-022-02488-0

22. Guo, Y., Zhang, Y., Zheng, D., Li, M., Yue J. Isolation and characterization of nanocellulose crystals via acid hydrolysis from agricultural waste-tea stalk. Int J Biol Macromol. 2020;163:927–33.

23. Lei N, Chai S, Xu M, Ji J, Mao H, Yan S, et al. Effect of dry heating treatment on multi-levels of structure and physicochemical properties of maize starch: A thermodynamic study. Int J Biol Macromol [Internet]. 2020 Mar;147:109–16. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141813019396187

24. Shah U, Naqash F, Gani A, Masoodi FA. Art and Science behind Modified Starch Edible Films and Coatings: A Review. Compr Rev Food Sci Food Saf. 2016;15(3):568–80.

25. Edhirej A, Sapuan SM, Jawaid M, Zahari NI. Effect of various plasticizers and concentration on the physical, thermal, mechanical, and structural properties of cassava-starch-based films. Starch - Stärke. 2017 Jan;69(1–2):1500366.

26. La Fuente CIA, do Val Siqueira L, Augusto PED, Tadini CC. Bio-Based Plastic Based on Ozonated Cassava Starch Produced by Extrusion. J Polym Environ. 2022 Jun;

27. Tampau A, González-Martínez C, Chiralt A. Biodegradability and disintegration of multilayer starch films with electrospun PCL fibres encapsulating carvacrol. Polym Degrad Stab. 2020 Mar;173:109100.

28. Hernández-García E, Vargas M, Chiralt A, González-Martínez C. Biodegradation of PLA-PHBV Blend Films as Affected by the Incorporation of Different Phenolic Acids. Foods [Internet]. 2022 Jan 17;11(2):243. Available from: https://www.mdpi.com/2304-8158/11/2/243

29. Cano AI, Cháfer M, Chiralt A, González-Martínez C. Biodegradation behavior of starch-PVA films as affected by the incorporation of different antimicrobials. Polym Degrad Stab [Internet]. 2016 Oct;132:11–20. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0141391016301136

30. Oliveira J, Belchior A, da Silva VD, Rotter A, Petrovski Ž, Almeida PL, et al. Marine Environmental Plastic Pollution: Mitigation by Microorganism Degradation and Recycling Valorization. Front Mar Sci [Internet]. 2020 Dec 17;7. Available from: https://www.frontiersin.org/articles/10.3389/fmars.2020.567126/full

31. Chandanasree D, Gul K, Riar CS. Effect of hydrocolloids and dry heat modification on physicochemical, thermal, pasting and morphological characteristics of cassava (Manihot esculenta) starch. Food Hydrocoll [Internet]. 2016 Jan;52:175–82. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0268005X15002726

32. Czaja-Jagielska N., & Melski K. Biodegradation of Starch-Based Films in Conditions of Nonindustrial Composting. Polym J Enviroment Stud. 2013;4:1039–44.

33. Trujillo-Hernández C. J-LA, Solorza-Feria J., J. T-J, García-Ibáñez S., Lorenzo-

592 Santiago A. Rendón-Villalobos J. Preparation and biodegradation analysis of
593 starch films reinforced with coconut bagasse cellulose (Cocos nucifera L.). Int J
594 Appl Environ Sci. 2021;16(1):1–12.
595 34. Chiu C, Solarek D. Modification of Starches. In: Starch [Internet]. Elsevier;
596 2009. p. 629–55. Available from:
597 https://linkinghub.elsevier.com/retrieve/pii/B9780127462752000173
598 35. Bettas Ardisson G, Tosin M, Barbale M, Degli-Innocenti F. Biodegradation of
599 plastics in soil and effects on nitrification activity. A laboratory approach. Front
600 Microbiol. 2014 Dec;5.
601 36. Bergo, P., Sobral, PJA., Prison J. Effect of glycerol on physical properties of
602 cassava starch films. J Food Process Preserv. 2008;34(2):401–10.
603 37. Colivet, J., Carvalho R. Hydrophilicity and physicochemical properties of
604 chemically modified cassava starch films. Ind Crops Prod. 2017;95:599–607.
605 38. López-Córdoba A, Medina-Jaramillo C, Piñeros-Hernandez D, Goyanes S.
606 Cassava starch films containing rosemary nanoparticles produced by solvent
607 displacement method. Food Hydrocoll [Internet]. 2017 Oct;71:26–34. Available
608 from: https://linkinghub.elsevier.com/retrieve/pii/S0268005X17300991
609 39. Seligra PG, Medina Jaramillo C, Famá L, Goyanes S. Biodegradable and non-
610 retrogradable eco-films based on starch–glycerol with citric acid as crosslinking
611 agent. Carbohydr Polym [Internet]. 2016 Mar;138:66–74. Available from:
612 https://linkinghub.elsevier.com/retrieve/pii/S0144861715011315
613 40. Ostadi H, Hakimabadi SG, Nabavi F, Vossoughi M, Alemzadeh I. Enzymatic and
614 soil burial degradation of corn starch/glycerol/sodium montmorillonite
615 nanocomposites. Polym from Renew Resour [Internet]. 2020 Feb 1;11(1–2):15–
616 29. Available from: http://journals.sagepub.com/doi/10.1177/2041247920952649

41. Mittal A, Garg S, Premi A, Giri AS. Synthesis of polyvinyl alcohol/modified starch-based biodegradable nanocomposite films reinforced with starch nanocrystals for packaging applications. Polym Polym Compos [Internet]. 2021 Jun 29;29(5):405–16. Available from: http://journals.sagepub.com/doi/10.1177/0967391120922429

42. Mittal A. Garg S. Bajpai S. Assessing Biodegradability of PVA/Starch Based Composite Films Reinforced With Long Chain Fatty Acid Grafted Barley Husk. Res Sq. 2021;

43. Dave H., Rao PVC., Desai JD. Biodegradation of starch–polyethylene films in soil and by microbial cultures. World J Microbiol Biotechnol. 1997;13:655–8.

44. Chevillard A, Angellier-Coussy H, Cuq B, Guillard V, César G, Gontard N, et al. How the biodegradability of wheat gluten-based agromaterial can be modulated by adding nanoclays. Polym Degrad Stab. 2011 Dec;96(12):2088–97.

45. Wang G, Huang D, Ji J, Völker C, Wurm FR. Seawater-Degradable Polymers—Fighting the Marine Plastic Pollution. Adv Sci [Internet]. 2021 Jan 23;8(1):2001121. Available from: https://onlinelibrary.wiley.com/doi/10.1002/advs.202001121

46. Wang Y, Lu L, Zheng Y, Chen X. Improvement in hydrophilicity of PHBV films by plasma treatment. J Biomed Mater Res Part A [Internet]. 2006 Mar 1;76A(3):589–95. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jbm.a.30575

Figure Caption

Figure 1. Morphological changes of native, DH2T and DH4T bio-based sheets collected at different times during the composting period. **Note.** Zoom images were obtained at 80x.

Figure 2. Scanning electron microscopy images of native, DH2T, and DH4T biobased sheets collected at different times during the composting period obtained at 5000x.

Figure 3. Weight loss of native, DH2T and DH4T biobased sheets collected at different times during the composting period at 58 ºC.

Figure 4. Mechanical properties of native, DH2T and DH4T bio-based sheets collected at different times during the composting period.

Figure 5. FTIR spectra of native, DH2T, and DH4T bio-based sheets collected at different times during the composting period.

Figure 6. Biodegradation **(A)** and biodegradation rate **(B)** of native, DH2T, and DH4T biobased sheets during the composting period. Symbols are experimental data points, solid lines correspond to the degradation adjusted curves with Hill's equation, and error bars represent standard deviation.

Table 1.

Solubility (%) and water uptake capacity (WUC) (%) at (25 and 58) ºC, water Content (*X*), thickness, and carbon content (*C*) of the native, DH2T, DH4T bio-based materials.

Material	Solubility (%)		WUC (%)		*X* (g/100 g d.b.)	Thickness (mm)	*C* (g/100g)
	25 ºC	58 ºC	25 ºC	58 ºC			
Native	27.7 ± 2.7[a]	36.1 ± 3.0[c]	468.2 ± 19.0[a]	1069.1 ± 54.0[a]	18.73 ± 0.43[a]	0.67 ± 0.17[a]	39.82 ± 0.11[a]
DH2T	30.8 ± 2.0[a]	54.4 ± 2.6[a]	380.2 ± 16.0[b]	1040.8 ± 55.0[a]	18.81 ± 0.38[a]	0.79 ± 0.08[a]	39.65 ± 0.01[a]
DH4T	28.3 ± 2.1[a]	44.1± 3.2[b]	290.0 ± 11.4[c]	724.6 ± 15.0[b]	20.02 ± 0.54[b]	0.82 ± 0.12[a]	39.55 ± 0.12[a]

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

659 **Table 2.**

660 Dried solids (*DS*) g/100 g, volatile solids (*VS*) g/100 g, the reduction of volatile solids

661 (*R*) %, and the disintegration after 45 days (D_{45}) of composting of native, DH2T, and

662 DH4T bio-based sheets.

Material	**Post-Composting**		***R* (%)**	**Disintegration**
	***DS* (g/100 g)**	***VS* (g/ 100 g)**		**D_{45} (%)**
Native	78.21 ± 8.61	86.38 ± 5.95	39.55 ± 3.27	89.8 ± 2.4 [a]
DH2T	49.40 ± 12.63	91.28 ± 2.21	45.20 ± 8.05	90.4 ± 2.5 [a]
DH4T	97.98 ± 1.20	89.96 ± 1.20	42.77 ± 4.30	90.7 ± 3.4 [a]

663 Means with different letters in the column are significantly different ($p<0.05$).

664 **Table 3.** Parameters of the Hill adjusted model $B(\%)_{max}$, k and, n is the curve radius of
665 the sigmoid function of native, DH2T and DH4T bio-based sheets collected at different
666 times during the composting period.

Material	***B* (%) *max.* (days)**	***K* (days)**	***n* (-)**	r^2
Native	94.80	10.20	2.47	0.99
DH2T	81.60	10.73	2.96	0.99
DH4T	98.94	10.09	2.65	0.99
CMC	80.74	11.28	2.71	0.99

667 **Note.** B (%) $_{max}$ is the percentage of biodegradation at infinite time, k is the time (days)
668 at which $B(\%) = 0.5\ B(\%)_{max}$ (days), and n is the curve radius of the sigmoid function.