



**HAL**  
open science

# 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

Bianca Maniglia, Dâmaris Lima, Manoel Matta Junior, Patricia Le-Bail, Alain Le-Bail, Pedro E.D. Augusto

## ► To cite this version:

Bianca Maniglia, Dâmaris Lima, Manoel Matta Junior, Patricia Le-Bail, Alain Le-Bail, et al.. 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 Research International, 2020, 128, pp.108803. 10.1016/j.foodres.2019.108803 . hal-02536289

**HAL Id: hal-02536289**

**<https://hal.science/hal-02536289v1>**

Submitted on 21 Jul 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Preparation of cassava starch hydrogels for application in 3D printing using**  
2 **dry heating treatment (DHT): a prospective study on the effects of DHT and**  
3 **gelatinization conditions**

4 Bianca C. MANIGLIA <sup>a,b,c,d,\*</sup>; Dâmaris C. LIMA <sup>d</sup>; Manoel D. MATTA JUNIOR <sup>d</sup>;  
5 Patricia LE-BAIL <sup>b,c</sup>; Alain LE-BAIL <sup>a,c,\*</sup>; Pedro E. D. AUGUSTO <sup>d,e</sup>

6 <sup>a</sup> ONIRIS-GEPEA UMR CNRS 6144 Nantes - France

7 <sup>b</sup> BIA-INRA UR 1268 Nantes – France

8 <sup>c</sup> SFR IBSM INRA CNRS 4202

9 <sup>d</sup> Department of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz, College of Agriculture  
10 (ESALQ), University of São Paulo (USP), Piracicaba, SP – Brazil

11 <sup>e</sup> Food and Nutrition Research Center (NAPAN), University of São Paulo (USP), São Paulo, SP, Brazil

12 \*bianca.chierigato-maniglia@oniris-nantes.fr; \*alain.lebail@oniris-nantes.fr

13 **Abstract**

14 3D printing is a technology capable of presenting creative, unique and intricate items in  
15 an attractive format, with specific compositions. However, novel ingredients must be  
16 developed to satisfy this new technological requirement. This work proposes dry heating  
17 treatment (DHT), a simple physical technique, as a method for modifying cassava starch,  
18 with particular focus on its use for 3D printing. DHT processing was conducted at 130 °C  
19 for 2 and 4 h (named DHT\_2h and DHT\_4h, respectively). Different gelatinization  
20 conditions (65, 75, 85, and 95 °C) were applied, and the hydrogels were evaluated  
21 considering different storage periods (1 and 7 days). Cassava starch properties were  
22 evaluated, focusing on the application of its hydrogels to 3D printing. The increase of  
23 DHT time produced a starch with higher carbonyl content and bigger granule size. It also  
24 reduced the water absorption index, increased the water solubility index, affected granule  
25 crystallinity and reduced molecular size. The longest storage period increased gel  
26 firmness. Increasing the temperature used in the gelatinization process reduced the gel  
27 strength of the native and DHT\_2h. DHT\_4h showed the lowest peak apparent viscosity  
28 and provided the strongest gels for all the evaluated conditions. Gels produced with DHT  
29 starches exhibited better printability than the native starch, mainly for the DHT\_4h. This  
30 treatment was chosen to print 3D stars, and displayed better resolution than the native  
31 gels. Therefore, by using DHT, it was possible to obtain hydrogels with enhanced pasting

32 properties, gel texture, and printability, thereby expanding the potential of applying  
33 cassava starch to 3D printing.

34 **Keywords:** starch modification, novel ingredients, starchy gels, printability, 3D food  
35 printing.

## 36 1. Introduction

37 Three-dimensional (3D) printing is an emerging technology in the food industry,  
38 with interesting possibilities. It consists in using computer-aided design (CAD) software  
39 to instruct a digital fabricating machine to shape 3D objects by the successive addition of  
40 material layers (Brunner, Delley, & Denkel, 2018). It is an interesting technique for  
41 overcoming certain common constraints and expanding possibilities in food processing.  
42 For example, Severini & Derossi, (2016) stated that 3D printing can produce nutritional  
43 meals personalized for each person, based on individual age, physical condition, and  
44 nutrition and energy requirements. Indeed, specific compositions, shapes and properties  
45 can be obtained that can be useful for specific diets such as those for the elderly, children  
46 and persons with special needs. Consequently, numerous studies on 3D-food printing  
47 have recently demonstrated the value of this technology in the food industry (Azam &  
48 Zhang, 2018; Chen, 2016; Godoi, Prakash, & Bhandari, 2016; Liu, Liang, Saeed, Lan, &  
49 Qin, 2019; Liu, Bhandari, Prakash, & Zhang, 2018; Yang, Guo, Zhang, Bhandari, & Liu,  
50 2019).

51 However, 3D-printing technology has generated new challenges for the food  
52 industry. In fact, it is necessary to better understand the relationships among the possible  
53 composition, structure (in different scales) and the obtained properties in order to develop  
54 3D-printed products with the desired characteristics (sensorial, physic-chemical,  
55 nutritional, etc). Therefore, most of the studies in the literature focused on factors  
56 influencing the rheology and printability of food material, with greater demand for new  
57 ingredients and formulations. Novel ingredients are needed to achieve this goal, in  
58 particular hydrogels that provide good printability, and 3D products with well-defined  
59 geometries and good resolution.

60 Starches, in particular modified ones, can be used to produce hydrogels with  
61 possible applications for 3D printing (Maniglia et al., 2019). Starch is widely used as a  
62 thickening and gelling agent in many food formulations to improve the convenience and  
63 quality of final products due to its gelatinization properties (Zheng, 2018). Starch-based  
64 materials have specific characteristics such as biodegradability, biocompatibility and non-  
65 toxicity, which are attractive for applications ranging from the food industry to material  
66 engineering (Llanos & Tadini, 2018). However, although simple and cheap, native

67 starches do not necessarily have the requisite properties to provide the application desired.  
68 As a result, starches are generally modified for industrial applications.

69 One interesting source of starch is cassava, a major ingredient used in the food  
70 industry. Brazil, one of the countries involved in this work, is the world's fifth largest  
71 producer of cassava, with 18.9 million tons produced in 2017 (FAO - Food and  
72 Agriculture Organization, 2019). Cassava presents positive characteristics because it is a  
73 starch-rich tuberous gluten-free root (interesting for people with celiac disease) (Liu et al.,  
74 2019), and highly tolerant to drought and harsh climatic conditions (Alves, 2002). It is  
75 available around-the-year, and it is considered as a reliable crop for food supply security  
76 (Liu et al., 2019; Zhu, 2015). Cassava starch shows high potential for application and it  
77 has been investigated by many researchers (Díaz, Dini, Viña, & García, 2018; Liu et al.,  
78 2019; Matta Junior, Castanha, dos Anjos, Augusto, & Sarmento, 2019). However, cassava  
79 starch is known for producing relatively “weak” gels that do not maintain their shape after  
80 being removed from the mold (Maniglia et al. 2019). Consequently, its modification is  
81 necessary for applications requiring strong gels.

82 Dry heating treatment (DHT) involves a simple physical technique to modify the  
83 structural and physicochemical properties of starch (Alcázar-alay, Angela, & Meireles,  
84 2015). DHT consists of heating samples at temperatures between 110–150 °C for 1–4 h,  
85 keeping the starch moisture level low (<10% w/w) (Oh, Bae, & Lee, 2018). The  
86 advantage of this treatment is that it is simple and safe (no chemical reagents are used). It  
87 does not generate effluents to be treated and the products obtained do not present traces of  
88 chemical components. Therefore, DHT is considered a “green technology” and bears a  
89 “clean label”. For these reasons, different studies have recently been performed to  
90 evaluate the effect of DHT on starch and, more specifically, starch and polysaccharide  
91 mixtures (Chandanasree, Gul, & Riar, 2016; Ji, Yu, Xu, & Zhang, 2016; Liu, Hao, Chen,  
92 & Gao, 2019; Pramodrao & Riar, 2014; Sun, Gong, Li, & Xiong, 2014; Zou, Xu, Tian, &  
93 Li, 2019).

94 Chandanasree, Gul, & Riar (2016) evaluated the effect of hydrocolloids and dry  
95 heating on the characteristics of cassava starch. The authors showed that DHT is capable  
96 of modifying the physicochemical, thermal, pasting and morphological properties of  
97 cassava starch. However, the final properties of the hydrogels produced with cassava

98 starch modified by DHT have not been studied. Indeed, very few works in the literature  
99 have focused in the final properties of the hydrogels obtained from pure starch processed  
100 by DHT (instead of flours and starch mixtures with other polysaccharides).

101 Our research team recently published a work involving cassava starch  
102 modification by ozone processing to obtain hydrogels with improved 3D printing  
103 properties (Maniglia et al., 2019). Investigations into the use of modified starches for this  
104 purpose are just beginning, and they augur the expansion of starch gel applications.  
105 Consequently, we believe DHT can be an interesting alternative to obtain starch hydrogels  
106 for the purpose of 3D printing.

107 In this context, the aim of this study was to investigate the effect of DHT and  
108 gelatinization conditions of cassava starch on its physical properties, focusing on its  
109 application for 3D printing. This work first dealt with the effect of dry heating treatment  
110 on starch structure (granule and molecular) and properties. Then, the hydrogels were  
111 evaluated for their paste and gel properties, including their printability, and the effects of  
112 gelatinization conditions and storage period were evaluated.

113

## 114 **2. Material and methods**

### 115 **2.1 Material and dry heating treatment (DHT)**

116 Regular native cassava starch (Amilogill 1500, moisture: 13.2 g/100 g, amylose  
117 content: 28.9 g/100 g and amylopectin content: 71.1 g/100 g) was provided by Cargill  
118 Agrícola - Brazil. All the chemicals were of analytical grade.

119 The DHT was based on the work of Chandanasree, Gul, & Riar (2016). A thin  
120 layer (~ 1 mm) of cassava starch (50 g) was distributed over a sheet of aluminum foil and  
121 covered with another sheet of aluminum foil. The lateral parts were closed with tape to  
122 form a closed envelope in order to avoid loss of material. The packed starch was then  
123 placed in a hot-air convective oven (MA 035, Marconi, São Paulo, Brazil), in which DHT  
124 was performed using air at 130 °C (air velocity  $1 \pm 0.1 \text{ m}\cdot\text{s}^{-1}$ ) for 2 and 4 h (named  
125 DHT\_2h and DHT\_4h, respectively), following the conditions described by  
126 Chandanasree, Gul, & Riar (2016). After processing, the starch was cooled and stored in  
127 glass containers for further analysis.

128

129 **2.2 Starch characterizations**

130 **2.2.1 Granule characterization: morphology, size distribution and crystallinity**

131 The starch granules (native as control and modified) were observed using a light  
132 microscope (model L1000, Bioval, Brazil; with a 20 W halogen lamp, magnification of 40  
133 x) and a digital camera of 5.1 megapixels (ECMOS C-Mount, Sony, Japão). The starch  
134 granules were dispersed in distilled water (1:1, v/v) and placed on a glass slide, which was  
135 covered by a glass cover slip and evaluated directly. A polarized light filter coupled to the  
136 system was used to observe the Maltese crosses.

137 Particle size distribution was determined using a Laser Analyser (Partica LA-  
138 950V2 Laser Particle Size Analyser HORIBA, Japan) and the LA-950 software for  
139 Windows (HORIBA, Japan). The samples were dispersed in ethanol (99.5%) and placed  
140 in an ultrasound bath for 5 min prior to analysis. The analysis was carried out eight times  
141 for each sample.

142 From the data obtained, the volume-based mean diameter ( $D[4,3]$ , Equation 1),  
143 and the area-based mean diameter ( $D[3,2]$ , Equation 2; where  $n_i$  = the number of particles  
144 with diameter  $d_i$ ), were calculated for each sample. Diameters  $d(0.1)$ ,  $d(0.5)$ , and  $d(0.9)$   
145 were also obtained, denoting the reduction in size, 10%, 50% and 90% respectively, of the  
146 particles.

147 
$$D[4,3] = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^3}$$
  
148 (1)

149 
$$D[3,2] = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2}$$
  
150 (2)

151 An X-ray diffractometer (Shimadzu XRD 7000, Tokyo, Japan) with copper  
152 radiation was used to evaluate granule crystallinity, using the following working  
153 conditions: angle  $2\theta$  ranging from 3 to  $40^\circ$ , scan rate of  $2^\circ \cdot \text{min}^{-1}$ , 40 kV and 30 mA. The  
154 starch samples were maintained in a desiccator containing saturated  $\text{BaCl}_2$  solution (25  
155  $^\circ\text{C}$ ,  $a_w = 0.900$ ) for 10 days to ensure constant water activity. Three runs for each sample  
156 were analyzed. The curves obtained were smoothed using the Origin software, version  
157 2018 (Microcal Inc., Northampton, MA, USA). The relative crystallinity was calculated

158 as the ratio of upper diffraction peak area to the total diffraction area, following the  
159 method described by (Nara & Komiya, 1983) and considering 2θ ranging from 3 to 36°.

160

### 161 **2.2.2 Molecular characterization: pH, molecular size distribution, carbonyl** 162 **and carboxyl groups**

163 All the analyses executed on this item were carried out in triplicate. The starch  
164 suspension (10.7 g starch / 100 g suspension) pH was measured using a potentiometer  
165 (Tecnal, model TEC-5 mode, Piracicaba – Brazil). Carbonyl and carboxyl contents were  
166 determined according to the method described by Smith (1967) and Chattopadhyay,  
167 Singhal, & Kulkarni (1997), respectively, with small modifications.

168 The carbonyl content was determined as follows: the starch sample (2 g) was  
169 dispersed in distilled water (100 mL) and heated in a bath with boiling water under  
170 constant stirring for 30 min. The sample was cooled to 40 °C, the pH was adjusted to 3.2  
171 using HCl solution (0.1 M), and 15 mL of hydroxylamine chloride solution (25 g of  
172 hydroxylamine hydrochloride diluted in 100 mL of NaOH 0.5 M) was added. The  
173 samples were incubated at 40 °C for 4 h, and then the excess hydroxylamine was titrated  
174 with 0.1 M HCl until pH 3.2 using an automatic titrator (Plus Titrino 848, Metrohm,  
175 Herisau, Switzerland). A blank was made without the sample but following the same  
176 procedures. The carbonyl content was calculated as the amount of carbonyl groups in  
177 relation to 100 units of glucose (CO/ 100 GU) according to Equation 3:

178

$$179 \quad \frac{CO}{100 GU} = \frac{(V_B - V_S) \cdot M \cdot 0.028 \cdot 100}{W} \quad (3)$$

180  
181 Where:

182  $V_s$  = the volume of NaOH required for the ozonated samples;

183  $V_b$  = the volume of NaOH used in the blank;

184  $M$  = the molarity of NaOH;

185  $W$  = the sample mass in dry basis.

186

187 The carboxyl content was determined as follows: the starch sample (2 g) was  
188 dispersed in 25 mL of HCl solution (0.1 M) under stirring at 25 °C for 30 min. The



189 residue was filtrated using a vacuum system with a qualitative filter paper (205 µm), and  
190 then washed with 400 mL of distilled water. Next, 300 mL of distilled water was added,  
191 and the dispersion was heated in a bath with boiling water under continuous stirring for 15  
192 min. While it was still hot and under stirring, the sample was titrated with 0.01 M NaOH  
193 until pH 8.3 using an automatic titrator (Plus Titrino 848, Metrohm, Herisau,  
194 Switzerland). A blank was made with the unmodified starch but following the same  
195 procedures. Carboxyl content was expressed as the amount of carboxyl groups in relation  
196 to 100 units of glucose (COOH / 100 GU), according to Equation 4:

197

$$\frac{COOH}{100GU} = \frac{(V_s - V_b) \cdot M \cdot 0.045 \cdot 100}{W} \quad (4)$$

199 Where:

200  $V_b$  = the volume of HCl used in the blank;

201  $V_s$  = the volume of HCl required for the sample;

202  $M$  = the molarity of HCl;

203  $W$  = the sample mass in dry basis.

204

205  
206 The starch molecular size distribution profile was determined using a gel  
207 permeation chromatography system, following the method of Song & Jane (2000), with  
208 some modifications. The system consisted of a glass column (GE Healthcare – 28988950  
209 XK 27/70) packed with Sepharose CL-2B gel (Sigma, Sweden). Firstly, a solution with  
210 0.1 g of starch with 10 mL of Dimethylsulfoxide (DMSO; 90%, Labsynth, Brazil) was  
211 prepared and heated in a bath with boiling water (1 h). Then, it was kept at 25 °C for 24 h  
212 under constant stirring. Next, 3 mL of this starch solution was mixed with 10 mL of  
213 absolute ethanol and centrifuged (30 min, 3000 g). The precipitated starch was dissolved  
214 in water (9 mL) and heated in a bath with boiling water (30 min). This starch solution (4  
215 mL) was added in the chromatographic column with an eluent solution (25 mmol·L<sup>-1</sup> of  
216 NaCl and 1 mmol·L<sup>-1</sup> of NaOH), at a rate of 1 mL·min<sup>-1</sup>. Aliquots of 4 mL were collected  
217 using a fraction collector (Gilson, model FC203B, Middleton, England) and then analyzed  
218 by the blue value method (Juliano, 1971), using a spectrophotometer at 620 nm of

219 wavelength (Femto, model 600S, São Paulo - Brazil). Glucose was used as marker to  
220 indicate the end of the analysis.

### 221 **2.2.3 Water absorption index (WAI) and water solubility index (WSI)**

222 The water absorption (WAI) and solubility (WSI) indexes were determined  
223 according to the methodology described by Anderson, Conway, & Peplinski (1970), with  
224 some modifications. To this end, 0.5 g (dry basis) of the material (MS, in Equations 5 and  
225 6) was weighed in a centrifuge tube, which was followed by the addition of 6 mL of  
226 distilled water. The suspension was heated at 65, 75, 85, and 95 °C in a water bath for 30  
227 min. The suspension was then centrifuged at 3000 × g for 10 min. To quantify the soluble  
228 starch fraction (SS, in Equations 5 and 6), the supernatant was removed and dried at 105  
229 °C in an oven until constant weight. The tubes containing the precipitate (PT, in Equations  
230 5 and 6) were weighed to determine the WAI, which is an indicator of starch swelling  
231 power and represents the amount of water that 1 g of insoluble starch (IS, in Equations 5  
232 and 6) is able to absorb and retain. The WAI and the WSI were calculated according to  
233 Equations (5) and (6), respectively:

$$234 \quad WAI \left( \frac{g \text{ water}}{g \text{ IS}} \right) = \frac{PT - (MS - SS)}{(MS - SS)} \quad (5)$$

$$236 \quad WSI (\%) = \frac{SS}{MS} \times 100 \quad (6)$$

### 239 **2.2.4 Pasting properties**

240 The starch pasting properties were determined using a Rapid Visco Analyzer -  
241 RVA-4 (Newport Scientific Pvt. Ltd., Australia, with ThermoLine for Windows software,  
242 version 3.0). The standard conditions for starch characterization were employed, using a  
243 suspension of 10.7 g starch / 100 g (correct to 14% moisture basis), following the  
244 procedure: keep at 50 °C for 1 min, then heat to 95 °C (6 °C.min<sup>-1</sup>), maintain at 95 °C for 5  
245 min), follow by cooling to 50 °C (6 °C.min<sup>-1</sup>), and finally maintain at 50 °C for 2 min.

246 Furthermore, the effect of the gelatinization condition on the pasting properties  
247 was also evaluated by following the same procedure, but changing the highest  
248 temperature to 65, 75, and 85 °C, instead of 95 °C.

249 According to Castanha, Matta Junior, & Augusto (2017), to eliminate any  
250 misinterpretation, the relative breakdown (RBD) and relative setback (RSB) were  
251 calculated. The Relative Breakdown (RBD) was calculated by the ratio between the  
252 values of Breakdown (BD) and the Peak Apparent Viscosity (PAV) to understand the  
253 facility of starch granule disruption. The relative setback (RSB) was calculated by the  
254 ratio between the Setback (SB) and the TAV (trough apparent viscosity) values, to  
255 understand the trend to retrogradation.

256

### 257 **2.2.5 Gel firmness**

258 Gel firmness was determined by a puncture assay using a texture analyzer TA TX  
259 Plus (Stable Micro Systems Ltd., Surrey, UK) with a load cell of 50 kgf (490 N).

260 The samples used to measure gel firmness consisted of the hydrogels produced in  
261 the RVA analysis, which were placed in plastic cups (40 mm diameter × 20 mm height)  
262 and stored for 1 and 7 days at  $5 \pm 2$  °C. The cups were kept in a desiccator with water at  
263 the bottom to avoid changes in moisture. The samples were then removed from the  
264 refrigerator and analyzed immediately to ensure a constant temperature.

265 The gels were penetrated to a distance of 4 mm using a cylindrical probe (P/0.5R)  
266 at  $1 \text{ mm} \cdot \text{s}^{-1}$ . The equipment measured the force as a function of the penetration depth. Gel  
267 firmness was evaluated by the energy required to penetrate the material (calculated by the  
268 area below the curve force versus the distance of penetration).

269 Furthermore, the effect of the gelatinization condition was evaluated by using the  
270 different **temperatures** described on the “Pasting properties” section.

271

### 272 **2.3 Hydrogel application: evaluating 3D printing**

273 Different hydrogels were produced with varying DHT **processing times**,  
274 gelatinization conditions and refrigerated storage times. They were evaluated through  
275 their potential to be used for 3D printing following two approaches.

276 Firstly, all the hydrogels were evaluated through a simple approach by extruding  
277 them with a syringe on a glass slide. **In a previous study (Maniglia et al., 2019), we**  
278 **described this analysis as good indication of the material’s printability, which had a good**  
279 **correlation with real 3D printing. The hydrogels** obtained from the RVA were stored and

280 then placed in a plastic syringe (die diameter: 1.5 mm). The gels were then extruded  
281 through the die over a glass slide (lines with ~ 6 cm), for a visual evaluation of the gel's  
282 consistency and moldability. The manual extrusion using the syringe was carried out ten  
283 times to ensure the reproducibility and veracity of this procedure. Pictures (Figure 8) were  
284 obtained based on the visual aspect that most predominated in the extruded material. This  
285 approach was used to select conditions for actual 3D printing.

286 The selected gels were then processed in a 3D printer Stampante 3D (3DRAG  
287 V1.2, Futura Elettronica, Italy). The gels were produced following the same conditions.  
288 The suspension was stirred and heated until reaching the desired gelatinization condition,  
289 then maintained in this condition for 5 min. The gels obtained were transferred to the  
290 printer syringes (60 mL), which were closed using parafilm plastic and then stored in a  
291 refrigerator ( $5 \pm 2$  °C) for 7 days (chosen condition after evaluating the other results). The  
292 syringes were then removed from the refrigerator and immediately processed in the 3D  
293 printer. A 0.8 mm diameter nozzle was used. The nozzle height was set at 18 mm, with a  
294 speed of 20 mm/s and an extrusion rate of 30 mm/s at 20 °C. The physical model (star)  
295 was created using the Repetier Host V2.0.1 and Slic3r software (Hot-World GmbH & Co.  
296 KG, Willich, Germany). The dimensions of the star shape were 5 cm x 5 cm x 2 mm  
297 (Length x Width x Height). Pictures (Figure 9) were obtained based on the visual aspect  
298 that most predominated in the printed material.

299

#### 300 ***2.4 Experimental design and Statistical analysis***

301 A completely randomized design was applied with four replicates for each  
302 processing condition. To evaluate differences, analysis of variance (ANOVA) and Tukey's  
303 test at a 5% significance level were carried out with the software *Statistic 13* (StatSoft,  
304 USA).

305

### 306 **3. Results and discussion**

#### 307 ***3.1 Starch characterizations***

##### 308 **3.1.1 Granule characterization: particle size distribution, morphology and** 309 **crystallinity**

310 Figure 1 shows the particle size distribution (A) and specific particle diameters (B)  
311 of the control and modified cassava starches. The effect of DHT enlarged the particle  
312 sizes of the granules (Figure 1A), which is consistent with the duration of the dry heating  
313 process: the D[4,3] varied from 17.8  $\mu\text{m}$  for the control starch to 19.3 and 27.3  $\mu\text{m}$   
314 DHT\_2h and DHT\_4h, respectively (Figure 1B). The dry heating process also increased  
315 the number of particles with bigger sizes considering the values  $d_{0.1}$ ,  $d_{0.5}$  and  $d_{0.9}$ .  
316 Moreover, the differences on D[4,3] were more pronounced than on D[3,2] (although both  
317 presented statistical differences).

318 According to Lopez-Sanchez et al. (2011), the D[4,3] value is more influenced by  
319 large particles, whereas D[3,2] is more influenced by the smaller ones. Therefore, the  
320 results indicate that the dry heating process led to an increase in the diameter of the larger  
321 starch granules, but had less influence on the smaller ones (which is consistent with the  
322 distribution – Figure 1A). Indeed, the moisture content of the cassava starch was reduced  
323 with the dry heating treatment (control: 13.2 g/100 g; DHT\_2h: 6.8 g/100 g and DHT\_4h:  
324 6.7 g/100 g), which indicated that part of the water content in the samples was vaporized.  
325 Therefore, the increase in granule size can be associated to a possible expansion process  
326 in the starch granules due to water vaporization. Even so, the changes in starch properties  
327 observed were not linked to the low moisture level, but to the associated thermal effect of  
328 DHT. In order to confirm it, we also performed starch drying at 35  $^{\circ}\text{C}$ , a temperature  
329 generally accepted for drying without causing changes, until the same moisture level  
330 described previously was reached. No change was observed on the starch dried this way.

331 Figure 2 shows the optical microscopy of the control and modified cassava  
332 starches using both nonpolarized and polarized light. The shapes of the cassava starch  
333 granules were polygonal, angular and truncated, as observed by Han et al. (2019).  
334 Polarized light microscopy shows all the granules with birefringent (Maltese cross is  
335 visible) and they seem to be intact. However, with higher magnification (1500 x) using  
336 scanning electron microscopy, Chandanasree et al. (2016) observed several fractures in  
337 the cassava starch modified in the same DHT conditions. Considering the fact that  
338 birefringence reflects the internal microstructure of starch granules, it shows that the dry  
339 heating treatment used did not significantly affect the internal microstructure of cassava

340 starch granules. Indeed, heat treatment lasting 2 or 4 hours did not alter the shape or  
341 promote degradation on the surface of the granules.

342 Another point is that particle aggregation was not observed (Figure 2).  
343 Consequently, the increase in particle dimensions (Figure 1) must be attributed to  
344 mechanisms other than aggregation, such as the expansion proposed. Further studies are  
345 needed to better understand this phenomenon.

346 The granule X-ray diffraction patterns are presented in Figure 3(A), and their  
347 relative crystallinity in Figure 3(B). All the samples showed peaks at 15°, 23°, 17°, and  
348 18° (2θ), representing a typical type-A starch pattern (Liu et al., 2019). In addition, no  
349 significant difference ( $p < 0.05$ ) was observed for the relative crystallinity between native  
350 starch and the DHT\_2h sample. However, the DHT\_4h sample showed significant  
351 reduction ( $p < 0.05$ ) of this parameter compared with control and DHT\_2h. Type-A starch  
352 polymorphs (with about six water molecules inside the helices) presented a more stable  
353 monoclinic structure (Chatpapamon, Wandee, Uttapap, Puttanlek, & Rungsardthong,  
354 2019); consequently, this crystalline arrangement is less prone to change by dry heating  
355 treatment. This indicates that the crystalline structure of the starch was affected only after  
356 4 h of dry heating treatment at 130 °C. Liu et al. (2019) indicated the decrease in  
357 crystallinity could be attributed to a reorientation of the double helices forming the  
358 crystalline array and/or crystallite disruption.

359 Sun, Xu, & Xiong (2014), Oh et al. (2018), and Liu et al. (2019) also observed the  
360 reduction of relative crystallinity when the DHT was applied in proso millet, high  
361 amylose rice and waxy potato starches. Therefore, DHT reduced the crystalline portion in  
362 different starch sources.

363 Therefore, the dry heating treatment changed the crystallinity (DHT\_4h) of  
364 cassava starch, but without bigger changes in the granule morphology. Consequently, it  
365 was necessary to also evaluate the possible changes in starch molecules, as described in  
366 the following.

367

368 **3.1.2 Molecular characterization: pH, molecular size distribution, carbonyl and**  
369 **carboxyl contents**

370 Table 1 shows the pH, carbonyl and carboxyl contents of the native (control) and  
371 the modified cassava starches.

372 The dry heating treatment was capable of oxidizing the starch molecules,  
373 generating carbonyl groups that increased with processing time. However, this oxidation  
374 was not drastic enough to produce carboxyl groups, which indicates a difference in pH.  
375 Indeed, Kurdziel, Łabanowska, Pietrzyk, Sobolewska-Zielińska, & Michalec (2019)  
376 described that atmospheric oxygen, even though a weak oxidizing agent, can initiate  
377 oxidation to a smaller extent during thermal treatment, leading to the formation of  
378 carbonyl groups in heated starches. Chávez-Murillo, Wang, & Bello-Pérez (2008)  
379 mentioned that starch heating can lead to new functional carbonyl and carboxyl groups  
380 formed mainly at the C-6 and C-2 or C-3 carbon atoms of glucose units in the starch  
381 structure.

382 [Figure 4 shows the molecular size distribution of the control and modified cassava](#)  
383 [starches](#). The dry heating treatment reduced the size of the molecules to intermediate size  
384 (Figure 4): the second peak increased and scrolled to the right, indicating that the  
385 molecules modified by dry heating were retained longer in the chromatographic column  
386 due to their lower size. DHT was thus responsible for cleaving the links present between  
387 the glucopyranose units (Haq et al., 2019). **In addition, the DHT samples** showed a narrow  
388 peak for the large molecules (first peak), reflecting that the treatment also affected the  
389 bigger molecules.

390 As observed, the DHT promoted different molecular changes in the cassava starch:  
391 the hydroxyl groups were oxidized to carbonyl groups (Table 1), and molecular  
392 depolymerization was also observed (Figure 4). Li et al. (2013) also observed that the dry  
393 heating method can break the intra- and inter-molecular hydrogen bonds of waxy rice  
394 starch. In addition, based in the results shown in Figure 4, this depolymerization occurred  
395 mainly for the intermediate size molecules, resulting in two groups of molecules that are  
396 more defined (large and small molecules size) than those of native starch (high fraction of  
397 molecules of intermediate size).

398 The dry heating treatment was capable of changing the molecular structure of  
399 cassava starch and displayed that it is an effective treatment for starch modification.  
400 Consequently, it can also change the properties of starch, which we evaluate in the  
401 following.

402

### 403 **3.1.3 Water absorption index (WAI) and water solubility index (WSI)**

404 Table 2 shows the water solubility index (WSI) and the water absorption index  
405 (WAI) of the control and modified cassava starches.

406 The WSI and WAI increased gradually from 65 °C for all the treatments. This  
407 temperature is related to the onset of gelatinization, which promotes hydrogen bond  
408 cleavage and irreversible and progressive water absorption (Pelissari, Andrade-Mahecha,  
409 Sobral, & Menegalli, 2012). Hydrogen bond cleavage and water fixation at higher  
410 temperatures gave rise to free hydroxyl groups and allow starch granules to continue  
411 swelling, thereby increasing starch solubility (Limberger, Da Silva, Emanuelli, Comarela,  
412 & Patias, 2008).

413 Starches treated by DHT showed lower WAI than the control at all the  
414 temperatures evaluated, and this became more pronounced as the processing time  
415 increased. This result can be associated with the starch depolymerization promoted by  
416 DHT (Figure 4). DHT reduced the capacity of the starch to maintain its integrity due to  
417 the weakening of the links between starch chains, whose glycosidic bonds were cleaved,  
418 leading to the reduction of starch granule swelling during gelatinization (Liu et al., 2019).  
419 The WSI was higher for the starches treated by DHT than the control, and it also became  
420 more pronounced as processing time increased. The molecular cleavage promoted by  
421 DHT easily disrupted the starch granules, especially at higher temperatures. Therefore, the  
422 longer the DHT time, the greater the molecular breakdown and, therefore, the higher the  
423 solubility of the granules.

424

### 425 **3.1.4 Pasting properties**

426 Figure 5 shows the RVA curves and Table 3 shows the associated pasting  
427 parameters in different **gelatinization conditions** (65, 75, 85, and 95 °C). The peak  
428 apparent viscosity was higher for the native starch than those treated by dry heating,



429 whatever the **gelatinization conditions**. In addition, a more significant reduction in the  
430 peak apparent viscosity was observed as processing time increased.

431 When the aqueous slurry of starch is heated, the granules can absorb water and  
432 swell; the swollen granules rub against each other under stirring in RVA, increasing the  
433 apparent viscosity (Hu et al., 2018). The peak viscosity represents the maximum  
434 resistance during gelatinization, demonstrating the swelling of the starch granules before  
435 disintegration (Gou et al., 2019). In Figure 5, the behavior observed indicates that the  
436 starch granules treated by DHT showed a reduction in their capacity to maintain their  
437 integrity. This was due to the weakening of the associations between starch chains, whose  
438 glycosidic bonds were cleaved (molecular depolymerization, Figure 4), thereby reducing  
439 the swelling power (WAI in the Table 2).

440 Similar behavior can be found in the literature. A reduction of the peak and final  
441 viscosities of millet, pea and corn starches was observed by Sun et al. (2014) and Sun,  
442 Sun, & Xiong (2013) after DHT at 130 °C for 2–4 h. Gou et al. (2019) also observed the  
443 reduction of the peak and final viscosities for sweet potato heat treated at 130 °C for 18 h.

444 The trough and final apparent viscosities showed similar tendencies to the peak  
445 viscosity, decreasing significantly ( $p < 0.05$ ) as exposure time increased. The same  
446 behavior was observed by (Sun et al., 2013) working with DHT on pea starch.

447 The relative breakdown (RB) increased according to the time of exposure to heat.  
448 This indicates that the new intermolecular association produced by DHT becomes easier  
449 when compared with the native starches.

450 The relative setback viscosity (RSB) is related to the potential for retrogradation in  
451 starches, and a higher RSB represents a greater chance for retrogradation (Gou et al.,  
452 2019). The RSB values of the DHT\_4h starch were higher than the control and the  
453 DHT\_2h starches (Table 3). Moreover, for the control and the DHT\_2h starches,  
454 increasing gelatinization temperature (up to 75 °C) reduced the RSB values; however, the  
455 variation of the temperature did not affect this parameter of the DHT\_4h starch.  
456 Therefore, the dry heating treatment and the gelatinization conditions cooperated  
457 differently regarding the retrogradation phenomenon. This can be explained by molecular  
458 depolymerization. As discussed before, the dry heating treatment led to a reduction of  
459 molecular size, in particular that of molecules of intermediate size, forming two well-

460 defined groups with larger and smaller molecules. Similar molecule sizes are more  
461 suitable for re-association due to better pairing; also, smaller molecules have higher  
462 mobility to re-associate (Castanha et al., 2017), explaining the behavior observed.

463 However, in addition to the changes in molecular size distribution (Figure 4), the  
464 starches processed using DHT showed new intermolecular associations due to the  
465 replacement of hydroxyl groups by carbonyl groups (Table 1). Consequently, other  
466 parameters also varied.

467 DHT for 2 h did not change the pasting temperature. However, the DHT\_4h  
468 samples showed slightly higher pasting temperatures (when the **gelatinization conditions**  
469 **were** 65, 75, or 85 °C) than the other samples. This might result in the presence of smaller  
470 molecules that need a higher energy level to gelatinize than large size molecules  
471 (Castanha et al., 2017).

472 It is interesting to note that **the behaviors of pasting properties of the DHT starches**  
473 **were similar to that observed for cassava starch modified by ozone in our last work**  
474 **(Maniglia et al., 2019). By comparison, the DHT modified starch method has the**  
475 **advantage of being simpler than the ozone method. However, the use of heating in the**  
476 **DHT implies an energy cost that must be evaluated in each case.**

477 **The results indicate that this method can produce starches with good properties for**  
478 **forming a strong gel and good printability. However, to confirm this, it is necessary to**  
479 **also evaluate the changes occurring in the hydrogels obtained. This was carried out as**  
480 **follows.**

481

### 482 **3.1.5 Gel firmness**

483 Figure 6 shows the gel firmness produced by the different DHT conditions,  
484 **gelatinization conditions** and storage periods at 5 °C. Figure 7 shows a photograph of each  
485 gel.

486 Dry heating treatment resulted in stronger hydrogels: for all the **gelatinization** and  
487 storage conditions, the starch processed through DHT for 4 h resulted in stronger gels  
488 when compared with the control and that processed for 2 h. The gels produced by  
489 DHT\_2h starch did not result in a behavior as pronounced as the DHT\_4h, but the gels  
490 were stronger than those produced by control starch. This is also highlighted in Figure 7.

491 This result is important as stronger gels are in general preferable in industrial applications  
492 – in particular considering 3D printing applications.

493 When gelatinized starch paste is subjected to cooling, the extent of the viscosity  
494 increase is mainly governed by the rapid re-association of linear amylose chains via the  
495 formation of a molecular matrix (Gunaratne & Corke, 2007). Starch retrogradation is not  
496 favored by chains that are too short or too long; there is an optimum size at which this  
497 process becomes favorable (Wang, Li, Copeland, Niu, & Wang, 2015). Therefore, even  
498 considering the molecular cleavage, the balance between molecular size and interactions  
499 (through the changes of hydroxyl to carbonyl groups) of DHT\_4h starch gave more  
500 favorable results with the formation of stronger gels. In another experiment conducted by  
501 our research group (Maniglia et al., 2019), we observed similar behavior for cassava  
502 starch processed by ozone technology: by changing the ozonation process conditions, both  
503 weaker and stronger gels could be obtained. In that case, the molecular interaction  
504 changes as a result of depolymerization and the presence of both carbonyl and carboxyl  
505 groups.

506 The gels processed by DHT were stronger after 7 days of refrigerated storage  
507 (Figure 6 and 7), which did not occur with the native starch. According to Wang et al.  
508 (2015), retrogradation is an ongoing process, which initially involves the rapid  
509 recrystallization of amylose molecules (short-term retrogradation) followed by the slow  
510 recrystallization of amylopectin molecules (long-term retrogradation). This behavior is  
511 expected to be more complex in modified starches, since both amylose and amylopectin  
512 change their size and interaction due to the newly formed carbonyl groups. The starches  
513 processed by DHT showed greater uniformity in the size of the smaller and larger  
514 molecules, although separated into two groups (Figure 4). Therefore, the association of  
515 the smaller (amylose) and larger (amylopectin) molecules was more effective for the  
516 modified starch, favoring short and long-term retrogradation.

517 The increase in hydrogel firmness is an important result, possibly reflecting the  
518 better formation of defined shapes during 3D printing – which is particularly important  
519 for cassava starch. Therefore, the 3D printing potential was evaluated.

520

### 521 **3.2 Hydrogel application: evaluating 3D printing potential**

522 Gel printability directly determines whether food can be produced from 3D  
523 printing and handled with dimensional stability capable of not spreading on surfaces, and  
524 of supporting its own weight during layer-by-layer deposition (Godoi et al., 2016; Liu et  
525 al., 2019).

526 Figure 8 shows images of the simulated printing, considering different dry heating  
527 treatment times, **gelatinization conditions and storage periods**. In general, all the gels  
528 produced could be extruded by a syringe and produce a straight line. However, different  
529 trends were observed when attempting to obtain a defined shape, which is important to  
530 ensure a good resolution of the printed object.

531 In all the **gelatinization conditions**, the DHT\_4h starch showed more defined lines  
532 with a thinner aspect, indicating that these gels presented stronger consistency (agreeing  
533 with the results obtained for the gel firmness), spreading less on the surface. On the other  
534 hand, the native starch appeared less adapted for 3D printing application when the gel was  
535 produced at temperatures up to 65 °C. Indeed, the “lines” produced by such gels were  
536 larger and spread more on the surface than the other gels. Indeed, cassava starch is known  
537 to produce relatively “weak” gels that do not maintain their shape after being removed  
538 from the mold.

539 The higher storage period (7 days) led to more defined lines that spread less. These  
540 results are in accordance with the gel strengths obtained, which indicates that this property  
541 is adequate for evaluating hydrogel printability.

542 In the conditions evaluated, DHT\_4h starch appeared as the best candidate for 3D  
543 printing application, as it could be used as a gelling agent. To evaluate if the dry heating  
544 treatment really improved the printability of this gel, star-shaped products were printed by  
545 a 3D printer using DHT\_4h gels produced at 65 and 95 °C. Figure 9 shows the result  
546 obtained, in comparison with the control (native starch) gel produced at the same  
547 temperatures.

548 In **Figure 9**, we can conclude that when the gels were produced using the lowest  
549 gelatinization temperature condition (65 °C), it was possible to print stars with both gels.  
550 However, it can be seen that the star produced with DHT\_4h gels gives higher resolution  
551 (well-defined star tips). Comparing the moldability of the gels produced in the highest

552 gelatinization temperature condition (95 °C), the control starch did not provide good  
553 printability, and spread over the surface. On the other hand, the DHT\_4h gels produced at  
554 this temperature showed good printability, resulting in a star with the best resolution.

555 Therefore, our results are very interesting since gels with lower apparent viscosity  
556 and the ability to form stronger gels can be obtained by altering the DHT process  
557 conditions. These characteristics imply that the use of cassava starch can be expanded to  
558 industrial applications, such as 3D food printing.

559 For 3D food printing, a material of sufficient viscosity is desirable for easy  
560 extrusion through a small nozzle and high enough to be cohesive without deformation as  
561 layers are deposited above (Chen, Xie, Chen, & Zheng, 2019).

562 Indeed, this work produced modified cassava starch with enhanced properties,  
563 bearing a “clean label”, using simple and safe physical treatment (DHT), that can be used  
564 as an ingredient for 3D printing.

565

#### 566 **4. Conclusion**

567 This work demonstrated that the simple and efficient technique of DHT can  
568 expand the application of cassava starch, in particular for 3D printing.

569 Using DHT to modify the molecular and granular characteristics of cassava,  
570 resulted in the reduction of the peak apparent viscosity and increased gel firmness. The  
571 temperature used to promote gelatinization and the storage period were parameters that  
572 affected each starch treatment and their hydrogels differently. Gels produced with starch  
573 treated by DHT for 4 h resulted in stronger hydrogels for all the gelatinization conditions  
574 (65, 75, 85, and 95 °C), notably in the case of storage for 7 days. Gel firmness was  
575 consistent with the profile of gel printability. Indeed, DHT\_4h treatment produced  
576 hydrogels with good printability, resulting in 3D products with well-defined geometries  
577 and good resolution.

578 Therefore, using DHT, we could obtain cassava starch with new and potentially  
579 interesting properties, thereby expanding the potential of using cassava starch in 3D  
580 printing.

581

#### 582 **5. Acknowledgements**

583 The authors are grateful to:

- 584 • the Région Pays de la Loire (France) / RFI “FOOD 4 TOMORROW” for funding the  
585 post-doctoral fellowship “STARCH-3D” of B. Maniglia;
- 586 • the São Paulo Research Foundation (FAPESP, Brazil) for funding project n°  
587 2016/18052-5 and 2019/05043-6;
- 588 • the National Council for Scientific and Technological Development (CNPq, Brazil)  
589 for funding the productivity grant of P.E.D. Augusto (306557/2017-7);
- 590 • the Technology Center of Cereal and Chocolate from the Food Technology Institute  
591 (ITAL, Brazil), in the name of Dr. Izabela Dutra Alvim, for the particle size  
592 distribution analysis;
- 593 • the “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil  
594 (CAPES)” - Finance Code 001, through the D.C. Lima Ph.D. scholarship for  
595 providing partial funding;
- 596 • Anthony OGE from ONIRIS-GEPEA for technical support in 3D printing  
597 applications.

598

## 599 **6. References**

- 600 Alcázar-alay, S. C., Angela, M., & Meireles, A. (2015). Physicochemical properties,  
601 modifications and applications of starches from different botanical sources. *Food*  
602 *Science and Technology*, 35(2), 215–236. <https://doi.org/10.1590/1678-457X.6749>
- 603 Alves, A. A. C. (2002). Cassava botany and physiology. *Cassava: Biology, Production*  
604 *and Utilization*, 1, 67–89.
- 605 Anderson, R. A., Conway, Hf., & Peplinski, A. J. (1970). Gelatinization of corn grits by  
606 roll cooking, extrusion cooking and steaming. *Starch-Stärke*, 22(4), 130–135.
- 607 Azam, S. M. R., & Zhang, M. (2018). Study on 3D printing of orange concentrate and  
608 material characteristics. *Journal of Food Process Engineering*, 41(5), 1–10.  
609 <https://doi.org/10.1111/jfpe.12689>
- 610 Brunner, T. A., Delley, M., & Denkel, C. (2018). Consumers’ attitudes and change of  
611 attitude toward 3D-printed food. *Food Quality and Preference*, 68, 389–396.  
612 <https://doi.org/10.1016/j.foodqual.2017.12.010>
- 613 Castanha, N., Matta Junior, M. D. da, & Augusto, P. E. D. (2017). Potato starch

614 modification using the ozone technology. *Food Hydrocolloids*, 66, 343–356.  
615 <https://doi.org/10.1016/j.foodhyd.2016.12.001>

616 Chandanasree, D., Gul, K., & Riar, C. S. (2016). Effect of hydrocolloids and dry heat  
617 modification on physicochemical, thermal, pasting and morphological characteristics  
618 of cassava (*Manihot esculenta*) starch. *Food Hydrocolloids*, 52, 175–182.

619 Chatpapamon, C., Wandee, Y., Uttapap, D., Puttanlek, C., & Rungsardthong, V. (2019).  
620 Pasting properties of cassava starch modified by heat-moisture treatment under  
621 acidic and alkaline pH environments. *Carbohydrate Polymers*, 215, 338–347.  
622 <https://doi.org/10.1016/j.carbpol.2019.03.089>

623 Chattopadhyay, S., Singhal, R. S., & Kulkarni, P. R. (1997). Optimisation of conditions of  
624 synthesis of oxidised starch from corn and amaranth for use in film-forming  
625 applications. *Carbohydrate Polymers*, 34(4), 203–212.  
626 [https://doi.org/10.1016/S0144-8617\(97\)87306-7](https://doi.org/10.1016/S0144-8617(97)87306-7)

627 Chávez-Murillo, C. E., Wang, Y., & Bello-Pérez, L. A. (2008). Morphological,  
628 Physicochemical and Structural Characteristics of Oxidized Barley and Corn  
629 Starches. *Starch - Stärke*, 60(11), 634–645. <https://doi.org/10.1002/star.200800016>

630 Chen, H., Xie, F., Chen, L., & Zheng, B. (2019). Effect of rheological properties of  
631 potato, rice and corn starches on their hot-extrusion 3D printing behaviors. *Journal*  
632 *of Food Engineering*, 244, 150–158. <https://doi.org/10.1016/j.jfoodeng.2018.09.011>

633 Chen, Z. (2016). Research on the Impact of 3D Printing on the International Supply  
634 Chain. *Advances in Materials Science and Engineering*, 1–16.  
635 <https://doi.org/10.1155/2016/4173873>

636 Díaz, A., Dini, C., Viña, S. Z., & García, M. A. (2018). Technological properties of sour  
637 cassava starches: Effect of fermentation and drying processes. *LWT - Food Science*  
638 *and Technology*, 93, 116–123. <https://doi.org/10.1016/j.lwt.2018.03.029>

639 FAO - Food and Agriculture Organization. (2019). Production crop data. Retrieved  
640 September 23, 2019, from <http://www.fao.org/faostat/en/#data/QC/visualize>

641 Godoi, F. C., Prakash, S., & Bhandari, B. R. (2016). 3d printing technologies applied for  
642 food design: Status and prospects. *Journal of Food Engineering*, 179, 44–54.  
643 <https://doi.org/10.1016/j.jfoodeng.2016.01.025>

644 Gou, M., Wu, H., Saleh, A. S. M., Jing, L., Liu, Y., Zhao, K., ... Li, W. (2019). Effects of

645 repeated and continuous dry heat treatments on properties of sweet potato starch.  
646 *International Journal of Biological Macromolecules*, 129, 869–877.  
647 <https://doi.org/10.1016/j.ijbiomac.2019.01.225>

648 Gunaratne, A., & Corke, H. (2007). Effect of hydroxypropylation and alkaline treatment  
649 in hydroxypropylation on some structural and physicochemical properties of heat-  
650 moisture treated wheat, potato and waxy maize starches. *Carbohydrate Polymers*,  
651 68(2), 305–313. <https://doi.org/10.1016/j.carbpol.2006.12.004>

652 Han, H., Hou, J., Yang, N., Zhang, Y., Chen, H., Zhang, Z., ... Guo, S. (2019). Insight on  
653 the changes of cassava and potato starch granules during gelatinization. *International*  
654 *Journal of Biological Macromolecules*, 126, 37–43.  
655 <https://doi.org/10.1016/j.ijbiomac.2018.12.201>

656 Haq, F., Yu, H., Wang, L., Teng, L., Haroon, M., Khan, R. U., ... Nazir, A. (2019).  
657 Advances in chemical modifications of starches and their applications. *Carbohydrate*  
658 *Research*, 476, 12–35. <https://doi.org/10.1016/j.carres.2019.02.007>

659 Hu, X., Guo, B., Liu, C., Yan, X., Chen, J., Luo, S., ... Wu, J. (2018). Modification of  
660 potato starch by using superheated steam. *Carbohydrate Polymers*, 198, 375–384.  
661 <https://doi.org/10.1016/j.carbpol.2018.06.110>

662 Ji, Y., Yu, J., Xu, Y., & Zhang, Y. (2016). Impact of dry heating on physicochemical  
663 properties of corn starch and lysine mixture. *International Journal of Biological*  
664 *Macromolecules*, 91, 872–876. <https://doi.org/10.1016/j.ijbiomac.2016.06.040>

665 Juliano, B. O. (1971). A simplified assay for milled-rice amylose. *Cereal Sci. Today*, 16,  
666 334–340.

667 Kurdziel, M., Łabanowska, M., Pietrzyk, S., Sobolewska-Zielińska, J., & Michalec, M.  
668 (2019). Changes in the physicochemical properties of barley and oat starches upon  
669 the use of environmentally friendly oxidation methods. *Carbohydrate Polymers*, 210,  
670 339–349. <https://doi.org/10.1016/j.carbpol.2019.01.088>

671 Li, Y., Zhang, H., Shoemaker, C. F., Xu, Z., Zhu, S., & Zhong, F. (2013). Effect of dry  
672 heat treatment with xanthan on waxy rice starch. *Carbohydrate Polymers*, 92(2),  
673 1647–1652. <https://doi.org/10.1016/j.carbpol.2012.11.002>

674 Limberger, V. M., Da Silva, L. P., Emanuelli, T., Comarela, C. G., & Patias, L. D. (2008).  
675 Modificação química e física do amido de quirera de arroz para aproveitamento na



676 indústria de alimentos. *Química Nova*, 31(1), 84–88. <https://doi.org/10.1590/S0100->  
677 40422008000100018

678 Liu, K., Hao, Y., Chen, Y., & Gao, Q. (2019). Effects of dry heat treatment on the  
679 structure and physicochemical properties of waxy potato starch. *International*  
680 *Journal of Biological Macromolecules*, 132, 1044–1050.  
681 <https://doi.org/10.1016/j.ijbiomac.2019.03.146>

682 Liu, R., Sun, W., Zhang, Y., Huang, Z., Hu, H., & Zhao, M. (2019). Preparation of starch  
683 dough using damaged cassava starch induced by mechanical activation to develop  
684 staple foods: Application in crackers. *Food Chemistry*, 271, 284–290.  
685 <https://doi.org/10.1016/j.foodchem.2018.07.202>

686 Liu, Y., Liang, X., Saeed, A., Lan, W., & Qin, W. (2019). Properties of 3D printed dough  
687 and optimization of printing parameters. *Innovative Food Science & Emerging*  
688 *Technologies*, 54, 9–18. <https://doi.org/10.1016/j.ifset.2019.03.008>

689 Liu, Z., Bhandari, B., Prakash, S., & Zhang, M. (2018). Creation of internal structure of  
690 mashed potato construct by 3D printing and its textural properties. *Food Research*  
691 *International*, 111(March), 534–543. <https://doi.org/10.1016/j.foodres.2018.05.075>

692 Llanos, J. H. R., & Tadini, C. C. (2018). Preparation and characterization of bio-  
693 nanocomposite films based on cassava starch or chitosan, reinforced with  
694 montmorillonite or bamboo nanofibers. *International Journal of Biological*  
695 *Macromolecules*, 107, 371–382. <https://doi.org/10.1016/j.ijbiomac.2017.09.001>

696 Lopez-Sanchez, P., Nijssse, J., Blonk, H. C. G., Bialek, L., Schumm, S., & Langton, M.  
697 (2011). Effect of mechanical and thermal treatments on the microstructure and  
698 rheological properties of carrot, broccoli and tomato dispersions. *Journal of the*  
699 *Science of Food and Agriculture*, 91(2), 207–217. <https://doi.org/10.1002/jsfa.4168>

700 Maniglia, B. C., Lima, D. C., Junior, M. D. M., Le-Bail, P., Le-Bail, A., & Augusto, P. E.  
701 D. (2019). Hydrogels based on ozonated cassava starch: Effect of ozone processing  
702 and gelatinization conditions on enhancing printability for 3D-printing applications.  
703 *International Journal of Biological Macromolecules*, 138, 1087–1097.

704 Matta Junior, M. D., Castanha, N., dos Anjos, C. B. P., Augusto, P. E. D., & Sarmiento, S.  
705 B. S. (2019). Ozone technology as an alternative to fermentative processes to  
706 improve the oven-expansion properties of cassava starch. *Food Research*

707 *International*. <https://doi.org/10.1016/j.foodress.2019.04.050>

708 Nara, S., & Komiya, T. (1983). Studies on the Relationship Between Water-saturated State  
709 and Crystallinity by the Diffraction Method for Moistened Potato Starch. *Starch -*  
710 *Stärke*, 35(12), 407–410. <https://doi.org/10.1002/star.19830351202>

711 Oh, I. K., Bae, I. Y., & Lee, H. G. (2018). Effect of dry heat treatment on physical  
712 property and in vitro starch digestibility of high amylose rice starch. *International*  
713 *Journal of Biological Macromolecules*, 108, 568–575.  
714 <https://doi.org/10.1016/j.ijbiomac.2017.11.180>

715 Pelissari, F. M., Andrade-Mahecha, M. M., Sobral, P. J. do A., & Menegalli, F. C. (2012).  
716 Isolation and characterization of the flour and starch of plantain bananas ( *Musa*  
717 *paradisiaca* ). *Starch - Stärke*, 64(5), 382–391.  
718 <https://doi.org/10.1002/star.201100133>

719 Pramodrao, K. S., & Riar, C. S. (2014). Comparative study of effect of modification with  
720 ionic gums and dry heating on the physicochemical characteristic of potato, sweet  
721 potato and taro starches. *Food Hydrocolloids*, 35, 613–619.  
722 <https://doi.org/10.1016/j.foodhyd.2013.08.006>

723 Severini, C., & Derossi, A. (2016). Could the 3D printing technology be a useful strategy  
724 to obtain customized nutrition? *Journal of Clinical Gastroenterology*, 50(2), 175–  
725 178.

726 Smith, R. J. (1967). Production and use of hypochlorite oxidized starches. *Starch*  
727 *Chemistry and Technology*. New York: Academic Press.

728 Song, Y., & Jane, J.-L. (2000). Characterization of barley starches of waxy, normal, and  
729 high amylose varieties. *Carbohydrate Polymers*, 41(4), 365–377.  
730 [https://doi.org/10.1016/S0144-8617\(99\)00098-3](https://doi.org/10.1016/S0144-8617(99)00098-3)

731 Sun, Q., Gong, M., Li, Y., & Xiong, L. (2014). Effect of dry heat treatment on the  
732 physicochemical properties and structure of proso millet flour and starch.  
733 *Carbohydrate Polymers*, 110, 128–134.  
734 <https://doi.org/10.1016/j.carbpol.2014.03.090>

735 Sun, Q., Sun, C., & Xiong, L. (2013). Mechanical, barrier and morphological properties  
736 of pea starch and peanut protein isolate blend films. *Carbohydrate Polymers*, 98(1),  
737 630–637. <https://doi.org/10.1016/j.carbpol.2013.06.040>

738 Sun, Q., Xu, Y., & Xiong, L. (2014). Effect of microwave-assisted dry heating with  
739 xanthan on normal and waxy corn starches. *International Journal of Biological*  
740 *Macromolecules*, 68, 86–91. <https://doi.org/10.1016/j.ijbiomac.2014.04.032>

741 Wang, S., Li, C., Copeland, L., Niu, Q., & Wang, S. (2015). Starch Retrogradation: A  
742 Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety*,  
743 14(5), 568–585. <https://doi.org/10.1111/1541-4337.12143>

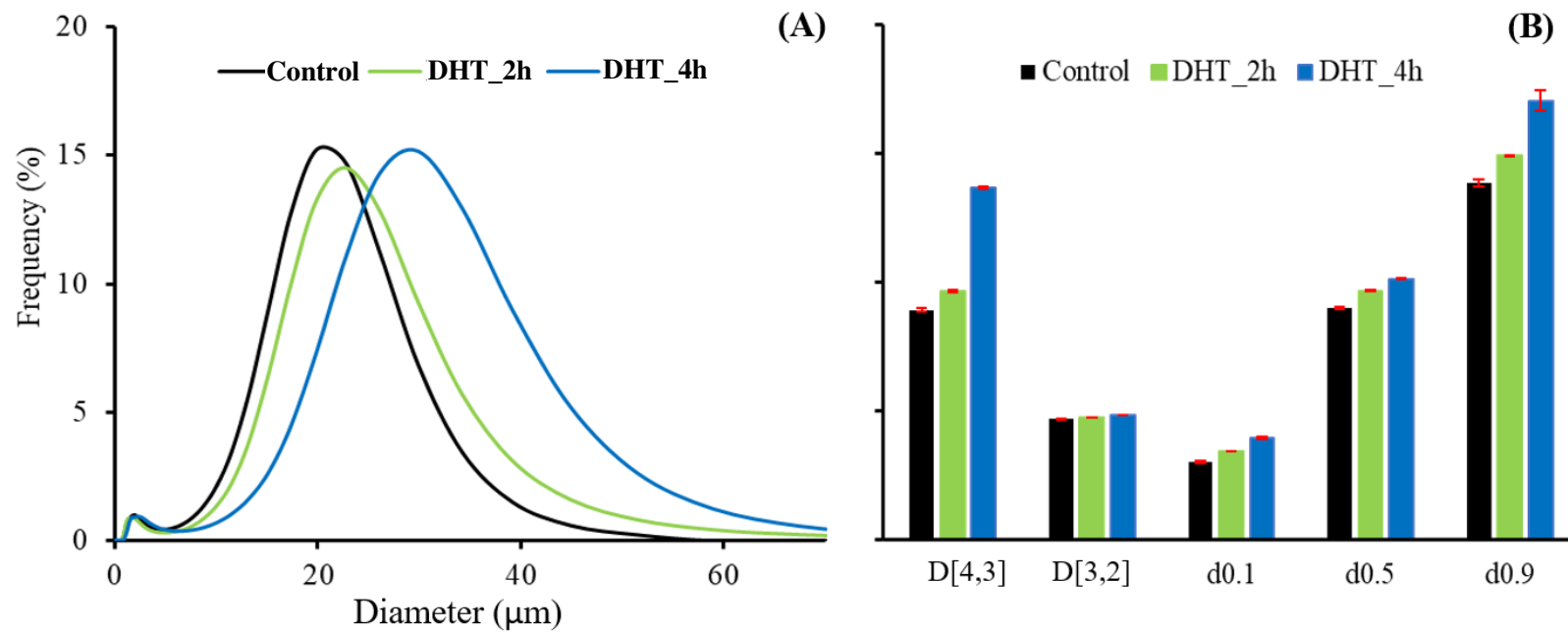
744 Yang, F., Guo, C., Zhang, M., Bhandari, B., & Liu, Y. (2019). Improving 3D printing  
745 process of lemon juice gel based on fluid flow numerical simulation. *LWT - Food*  
746 *Science and Technology*, 102, 89–99. <https://doi.org/10.1016/j.lwt.2018.12.031>

747 Zheng, H. (2018). Polymers for Structure Design of Dairy Foods. In *Polymers for Food*  
748 *Applications* (pp. 509–528). Springer.

749 Zhu, F. (2015). Composition, structure, physicochemical properties, and modifications of  
750 cassava starch. *Carbohydrate Polymers*, 122, 456–480.  
751 <https://doi.org/10.1016/j.carbpol.2014.10.063>

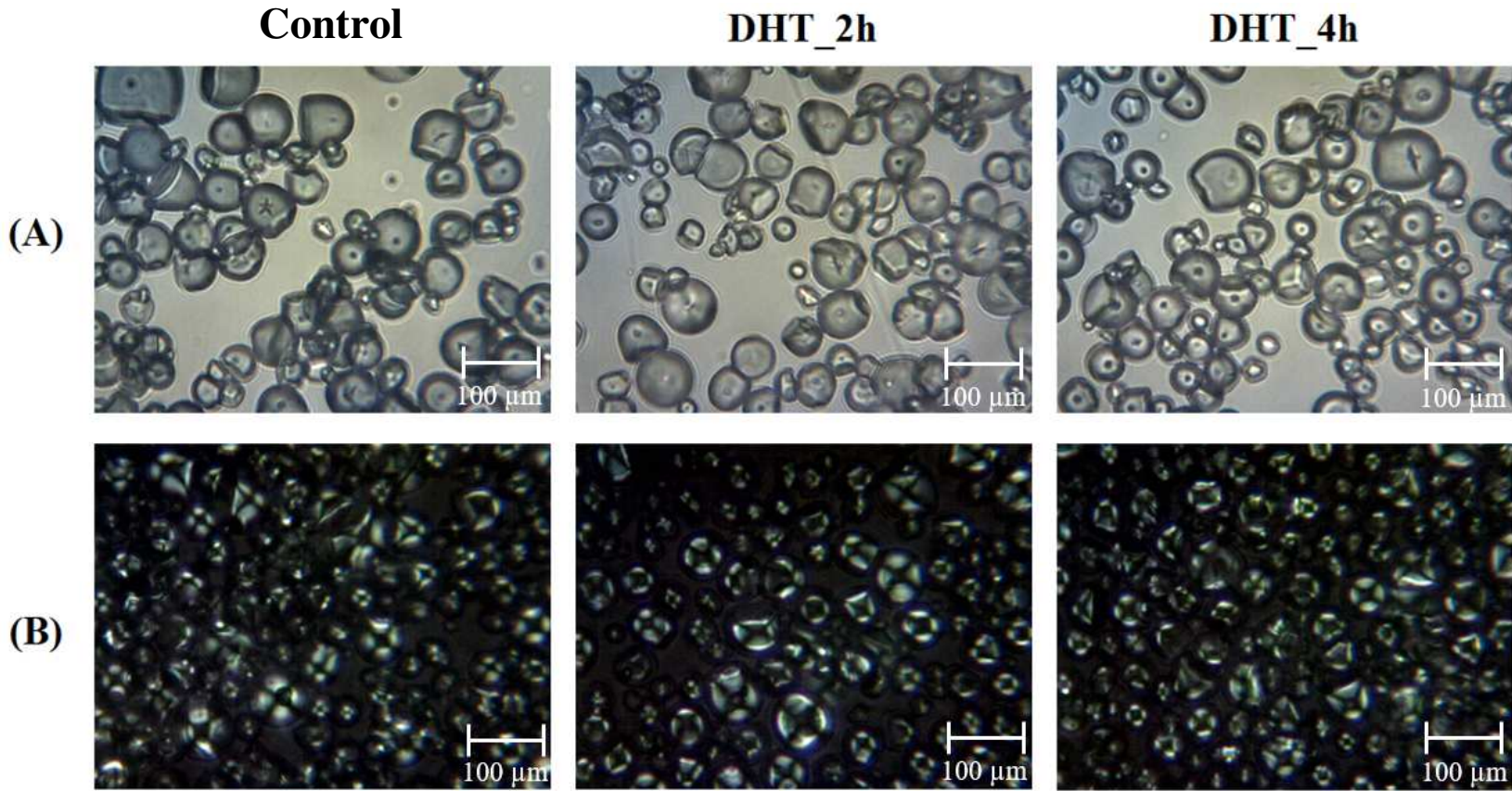
752 Zou, J., Xu, M., Tian, J., & Li, B. (2019). Impact of continuous and repeated dry heating  
753 treatments on the physicochemical and structural properties of waxy corn starch.  
754 *International Journal of Biological Macromolecules*, 135, 379–385.

755



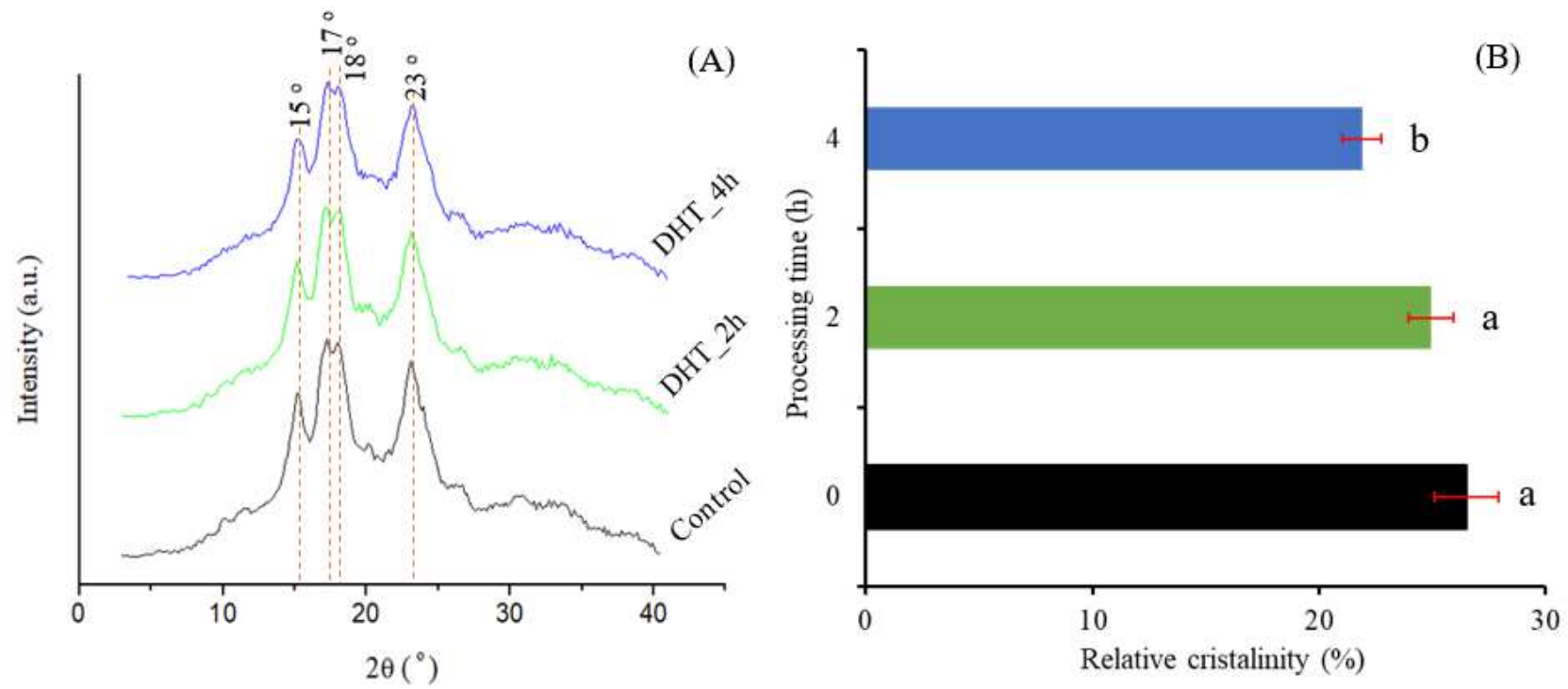
1  
2  
3

**Figure 1.** Starch granule size of the control and modified cassava starches by dry heating: (A) Particle size distribution. (B) Specific diameters (D[4,3], D[3,2], d0.1, d0.5, d0.9) – vertical red bars are the standard deviations.



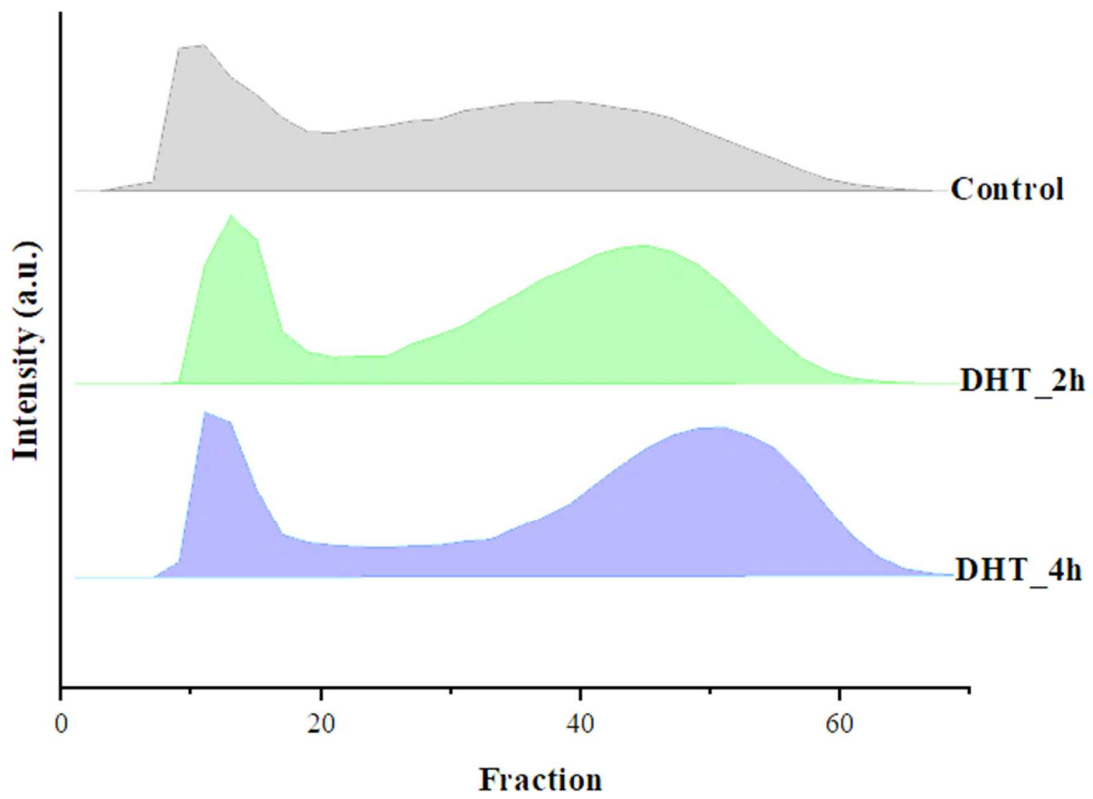
4  
5  
6  
7

**Figure 2.** Photographs from light microscopy (40×) using (A) nonpolarized and (B) polarized light of the control and modified cassava starches by dry heating treatment.



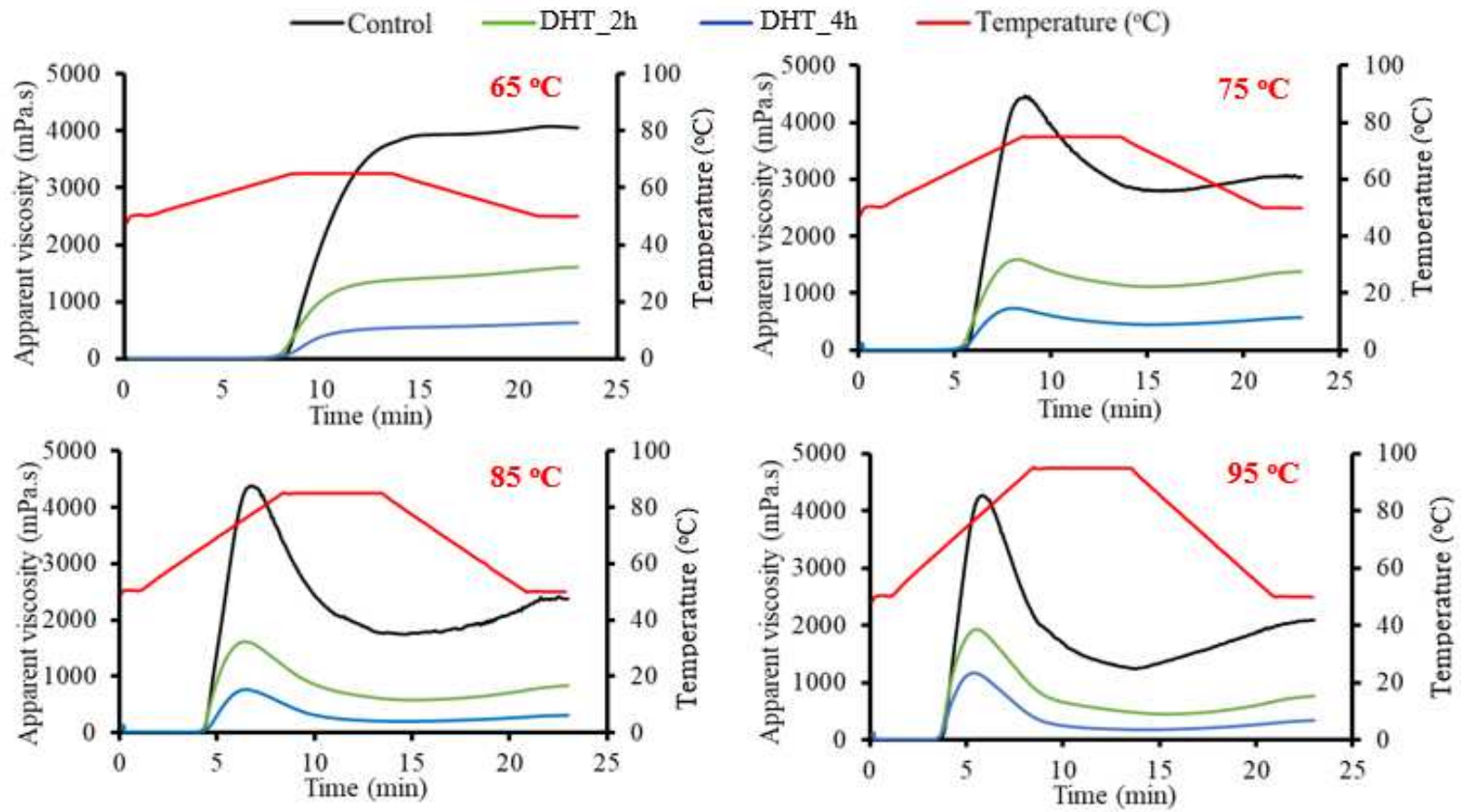
8  
9  
10  
11

**Figure 3.** (A) Diffractogram and (B) relative crystallinity of the starch samples (control, DHT\_2h, and DHT\_4h) – horizontal red bars are the standard deviations; different small caps indicate significant difference among treatments, as revealed by Tukey's test,  $p < 0.05$ .



12  
13  
14

**Figure 4.** Molecular size distribution of the starch samples (control, DHT\_2h, and DHT\_4h).



15

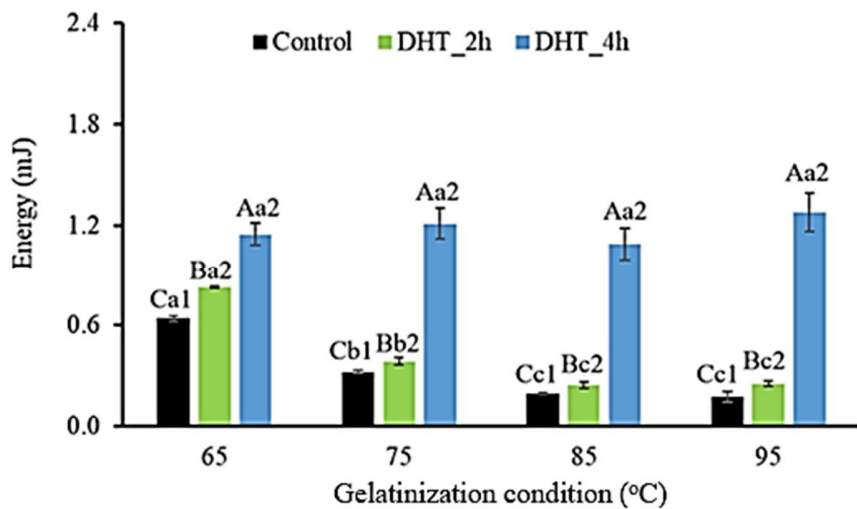
16

**Figure 5.** RVA curves of the control and cassava starches treated by dry heating in different gelatinization conditions

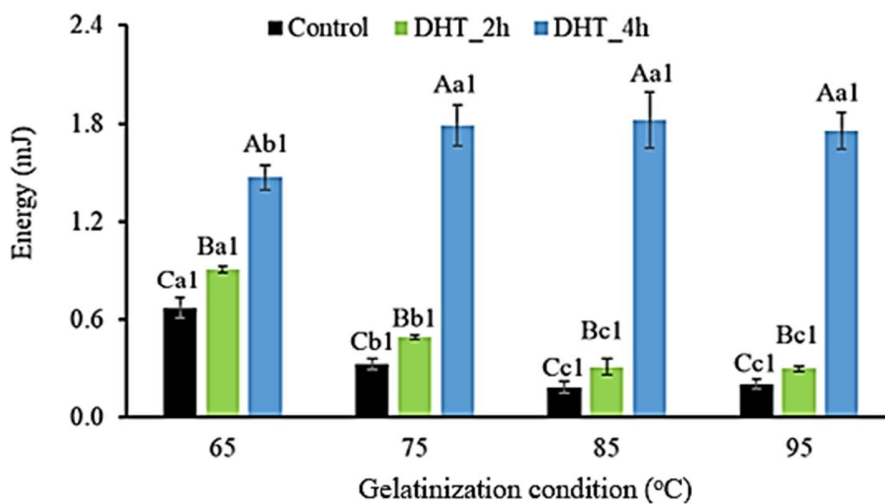
17

(65, 75, 85 and 95 °C).





1 day of storage



7 days of storage

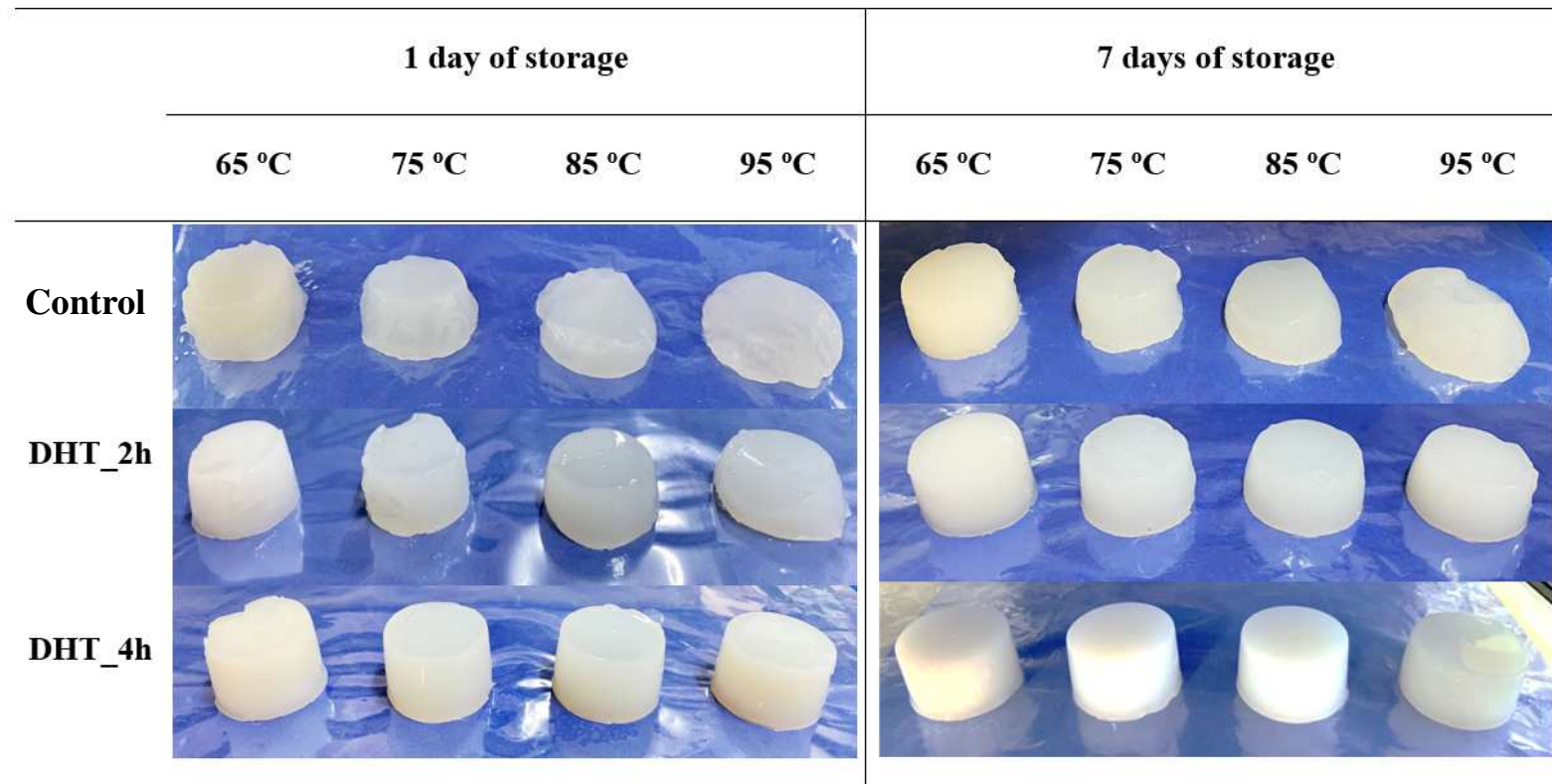
18 **Figure 6.** Firmness (measured as the energy necessary for a punctual assay) of the gels  
 19 produced with starch samples (control, DHT\_2h, and DHT\_4h) in different gelatinization  
 20 condition and storage period.  
 21

22 Statistical evaluation by Tukey's test ( $p < 0.05$ ):

23 A – C: different big caps indicate significant difference among DHT treatments in the same gelatinization  
 24 condition;

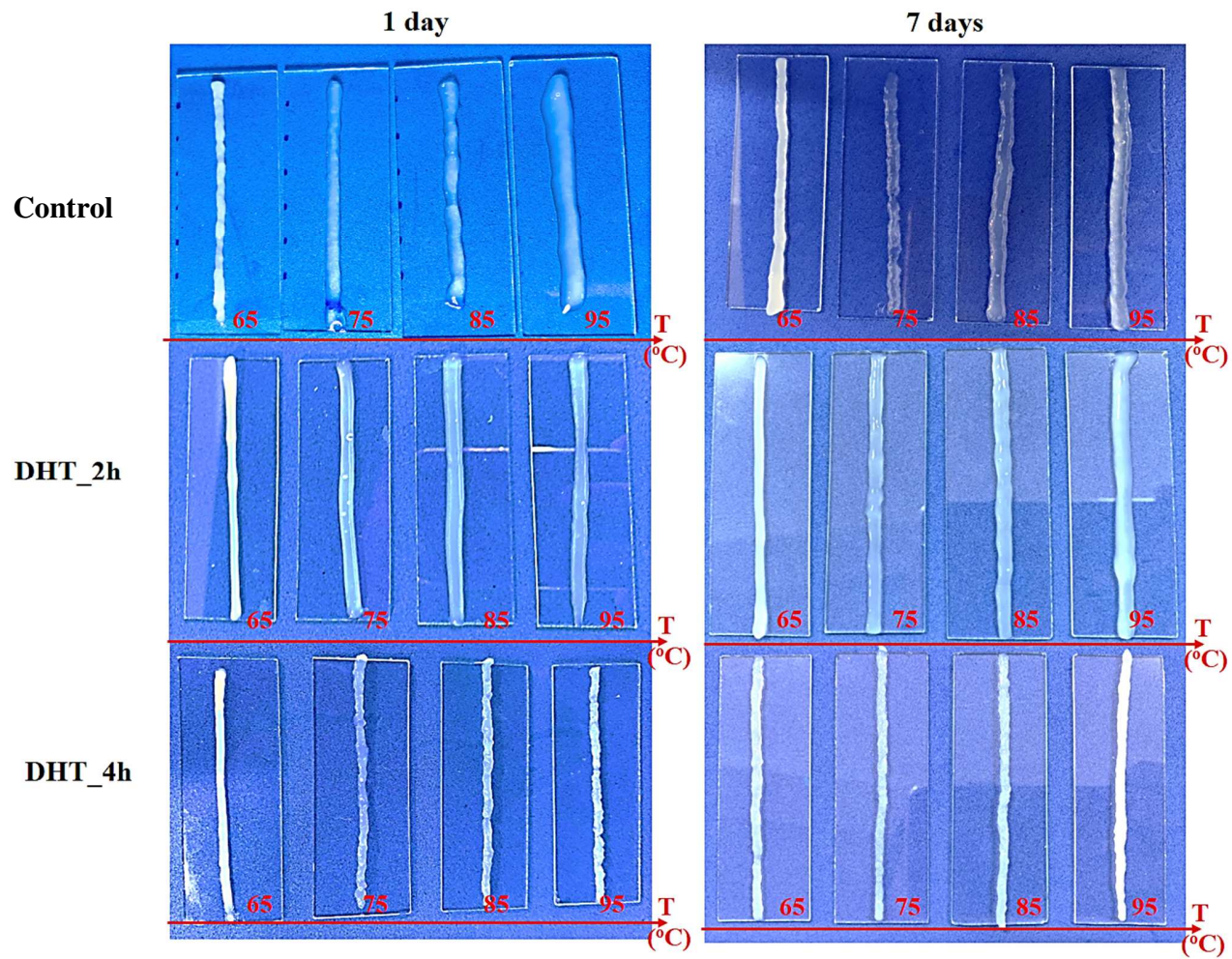
25 a - c: different small caps indicate significant difference among different gelatinization condition, by the  
 26 same starch DHT sample;

27 1 - 2: different numbers indicate significant difference among the same starch DHT sample, in the same  
 28 gelatinization condition, but with different storage period.



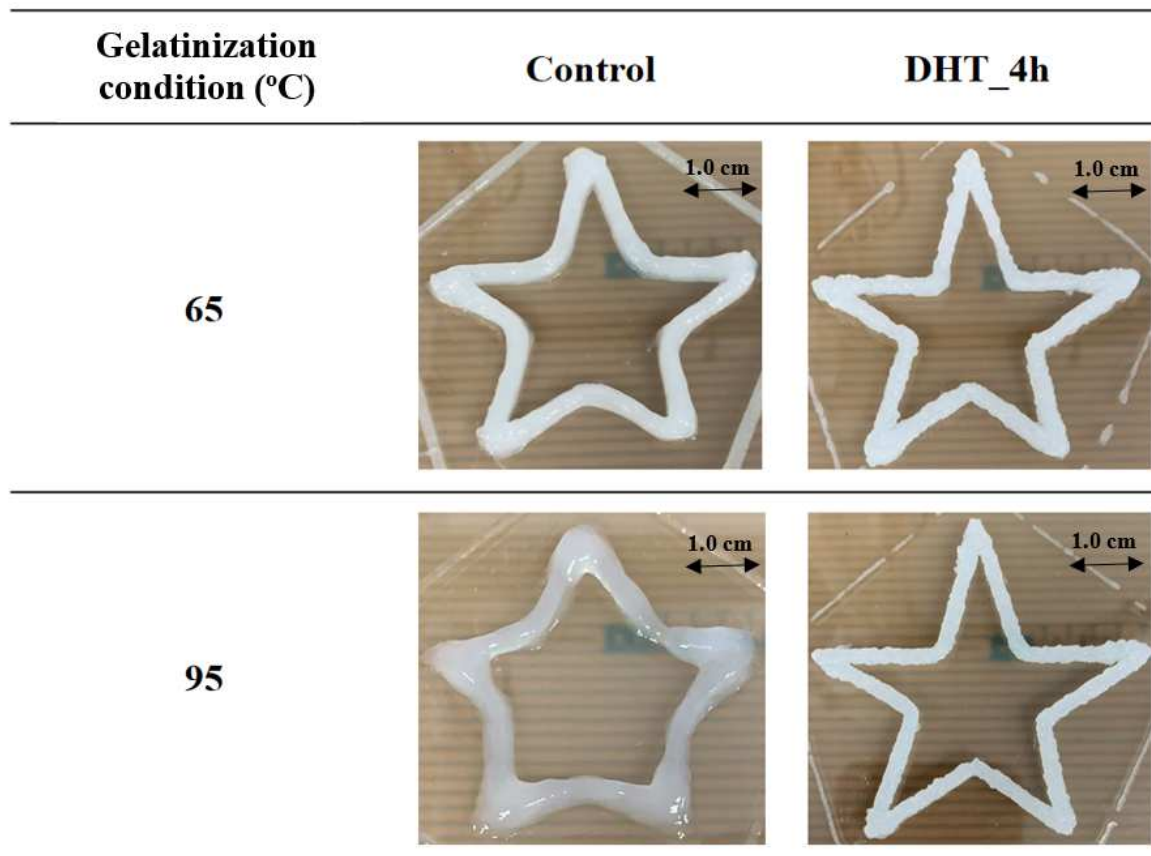
**Figure 7.** The visual aspect of the gels produced with starch samples (control, DHT\_2h, and DHT\_4h) in different gelatinization conditions and storage periods.

29  
30  
31  
32  
33



34  
35  
36

**Figure 8.** Images of simulated printing with the cassava starch gels produced with the different starch samples (control, DHT\_2h, and DHT\_4h) in different gelatinization conditions and storage period.



**Figure 9.** Images of the stars printed by 3D printing based on control and DHT\_4h gels produced using gelatinization condition of 65 and 95 °C.

1 **Table 1.** pH, carbonyl and carboxyl contents of the control and modified cassava starches  
 2 by dry heating treatment (average  $\pm$  standard deviation)

Samples	pH	Carbonyl content (CO/ 100 GU)	Carboxyl content (COOH / 100 GU)
<i>Control</i>	5.45 $\pm$ 0.19 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>c</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
<i>DHT_2h</i>	5.23 $\pm$ 0.11 <sup>a</sup>	0.06 $\pm$ 0.01 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
<i>DHT_4h</i>	5.32 $\pm$ 0.17 <sup>a</sup>	0.22 $\pm$ 0.03 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>

3 a, b, c: different letters in the same column indicates significant difference among treatments, as revealed by  
 4 Tukey's test,  $p < 0.05$ .

5  
 6  
 7  
 8 **Table 2.** Water absorption index (WAI) and water solubility index (WSI) of the control and  
 9 modified cassava starches by dry heating treatment (average  $\pm$  standard deviation)

WAI				
Samples	65 °C	75 °C	85 °C	95 °C
<i>Control</i>	8.74 $\pm$ 0.37 <sup>Ca</sup>	9.47 $\pm$ 0.25 <sup>Ca</sup>	10.59 $\pm$ 0.47 <sup>Ba</sup>	11.83 $\pm$ 0.33 <sup>Aa</sup>
<i>DHT_2h</i>	4.39 $\pm$ 0.34 <sup>Db</sup>	5.90 $\pm$ 0.13 <sup>Cb</sup>	6.96 $\pm$ 0.21 <sup>Bb</sup>	8.01 $\pm$ 0.14 <sup>Ab</sup>
<i>DHT_4h</i>	3.83 $\pm$ 0.24 <sup>Db</sup>	4.55 $\pm$ 0.26 <sup>Cc</sup>	5.90 $\pm$ 0.59 <sup>Bc</sup>	6.92 $\pm$ 0.22 <sup>Ac</sup>
WSI				
Samples	65 °C	75 °C	85 °C	95 °C
<i>Control</i>	1.02 $\pm$ 0.20 <sup>Bb</sup>	2.44 $\pm$ 0.34 <sup>Ac</sup>	2.52 $\pm$ 0.16 <sup>Ac</sup>	2.75 $\pm$ 0.17 <sup>Ac</sup>
<i>DHT_2h</i>	2.36 $\pm$ 0.37 <sup>Da</sup>	8.86 $\pm$ 0.64 <sup>Cb</sup>	12.16 $\pm$ 0.84 <sup>Bb</sup>	21.83 $\pm$ 1.68 <sup>Ab</sup>
<i>DHT_4h</i>	2.39 $\pm$ 0.35 <sup>Da</sup>	13.72 $\pm$ 1.13 <sup>Ca</sup>	19.41 $\pm$ 1.72 <sup>Ba</sup>	26.93 $\pm$ 1.72 <sup>Aa</sup>

10 A, B, C: different big caps in the same column indicates significant difference among treatments in the same  
 11 temperature (Tukey's test,  $p < 0.05$ ). a, b, c: different small caps in the same line indicates significant difference  
 12 in each treatment among different temperatures (Tukey's test,  $p < 0.05$ ).



**Table 3.** Pasting parameters of the control and modified cassava starches by dry heating treatment in different gelatinization conditions (average  $\pm$  standard deviation)

Sample	GC (°C)	PAV (mPa.s)	TAV (mPa.s)	RBD (mPa.s)	FAV (mPa.s)	RSB (mPa.s)	PT (°C)
<i>Control</i>	65	3699.00 $\pm$ 31.08 <sup>bA</sup>	2670.75 $\pm$ 28.37 <sup>aA</sup>	0.00 $\pm$ 0.00 <sup>A</sup>	4061.25 $\pm$ 11.38 <sup>aA</sup>	52.08 $\pm$ 3.56 <sup>aB</sup>	64.09 $\pm$ 0.13 <sup>dB</sup>
	75	4418.25 $\pm$ 44.70 <sup>aA</sup>	2110.50 $\pm$ 77.57 <sup>bA</sup>	33.41 $\pm$ 0.54 <sup>cC</sup>	3031.00 $\pm$ 42.79 <sup>bA</sup>	46.74 $\pm$ 2.98 <sup>aB</sup>	64.55 $\pm$ 0.05 <sup>cB</sup>
	85	4451.75 $\pm$ 84.24 <sup>aA</sup>	1750.50 $\pm$ 31.13 <sup>cA</sup>	60.68 $\pm$ 0.37 <sup>bC</sup>	2471.00 $\pm$ 86.02 <sup>cA</sup>	41.15 $\pm$ 2.63 <sup>bB</sup>	65.51 $\pm$ 0.02 <sup>bB</sup>
	95	4412.00 $\pm$ 73.54 <sup>aA</sup>	1261.00 $\pm$ 26.87 <sup>dA</sup>	70.76 $\pm$ 0.12 <sup>aC</sup>	1850.00 $\pm$ 70.71 <sup>dA</sup>	44.80 $\pm$ 1.74 <sup>bB</sup>	66.33 $\pm$ 0.04 <sup>aA</sup>
<i>DHT_2h</i>	65	1398.50 $\pm$ 44.01 <sup>bB</sup>	1086.50 $\pm$ 68.63 <sup>aB</sup>	0.00 $\pm$ 0.00 <sup>dA</sup>	1646.75 $\pm$ 54.06 <sup>aB</sup>	52.82 $\pm$ 1.86 <sup>aB</sup>	63.91 $\pm$ 0.08 <sup>dB</sup>
	75	1622.50 $\pm$ 38.04 <sup>aB</sup>	930.00 $\pm$ 17.09 <sup>bB</sup>	35.34 $\pm$ 1.08 <sup>cB</sup>	1384.75 $\pm$ 36.43 <sup>bB</sup>	46.91 $\pm$ 1.40 <sup>aB</sup>	64.75 $\pm$ 0.04 <sup>cB</sup>
	85	1668.50 $\pm$ 39.03 <sup>aB</sup>	593.00 $\pm$ 27.14 <sup>cB</sup>	63.68 $\pm$ 0.26 <sup>bB</sup>	871.50 $\pm$ 24.03 <sup>cB</sup>	42.14 $\pm$ 2.56 <sup>bB</sup>	65.54 $\pm$ 0.05 <sup>bB</sup>
	95	1677.00 $\pm$ 14.14 <sup>aB</sup>	270.00 $\pm$ 14.14 <sup>dB</sup>	82.20 $\pm$ 0.03 <sup>aB</sup>	384.50 $\pm$ 6.36 <sup>dB</sup>	42.54 $\pm$ 1.11 <sup>bB</sup>	66.35 $\pm$ 0.06 <sup>aA</sup>
<i>DHT_4h</i>	65	570.25 $\pm$ 29.41 <sup>bC</sup>	406.00 $\pm$ 11.89 <sup>aC</sup>	0.00 $\pm$ 0.00 <sup>dA</sup>	780.75 $\pm$ 8.38 <sup>aC</sup>	89.87 $\pm$ 3.79 <sup>bA</sup>	64.91 $\pm$ 0.02 <sup>cA</sup>
	75	752.50 $\pm$ 12.04 <sup>aC</sup>	347.75 $\pm$ 10.81 <sup>bC</sup>	38.44 $\pm$ 0.37 <sup>cA</sup>	655.50 $\pm$ 23.30 <sup>bC</sup>	90.93 $\pm$ 3.84 <sup>aA</sup>	65.85 $\pm$ 0.20 <sup>aA</sup>
	85	787.00 $\pm$ 25.13 <sup>aC</sup>	198.00 $\pm$ 6.06 <sup>cC</sup>	74.83 $\pm$ 0.75 <sup>bA</sup>	353.50 $\pm$ 32.60 <sup>cC</sup>	90.47 $\pm$ 2.98 <sup>aA</sup>	66.08 $\pm$ 0.32 <sup>aA</sup>
	95	754.00 $\pm$ 27.50 <sup>aC</sup>	103.67 $\pm$ 1.53 <sup>dC</sup>	85.67 $\pm$ 0.35 <sup>aA</sup>	200.33 $\pm$ 4.16 <sup>dC</sup>	93.26 $\pm$ 3.80 <sup>aA</sup>	66.47 $\pm$ 0.25 <sup>aA</sup>

GC: gelatinization condition, Peak Apparent Viscosity (PAV), Through Apparent Viscosity (TAV), Relative Breakdown (RBD), Final Apparent Viscosity (FAV), Relative Setback (RSB) and Pasting Temperature (PT).

a - d: different small caps in the same column indicate significant difference among different gelatinization temperatures for each DHT treatment, as revealed by Tukey's test,  $p < 0.05$ .

A - C: different big caps in the same column indicate significant difference among DHT treatments (0, 2, and 4 hours of dry heating) and considering the same temperature of gelatinization, as revealed by Tukey's test,  $p < 0.05$ .

