

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

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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
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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 $^{\rm o}{\rm C}$
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 applying33 cassava starch to 3D printing.
- 34 Keywords: starch modification, novel ingredients, starchy gels, printability, 3D food
- 35 printing.

36 **1. Introduction**

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

However, 3D-printing technology has generated new challenges for the food 51 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 3D-printed products with the desired characteristics (sensorial, physic-chemical, 54 nutritional, etc). Therefore, most of the studies in the literature focused on factors 55 influencing the rheology and printability of food material, with greater demand for new 56 ingredients and formulations. Novel ingredients are needed to achieve this goal, in 57 particular hydrogels that provide good printability, and 3D products with well-defined 58 geometries and good resolution. 59

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

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

One interesting source of starch is cassava, a major ingredient used in the food 69 industry. Brazil, one of the countries involved in this work, is the world's fifth largest 70 71 producer of cassava, with 18.9 million tons produced in 2017 (FAO - Food and Agriculture Organization, 2019). Cassava presents positive characteristics because it is a 72 73 starch-rich tuberous gluten-free root (interesting for people with celiac disease) (Liu et al., 2019), and highly tolerant to drought and harsh climatic conditions (Alves, 2002). It is 74 75 available around-the-year, and it is considered as a reliable crop for food supply security (Liu et al., 2019; Zhu, 2015). Cassava starch shows high potential for application and it 76 has been investigated by many researchers (Díaz, Dini, Viña, & García, 2018; Liu et al., 77 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 being removed from the mold (Maniglia et al. 2019). Consequently, its modification is 80 81 necessary for applications requiring strong gels.

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

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

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

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

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

The DHT was based on the work of Chandanasree, Gul, & Riar (2016). A thin 119 120 layer (~ 1 mm) of cassava starch (50 g) was distributed over a sheet of aluminum foil and covered with another sheet of aluminum foil. The lateral parts were closed with tape to 121 122 form a closed envelope in order to avoid loss of material. The packed starch was then placed in a hot-air convective oven (MA 035, Marconi, São Paulo, Brazil), in which DHT 123 was performed using air at 130 °C (air velocity $1 \pm 0.1 \text{ m.s}^{-1}$) for 2 and 4 h (named 124 DHT_2h and DHT_4h, respectively), following the conditions described by 125 Chandanasree, Gul, & Riar (2016). After processing, the starch was cooled and stored in 126 glass containers for further analysis. 127

129 2.2 Starch characterizations

130 2.2.1 Granule characterization: morphology, size distribution and crystallinity

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

Particle size distribution was determined using a Laser Analyser (Partica LA950V2 Laser Particle Size Analyser HORIBA, Japan) and the LA-950 software for
Windows (HORIBA, Japan). The samples were dispersed in ethanol (99.5%) and placed
in an ultrasound bath for 5 min prior to analysis. The analysis was carried out eight times
for each sample.

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

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

148

149

$$D[3,2] = \frac{\sum_{i} n_i d_i^3}{\sum_{i} n_i d_i^2}$$
(2)

150

An X-ray diffractometer (Shimadzu XRD 7000, Tokyo, Japan) with copper radiation was used to evaluate granule crystallinity, using the following working conditions: angle 20 ranging from 3 to 40°, scan rate of $2^{\circ} \cdot \min^{-1}$, 40 kV and 30 mA. The starch samples were maintained in a desiccator containing saturated BaCl₂ solution (25 °C, aw = 0.900) for 10 days to ensure constant water activity. Three runs for each sample were analyzed. The curves obtained were smoothed using the Origin software, version 2018 (Microcal Inc., Northampton, MA, USA). The relative crystallinity was calculated

(1)

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

- 160
- 161

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

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

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

178

179
$$\frac{CO}{100 \ GU} = \frac{(V_B - V_S). \ M, \ 0.028.100}{W}$$

180

Where: 181

Vs = the volume of NaOH required for the ozonated samples; 182

Vb = the volume of NaOH used in the blank; 183

184 M = the molarity of NaOH;

W = the sample mass in dry basis. 185

186

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

(3)

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

197

198

$$\frac{COOH}{100GU} = \frac{(V_S - V_b).\,M.\,0.045.100}{W}$$

199

200 Where:

201 Vb = the volume of HCl used in the blank;

Vs = the volume of HCl required for the sample;

M =the molarity of HCl;

W =the sample mass in dry basis.

205

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

(4)

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

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

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

234
$$WAI\left(\frac{g \ water}{g \ IS}\right) = \frac{PT - (MS - SS)}{(MS - SS)}$$
235 (5)

 $WSI\ (\%) = \frac{SS}{MS}X\ 100$

236

237

238

239 2.2.4 Pasting properties

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

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

(6)

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

256

257 **2.2.5 Gel firmness**

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

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

The gels were penetrated to a distance of 4 mm using a cylindrical probe (P/0.5R) at 1 mm·s⁻¹. The equipment measured the force as a function of the penetration depth. Gel firmness was evaluated by the energy required to penetrate the material (calculated by the area below the curve force versus the distance of penetration).

Furthermore, the effect of the gelatinization condition was evaluated by using the different temperatures described on the "Pasting properties" section.

271

272 2.3 Hydrogel application: evaluating 3D printing

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

Firstly, all the hydrogels were evaluated through a simple approach by extruding them with a syringe on a glass slide. In a previous study (Maniglia et al., 2019), we described this analysis as good indication of the material's printability, which had a good correlation with real 3D printing. The hydrogels obtained from the RVA were stored and then placed in a plastic syringe (die diameter: 1.5 mm). The gels were then extruded through the die over a glass slide (lines with ~ 6 cm), for a visual evaluation of the gel's consistency and moldability. The manual extrusion using the syringe was carried out ten times to ensure the reproducibility and veracity of this procedure. Pictures (Figure 8) were obtained based on the visual aspect that most predominated in the extruded material. This approach was used to select conditions for actual 3D printing.

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

299

300 2.4 Experimental design and Statistical analysis

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

Figure 1 shows the particle size distribution (A) and specific particle diameters (B) 310 of the control and modified cassava starches. The effect of DHT enlarged the particle 311 sizes of the granules (Figure 1A), which is consistent with the duration of the dry heating 312 process: the D[4,3] varied from 17.8 µm for the control starch to 19.3 and 27.3 µm 313 DHT 2h and DHT 4h, respectively (Figure 1B). The dry heating process also increased 314 the number of particles with bigger sizes considering the values d0.1, d0.5 and d0.9. 315 Moreover, the differences on D[4,3] were more pronounced than on D[3,2] (although both 316 317 presented statistical differences).

According to Lopez-Sanchez et al. (2011), the D[4,3] value is more influenced by 318 large particles, whereas D[3,2] is more influenced by the smaller ones. Therefore, the 319 results indicate that the dry heating process led to an increase in the diameter of the larger 320 321 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: 6.7 g/100 g), which indicated that part of the water content in the samples was vaporized. 324 325 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 °C, a temperature generally accepted for drying without causing changes, until the same moisture level 329 described previously was reached. No change was observed on the starch dried this way. 330

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 granules were polygonal, angular and truncated, as observed by Han et al. (2019). 333 334 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 the cassava starch modified in the same DHT conditions. Considering the fact that 337 birefringence reflects the internal microstructure of starch granules, it shows that the dry 338 heating treatment used did not significantly affect the internal microstructure of cassava 339

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

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

346 The granule X-ray diffraction patterns are presented in Figure 3(A), and their relative crystallinity in Figure 3(B). All the samples showed peaks at 15°, 23°, 17°, and 347 18° (20), representing a typical type-A starch pattern (Liu et al., 2019). In addition, no 348 349 significant difference (p < 0.05) was observed for the relative crystallinity between native starch and the DHT_2h sample. However, the DHT_4h sample showed significant 350 reduction (p < 0.05) of this parameter compared with control and DHT_2h. Type-A starch 351 polymorphs (with about six water molecules inside the helices) presented a more stable 352 353 monoclinic structure (Chatpapamon, Wandee, Uttapap, Puttanlek, & Rungsardthong, 354 2019); consequently, this crystalline arrangement is less prone to change by dry heating treatment. This indicates that the crystalline structure of the starch was affected only after 355 4 h of dry heating treatment at 130 °C. Liu et al. (2019) indicated the decrease in 356 357 crystallinity could be attributed to a reorientation of the double helices forming the crystalline array and/or crystallite disruption. 358

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

Therefore, the dry heating treatment changed the crystallinity (DHT_4h) of cassava starch, but without bigger changes in the granule morphology. Consequently, it was necessary to also evaluate the possible changes in starch molecules, as described in the following.

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

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

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

382

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

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

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

402

403

3.1.3 Water absorption index (WAI) and water solubility index index (WSI)

404 Table 2 shows the water solubility index (WSI) and the water absorption index405 (WAI) of the control and modified cassava starches.

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

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

424

425 **3.1.4 Pasting properties**

Figure 5 shows the RVA curves and Table 3 shows the associated pasting parameters in different gelatinization conditions (65, 75, 85, and 95 °C). The peak apparent viscosity was higher for the native starch than those treated by dry heating, whatever the gelatinization conditions. In addition, a more significant reduction in thepeak apparent viscosity was observed as processing time increased.

When the aqueous slurry of starch is heated, the granules can absorb water and 431 swell; the swollen granules rub against each other under stirring in RVA, increasing the 432 433 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 starch granules treated by DHT showed a reduction in their capacity to maintain their 436 437 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 the swelling power (WAI in the Table 2). 439

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

The relative breakdown (RB) increased according to the time of exposure to heat.
This indicates that the new intermolecular association produced by DHT becomes easier
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., 2019). The RSB values of the DHT 4h starch were higher than the control and the 452 453 DHT_2h starches (Table 3). Moreover, for the control and the DHT_2h starches, increasing gelatinization temperature (up to 75 °C) reduced the RSB values; however, the 454 variation of the temperature did not affect this parameter of the DHT_4h starch. 455 Therefore, the dry heating treatment and the gelatinization conditions cooperated 456 differently regarding the retrogradation phenomenon. This can be explained by molecular 457 depolymerization. As discussed before, the dry heating treatment led to a reduction of 458 459 molecular size, in particular that of molecules of intermediate size, forming two welldefined groups with larger and smaller molecules. Similar molecule sizes are more
suitable for re-association due to better pairing; also, smaller molecules have higher
mobility to re-associate (Castanha et al., 2017), explaining the behavior observed.

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

DHT for 2 h did not change the pasting temperature. However, the DHT_4h samples showed slightly higher pasting temperatures (when the gelatinization conditions were 65, 75, or 85 °C) than the other samples. This might result in the presence of smaller molecules that need a higher energy level to gelatinize than large size molecules (Castanha et al., 2017).

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

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

481

482 **3.1.5 Gel firmness**

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

Dry heating treatment resulted in stronger hydrogels: for all the gelatinization and storage conditions, the starch processed through DHT for 4 h resulted in stronger gels when compared with the control and that processed for 2 h. The gels produced by DHT_2h starch did not result in a behavior as pronounced as the DHT_4h, but the gels 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.

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

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

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).

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

In all the gelatinization conditions, the DHT_4h starch showed more defined lines 531 with a thinner aspect, indicating that these gels presented stronger consistency (agreeing 532 with the results obtained for the gel firmness), spreading less on the surface. On the other 533 hand, the native starch appeared less adapted for 3D printing application when the gel was 534 535 produced at temperatures up to 65 °C. Indeed, the "lines" produced by such gels were larger and spread more on the surface than the other gels. Indeed, cassava starch is known 536 to produce relatively "weak" gels that do not maintain their shape after being removed 537 538 from the mold.

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

In the conditions evaluated, DHT_4h starch appeared as the best candidate for 3D printing application, as it could be used as a gelling agent. To evaluate if the dry heating treatment really improved the printability of this gel, star-shaped products were printed by a 3D printer using DHT_4h gels produced at 65 and 95 °C. Figure 9 shows the result obtained, in comparison with the control (native starch) gel produced at the same temperatures.

In Figure 9, we can conclude that when the gels were produced using the lowest gelatinization temperature condition (65 °C), it was possible to print stars with both gels. However, it can be seen that the star produced with DHT_4h gels gives higher resolution (well-defined star tips). Comparing the moldability of the gels produced in the highest gelatinization temperature condition (95 °C), the control starch did not provide good printability, and spread over the surface. On the other hand, the DHT_4h gels produced at this temperature showed good printability, resulting in a star with the best resolution.

Therefore, our results are very interesting since gels with lower apparent viscosity and the ability to form stronger gels can be obtained by altering the DHT process conditions. These characteristics imply that the use of cassava starch can be expanded to 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).

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

565 566

4. Conclusion

567 This work demonstrated that the simple and efficient technique of DHT can568 expand the application of cassava starch, in particular for 3D printing.

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

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- 598

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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.



- 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.



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.







16

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

(65, 75, 85 and 95 °C).



18 19 Figure 6. Firmness (measured as the energy necessary for a punctual assay) of the gels produced with starch samples (control, DHT_2h, and DHT_4h) in different gelatinization 20 21 condition and storage period. 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 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.

Gelatinization condition (°C)	Control	DHT_4h
65	L0 em	1.0 cm
95	1.0 cm	1.0 cm

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

	11	Carbonyl content	Carboxyl content
Samples	рн	(CO/ 100 GU)	(COOH / 100 GU)
Control	5.45 ± 0.19^{a}	$0.00 \pm 0.00^{\circ}$	0.00 ± 0.00^{a}
DHT_2h	5.23 ± 0.11^{a}	0.06 ± 0.01^{b}	0.00 ± 0.00^{a}
DHT_4h	5.32 ± 0.17^{a}	0.22 ± 0.03^{a}	$0.00 \pm 0.00^{\mathrm{a}}$

2 by dry heating treatment (average ± standard deviation)

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

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

9 modified cassava starches by dry heating treatment (average ± standard deviation)

10 A, B, C: different big caps in the same column indicates significant difference among treatments in the same

temperature (Tukey's test, p < 0.05). a, b, c: different small caps in the same line indicates significant difference in each tractment among different temperatures (Tukey's test, p < 0.05).

12 in each treatment among different temperatures (Tukey's test, p < 0.05).

Sample	GC	$\mathbf{PAV}(\mathbf{mPas})$	$\mathbf{TAV}(\mathbf{mPas})$	$\mathbf{D}\mathbf{R}\mathbf{D}$ (m \mathbf{P}_{0} s)	$\mathbf{FAV}(\mathbf{mPos})$	PSR (mPa a)	PT (% C)
Sample	(°C)			KDD (IIII a.s)	$\mathbf{F}\mathbf{A}\mathbf{V}$ (IIII a. 5)	KSD (IIII a.s)	$\mathbf{H}(\mathbf{C})$
	65	3699.00 ± 31.08^{bA}	2670.75 ± 28.37^{aA}	$0.00 \pm 0.00^{\rm A}$	4061.25 ± 11.38^{aA}	$52.08 \pm 3.56^{\mathrm{aB}}$	$64.09 \pm 0.13^{\mathrm{dB}}$
Control	75	4418.25 ± 44.70^{aA}	2110.50 ± 77.57^{bA}	33.41 ± 0.54^{cC}	3031.00 ± 42.79^{bA}	$46.74 \pm 2.98^{\mathrm{aB}}$	$64.55 \pm 0.05^{\text{cB}}$
Control	85	4451.75 ± 84.24^{aA}	1750.50 ± 31.13^{cA}	60.68 ± 0.37^{bC}	2471.00 ± 86.02^{cA}	41.15 ± 2.63^{bB}	65.51 ± 0.02^{bB}
	95	4412.00 ± 73.54^{aA}	1261.00 ± 26.87^{dA}	70.76 ± 0.12^{aC}	1850.00 ± 70.71^{dA}	44.80 ± 1.74^{bB}	66.33 ± 0.04^{aA}
	65	$1398.50 \pm 44.01^{\text{bB}}$	$1086.50 \pm 68.63^{\mathrm{aB}}$	0.00 ± 0.00^{dA}	$1646.75 \pm 54.06^{\mathrm{aB}}$	$52.82 \pm 1.86^{\mathrm{aB}}$	$63.91 \pm 0.08^{\mathrm{dB}}$
DHT_2h	75	$1622.50 \pm 38.04^{\mathrm{aB}}$	930.00 ± 17.09^{bB}	$35.34 \pm 1.08^{\text{cB}}$	$1384.75 \pm 36.43^{\text{bB}}$	$46.91 \pm 1.40^{\mathrm{aB}}$	$64.75 \pm 0.04^{\text{cB}}$
	85	$1668.50 \pm 39.03^{\mathrm{aB}}$	$593.00 \pm 27.14^{\text{cB}}$	$63.68 \pm 0.26^{\mathrm{bB}}$	$871.50 \pm 24.03^{\text{cB}}$	42.14 ± 2.56^{bB}	$65.54 \pm 0.05^{\mathrm{bB}}$
	95	$1677.00 \pm 14.14^{\mathrm{aB}}$	$270.00 \pm 14.14^{\mathrm{dB}}$	82.20 ± 0.03^{aB}	$384.50 \pm 6.36^{\mathrm{dB}}$	$42.54 \pm 1.11^{\text{bB}}$	66.35 ± 0.06^{aA}
	65	570.25 ± 29.41^{bC}	$406.00 \pm 11.89^{\mathrm{aC}}$	0.00 ± 0.00^{dA}	$780.75 \pm 8.38^{\mathrm{aC}}$	89.87 ± 3.79^{bA}	64.91 ± 0.02^{cA}
DUT 41	75	$752.50 \pm 12.04^{\mathrm{aC}}$	347.75 ± 10.81^{bC}	38.44 ± 0.37^{cA}	655.50 ± 23.30^{bC}	90.93 ± 3.84^{aA}	65.85 ± 0.20^{aA}
DH1_4N	85	$787.00 \pm 25.13^{\mathrm{aC}}$	198.00 ± 6.06^{cC}	74.83 ± 0.75^{bA}	$353.50 \pm 32.60^{\circ C}$	90.47 ± 2.98^{aA}	66.08 ± 0.32^{aA}
	95	$754.00 \pm 27.50^{\mathrm{aC}}$	$103.67 \pm 1.53^{\rm dC}$	85.67 ± 0.35^{aA}	200.33 ± 4.16^{dC}	93.26 ± 3.80^{aA}	66.47 ± 0.25^{aA}

 Table 3. Pasting parameters of the control and modified cassava starches by dry heating treatment in different gelatinization conditions

(average ± standard deviation)

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.

Cassava starch (Native)	Dry heating treatment (DHT)	Modified starch DHT_2h DHT_4h
	Gelatinization condition T = 65, 75, 85, or 95 °C)	
	Storage period (1 or 7 days)	

Gels storage for 7 days

+ Hydrogels with higher printability (high gel strength and less spread in the surface)

