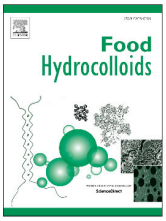

Modification of structural and physicochemical properties of cowpea (*Vigna unguiculata*) starch by hydrothermal and ultrasound treatments

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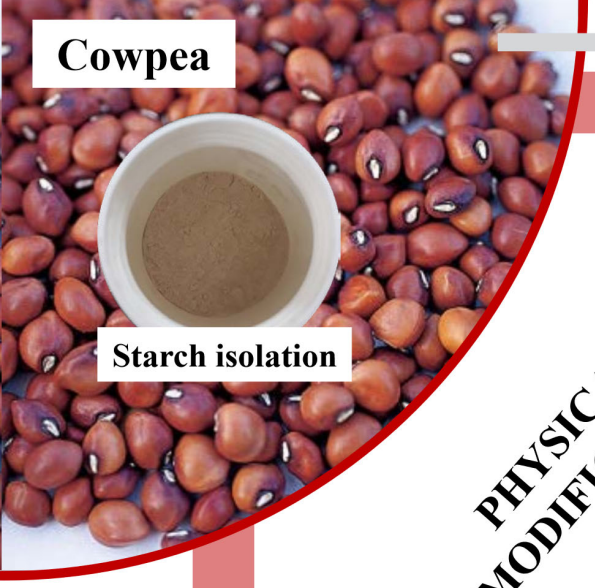
Author contributions

Belen A. Acevedo and Felicidad Ronda conceived and designed the experiments.

Belen A. Acevedo, Marina Villanueva, María G. Chaves and María V. Avanza performed the experiments; analysed and interpreted the data and wrote/reviewed the paper

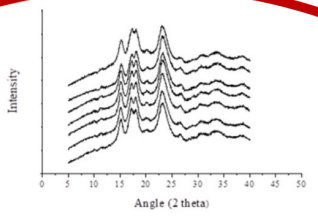
Felicidad Ronda: Funding acquisition, Conceptualization, Methodology, Resources, Investigation, Visualization, Supervision, Writing - review & editing, Project administration.

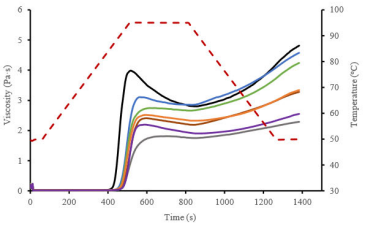


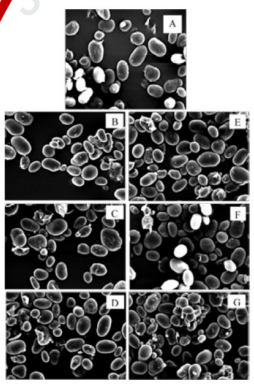
HMT

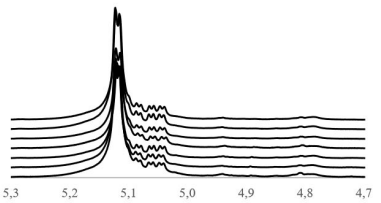
20% moisture content
2h, 5h, 12h at 100ºC

PHYSICAL MODIFICATION









HMT+US

20% moisture content
2h, 5h, 12h at 100ºC
Suspension 10% w/v
20 kHz (80% amp)
30 min (2s on – 2s off)

Modification of structural and physicochemical properties of cowpea (*Vigna unguiculata*) starch by hydrothermal and ultrasound treatments

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Abstract

Cowpea (*Vigna unguiculata*) starch was physically modified by heat moisture treatment (HMT) or HMT followed by ultrasound treatment (UST). The modifications of starch crystallinity, morphology and digestibility as well as thermal, and pasting characteristics were evaluated. HMT was performed at 20% moisture content, for 2, 5 and 12 h at 100 °C (HMT2, HMT5, HMT12). UST was performed at 80% amplitude (20 kHz) for 30 min (2 s on-2 s off). The granule shape and XRD patterns were not modified by any of the applied treatments. ^{1}H NMR revealed that HMT and HMT-UST caused a decrease of amylopectin branching degree. Thermal stability increased more in heat-moisture-treated-starch, while gelatinization enthalpy was not affected by the treatments applied. The resistant starch content increased up to 11% with HMT, whereas an increase in slow digestible starch (SDS) (+30%) was observed in HMT2-UST and HMT5-UST. The decrease in pasting viscosity of treated starch samples increased incrementally with heating time. The results demonstrate that HMT and HMT-UST expand the opportunities and potential of using modifying cowpea starches as ingredients in several food applications.

Keyword: cowpea starch; physical modification; functional properties; *in vitro* starch digestibility; structural properties.

1. Introduction

Cowpea (*Vigna unguiculata*) is an annual legume that belongs to the Fabaceae family and it is an African native plant (Rengadu, Gerrano, & Mellem, 2020), which grows in a tropical or sub-tropical climate. Cowpea seeds contain proteins (28% w/w) and carbohydrates (52% w/w) (Avanza, Chaves, Acevedo, & Añón, 2013), mainly enriched in starch (36 - 50%) as in other legumes (Acevedo, Avanza, Chaves, & Ronda, 2013).

Starch is a polysaccharide synthesized by plant tissues as the main storage carbohydrate and represents a versatile raw material for a wide range of applications. However, in the food industry, native starches are not widely used due to their limited functional properties: poor thermal, shear and acid stability, and high rates and extent of retrogradation (Hoover, 2010). These limitations can be overcome by modifying the starch native structure with different treatments. Recently, the food industry has been using physically modified starches, which are considered as a natural material and a highly safe ingredient.

Heat moisture treatment (HMT) is widely used to physically modify starch by heating for a defined period (15 min to 16 h), up to a temperature above the glass transition, keeping moisture levels below 35% (Colussi et al., 2020). Under these conditions, the intermolecular starch associations, both in the amorphous and crystalline regions, are modified without destroying the granular structure (Hoover & Vasanthan, 1994), but modifying the thermal and functional properties of starches (Kaur & Singh, 2019). The magnitude of these changes depends on the moisture content (Sui et al., 2015), the heating period (Arns et al., 2015; Sui et al., 2015) and the starch source (Villanueva, De Lamo, Harasym & Ronda, 2018a). Several authors studied the effect of HMT on legume starches (pea, lentil, pinto bean, black bean, field pea, pigeon pea) at 100 °C for 16 h and 30% of moisture content (Hoover & Vasanthan,

1994; Hoover & Manuel, 1996). Therefore, we consider it important to evaluate shorter heating periods to decrease energy consumption and broaden the versatility of the process.

Ultrasound is a physical treatment (UST) also used to modify native starches, since its effects are due to the phenomenon of acoustic cavitation, collapsing cavitation bubbles (Soria & Villamiel, 2010) which has an impact on the structure of the starch granules, affecting their physicochemical and functional properties (Luo et al., 2008; Monroy, Rivero, & García, 2018; Kaur & Gill, 2019). This technology is considered simple, cheap and an energy saving treatment (Majeed, Wani, & Hussain, 2017).

Dual physical modifications, as HMT-UST, are a promising alternative to other structural modifications of starch, as they produce physical changes in the starch granules without the use of chemical reagents (Colussi et al., 2020) and has been applied to corn starch (Flores Silva, Alvarez Ramírez & Bello Perez, 2018) and taro starch (Thomaz et al., 2020) to modify their digestibility and thermal properties, respectively. However, currently, there is no information, or reports found associated with the use of these treatments in cowpea starches. Therefore, the objective of this work was to evaluate and compare the effect of heat moisture treatments (HMT) at short heating periods (2, 5 and 12 h) with dual modifications by HMT-UST on the structural, techno-functional, thermal and nutritional properties of cowpea starches.

2. Materials and Methods

2.1 Starch isolation

The seeds were provided by Estación Experimental “El Sombrero-Corrientes” (Instituto Nacional de Tecnología Agropecuaria-INTA) Argentina. Starch was extracted from cowpea seeds by using the method of Schoch & Maywald (1968), with some modifications. Healthy

seeds (100 g) were soaked in 335 mL of NaOH 0.2% (p/v) for 2h and then washed with distilled water. Subsequently, they were ground (wet grinding) with a Butt Decalab Grinder (Decalab SRL, Argentina), at low speed in an ice bath. The paste obtained was initially filtered (ASTM 18-129) and washed repeatedly with small portions of distilled water. The filtrate was left to settle, and the supernatant was removed taking care not to drag the decanted starch. Then the decanted starch was filtered (ASTM 18-140), washed repeatedly with small portions of distilled water and centrifuged (15 min, 2000 rpm). The starch obtained was dried (24 h, 40 °C) in an oven (San-Jor, Argentina), grounded in glass mortar, sieved (ASTM 18-140) and kept in plastic tubes (4 °C), for further analysis. The extraction of the starch was performed in duplicate.

The chemical composition of starch, determined with the standard methods of AOAC (AOAC, 1990), was: 1.2±0.20% of protein, 0.12±0.01% fat and 1.23±0.13% ash. The amylose content, which was determined with the Megazyme assay kit (Megazyme International Ireland Ltd., Bray, Ireland), was 24.33±0.22%.

2.2 Treatments

2.2.1 Heat moisture treatment (HMT)

Starch samples were placed within hermetic glass containers and distilled water was added, considering its initial moisture content, to moisten them to 20% water content. The samples were left for 24 hours at room temperature to equilibrate their water content. Subsequently they were placed in a convection stove (TDSF50, Tecnodalvo, Argentina) at 100 °C for 2, 5 and 12 h. Treated samples were then dried at 40 °C for 24 h and named as follows: HMT2 (2 h), HMT5 (5 h) and HMT12 (12 h).

2.2.2 Ultrasound treatment (UST)

One portion of HMT starches (HMT2, HMT5 and HMT12) was suspended in water (10% w/v; 100 mL), and treated in an ultrasound equipment (VCX500, Sonics & Material Inc, USA) with 80% amplitude (20 kHz) for 30 min in cycles of 2 s on and 2 s off (at 50% on-off pulse), using an ultrasound probe (220-B, CV334 model, Sonics, USA) with 13 mm tip diameter. During the treatment the starch dispersions were stirred with a magnetic stirrer. The overheating of the sample was prevented by placing the beaker with the starch dispersion in an ice bath. The temperature, recorded with a probe immersed in the starch dispersion, ranged between 25 and 30°C. Finally, samples were dried at 40 °C for 24 h and named HMT2-UST, HMT5-UST and HMT12-UST.

2.3 Morphological properties

All samples were observed under a scanning electron microscope (model JEOL, 5800 LV, Tokio, Japan) at 15 kV as described by Acevedo, Villanueva, Chaves, Avanza, & Ronda (2020).

2.4. Nuclear magnetic resonance (NMR)

The NMR samples were prepared as follows: 7 mg of sample were placed into a small vial and 600 µL of deuterated dimethyl sulfoxide (DMSO-d_6), 50 µL of deuterated trifluoroacetic acid (TFA-d) and 3 mg of lithium bromide (LiBr) were added. Each sample was then transferred into a NMR tube. The ^{1}H experiments were recorded on 500 MHz Agilent instruments (Santa Clara, USA) equipped with OneNMR probe, at 70 ºC, 45º pulse width, 5 s relaxation delay between transient, spectral width of 8012.8 Hz, a total of 400 transients and 2.004 s acquisition time. ^{1}H chemical shifts (δ) were reported in parts per million (ppm) and referenced to DMSO, using the solvent residual peak as an internal reference. The final

spectra were analyzed using MestReNova software v.12 (Mestrelab Research Co., Spain). The degree of branching (DB) of starch was calculated according the following equation:

$$DB\ (\%) = \frac{I_{\alpha-(1,6)}}{I_{\alpha-(1,4)} + I_{\alpha-(1,6)}} \times 100$$

where $I_{\alpha\text{-}(1,6)}$ is the ^{1}H NMR integral of peak for α-(1,6)-glucosidic bonds at ~ 4.80 ppm and $I_{\alpha\text{-}(1,4)}$ is the ^{1}H NMR integral of peak for α-(1,4)-glucosidic bonds at ~ 5.12 ppm (Sweedman, Hasjim, Tizzotti, Schäfer, & Gilbert, 2013). These analyses were carried out in duplicate.

2.5 X-ray diffraction

The crystalline order in starch samples was determined using a Bruker-D8-Discover-A25 diffractometer (Bruker AXS, Rheinfelden, Germany) equipped with a copper tube operating at 40 kV and 40 mA, with CuKα radiation of 0.154 nm wavelength as described by Villanueva, Harasym, Muñoz, & Ronda (2018b). The "search-match" software DifracEVA with PDF2-2004 and COD database was used for this purpose. Before measurement, all starch samples were equilibrated to 15% moisture content after exposure to a saturated humidity ICP260 incubator at 15°C (Memmert GmbH, Germany) for one day.

2.6 Thermal properties

Thermograms of starch samples were determined using a differential scanning calorimeter (DSC3, STAR^{e} System, Mettler Toledo, Switzerland) calibrated for temperature and heat flow using Indium. Samples (~6 mg) were weighed into aluminum pans, and distilled water (~14 µL) was added. The sample pans were kept for 1 h at room temperature for moisture equilibration and then scanned from 0 to 120 °C at 5 C/min, then cooled from 120 to 0 °C at 5 °C/min and then again heated from 0 to 120 °C at 5 °C/min, using an empty sealed pan as a reference. The retrogradation of starch was evaluated in the samples previously gelatinized

and stored in the DSC pans for 7 days at 4±2 °C following the same temperature scan. The enthalpy (ΔH), onset temperature (To), end-set temperature (Te), peak temperature (Tp) and the difference Te−To (ΔT) were established in both scans, at 0 and 7 days. Assays were carried out in duplicate.

2.7 *In vitro* digestibility of starch

In vitro starch digestibility was measured according to the Englyst method (Englyst, Hudson & Englist, 2000) as described by Abebe, Collar, & Ronda (2015). The glucose released at 20 min (G_{20}) and 120 min (G_{120}) and the total glucose (TG) were determined by the glucose oxidase colorimetric method. Rapidly digestible starch RDS=0.9·(G20−FGS) (FGS: free glucose + glucose from sucrose), slowly digestible starch SDS=0.9·(G120−G20), resistant starch RS=0.9·(TG−G120), total starch TS=0.9·(TG−FGS) and rapidly available glucose RAG=G20 were calculated. Starch digestion rate index (SDRI), which expresses the amount of RDS in the sample as a percentage of the TS content, was also computed. These analyses were carried out in quadruplicate.

2.8 Pasting properties

Samples (3.5 g, 14% moisture basis) were transferred into canisters and 25 ± 0.1mL of distilled water were added and processed following the AACC International Method 76–21.02 Standard 2 (AACC, 2017) using a Kinexus Pro+ rheometer (Malvern Instruments Ltd, Malvern, UK) supplied with starch pasting cell and controlled by rSpace software. Pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown viscosity (BV) and setback viscosity (SV) were calculated from the pasting curve (Acevedo et al., 2020). For each viscometric measurement, three samples were used.

2.9 Statistical analysis

Measurements were performed at least in duplicate. Differences between native and treated starch samples were established using analysis of variance (ANOVA) by applying Least significant difference (LSD) ($p<0.05$). Statistical analysis was performed using the Infostat software (Di Rienzo et al., 2008).

3. Results and discussion

3.1 Morphological properties

The morphology of the native and treated starch granules is shown in Figure 1. The native starch granules are oval shaped with a smooth surface that is typical of starch granules from legumes (Acevedo et al., 2020). The different HMT times applied did not alter the shape or the surface of starch granules (Figure 1B, 1C and 1D), which is consistent with the results from a study by Hoover (2010) when HMT was performed at temperatures below 110 °C, regardless of starch origin. However, Kaur & Singh (2019) reported the appearance of some pores on oat starch granule surface due to partial gelatinization when granules were exposed to high temperatures at a high moisture content (100 °C, 30% and 12 h). On the other hand, cowpea starch treated by HMT-UST, showed starch granules with no modifications of its shape (Figure 1E, 1F and 1G), but with a rough and irregular surface that could facilitate the small pores formation. Luo et al. (2008) informed the presence of pores and fissures at the corn starch granule surface after UST. Pore formation may be attributed to the mechanical damage by collapse of cavitation bubbles during UST because of the high shear forces that were capable of breaking polymer chains and damaging granules (Kaur & Gill, 2019). Several authors reported cracks and depressions as the main effect of UST on the morphology of rice (Vela, Villanueva, Solaesa & Ronda, 2021) and cassava starch granules (Monroy et al., 2018).

3.2 NMR

To characterize the structural features of native and treated starches, the resonances of the anomeric protons involved in α−(1,4) and α−(1,6) glycosidic bonds were assessed using ^{1}H NMR spectroscopy. Figure 2 shows a partial ^{1}H NMR spectra of the native, HMT and HMT-UST starches and their degree of branching (DB, %). The anomeric signals α-(1,4) and α-(1,6) were clearly visible at 5.12 and 4.80 ppm, respectively, which is in agreement with those reported by Tizzotti, Sweedman, Tang, Schaefer, & Gilbert, (2011) for corn starch.

HMT2 and HMT12 samples presented a decrease of DB (-25% and -4%, respectively) with respect to native starch. This might suggest a first and a greater rupture α-(1,6)-glycosidic bonds from amylopectin and a later and slower degradation of α-(1,4)-glycosidic bonds from amylose and amylopectin (Chen et al., 2017). This could be due to a major susceptibility of α-(1,6)- bonds to HMT since the steric hindrance around the α-(1,4)- bonds is stronger than that around α-(1,6) bonds in starch (Yang et al., 2017).

All HMT-UST samples, regardless of HMTs applied (2, 5, 12 h), showed similar DB values and significantly lower than native starch ($p<0.05$). In addition, the DB obtained at HMT5-UST and HMT12-UST were lower than those obtained at HMT5 and HMT12 suggesting that UST was responsible for this decrease, associated to the decrease of the anomeric signal α-(1,6) (Figure 2). Zheng et al. (2013) reported that sweet potato starch treated by ultrasonic treatment showed higher amylose content as result of the destruction of amylopectin branches.

3.3 X-Ray Diffraction

The X-ray diffraction pattern of the native and treated starches are shown in Figure 3. The native starch presented a diffraction pattern compatible with the C-type pattern, with two

well-defined peaks at 15.2 and 23° and dual peaks at 17 and 18°. These results were similar to those reported for Korean cowpea starch (Kim, Woo, & Chung, 2018). The peak related to the amylose-lipid complex at 20 ° was observed in all samples. The diffraction patterns of the treated starches were similar to native starch showing that their crystalline structure was preserved, regardless of the treatment applied (HMT and HMT-UST). These results agree with those of Hoover & Manuel (1996) since hydrogen bonds within the crystalline regions are not modified by HMT when the moisture content is limited to 30%. Hoover & Vasanthan (1994) informed changes from B type to A type diffraction pattern when potato starch was treated by HMT. Therefore, the influence of HMT may depend on the origin of the starch and moisture content (Hoover & Vasanthan, 1994). Monroy et al. (2018) and Luo et al., (2008) did not report any diffraction pattern modification in sonicated cassava and corn starches, as was observed for cowpea starch in the current study.

The relative crystallinity of native and treated starches is presented in Figure 3 and the major increment was observed in HMT2 (6%), which could be due to the minor DB observed by NMR, leading to a greater rearrangement of starch chains. The increase in relative crystallinity may be due to the enhanced associations between starch chains and to the rearrangement of the disrupted double helices within the crystalline regions that led to an increase in crystal perfection or the formation of new crystallite (Yang et al., 2017). The relative crystallinity of HMT12-UST increased 10% with respect to native starch, which denotes UST as capable of rearranging starch chains from amorphous zones promoting the formation of crystalline structure (Thomaz et al., 2020).

3.4 Thermal properties

The gelatinization parameters of native and treated starches are summarized in Table 1. The native starch exhibited To, Tp and Te values similar to those reported for cowpea starch by

Huang et al. (2007), but higher than other legume starches (Acevedo et al., 2020). This can be attributed to differences in starch crystallinity depending on its origin (Chung, Liu, & Hoover, 2009). HMT increased To (2.7-8.5%), Tp (2.3-5.6%) and Te (4.6-5.3%), reaching maximum values with the longest heating time (12 h), suggesting that more ordered crystals are obtained (Sui et al., 2015). In this sense, Hoover (2010) reported that HMT facilitates amylose-amylose and amylose-amylopectin interactions that limit the mobility of starch chains in the amorphous region requiring higher temperatures for the granule swelling and consequently higher starch phase transition temperatures.

The impact of HMT on ΔT_{gel} varied depending on the heating time. ΔT_{gel} increased by 16% in HMT2 sample with respect to the native starch evidencing its major heterogeneity of crystalline regions within the starch granule (Zeng, Ma, Kong, Gao, & Yu, 2015), while decreased by 13% in HMT12, suggesting a more homogeneous structure of starch and denoting more perfect amylopectin crystallites (Villanueva et al., 2018b).

The enthalpy of gelatinization, ΔH_{gel}, did not show significant variations ($p>0.05$) during the HMT of cowpea starches, which is in agreement with a study by Hoover & Manuel (1996) for other legume starches. This may be attributed to the starch moisture content (20%) used, being not high enough to contribute to a ΔH_{gel} reduction (starch partial pre-gelatinization) during heat treatments (Villanueva et al., 2018b). However, studies have shown a decrease in ΔH_{gel} of corn (Sui et al., 2015) and rice (Arns et al., 2015) starches reported when HMT was applied. The influence of HMT depends on the botanical source, level of moisture content and amylose content (Hoover, 2010) and by the length of heating (Sui et al., 2015).

Dual HMT-UST increased To (1.9 - 5.3%), Tp (1.5 – 3.2%) and Te (1.8- 4.8%), but to a less extent compared to the individual HMT. This means UST partially reduced the order in the

crystals obtained with HMT. A decrease of starch gelatinization temperatures (Tp and Te) was also reported by Vela et al., (2021) in sonicated rice flour.

Amylopectin retrogradation was evaluated after storing the gelatinized starches at 4 °C for 7 days (Table 1). The Tp of the recrystallized amylopectin decreased to 23.3ºC - 26.4°C with respect to that of native (76.18 °C) and treated (77.33 - 80.41 °C) starches, showing, however, no differences between the two types of treatment (HMT and HMT-UST). The retrogradation enthalpy, ΔH_{ret} reflects the unraveling and melting of double helices formed during storage of gelatinized starch, which is a function of amylopectin unit chain length distribution (Lai, Lu & Lii, 2000). All treated samples presented a greater degree of retrogradation in comparison to the native starch, leading to increases in ΔH_{ret} up to 16.5% in the HMT5 sample. Villanueva et al. (2018b) also reported a significant increase in ΔH_{ret} in microwaved-rice flour. The degree of retrogradation, or ΔH_{ret} /ΔH_{gel} ratio, always increased with treatments, going from 0.65 in the native starch, up to 0.76 in HMT2-UST and HMT5-UST.

3.5 Starch *in vitro* digestibility

Starch fractions of native and treated starches obtained by digestive enzymatic hydrolysis are presented in Table 2. The native starch showed similar values to those obtained for other legume starches (RDS: 8.2%; SDS: 18.6%; RS: 60.6%) (Acevedo et al., 2020). The low total digestibility (RDS + SDS) could be due to the higher content of amylose in cowpea starch in comparison to that of cereal starches (Hoover & Sosulski, 1985; Socorro, Levy-Benshimol, Tovar, 1989; Acevedo et al., 2020).

The starch fractions obtained in HMT2 showed non-significant difference with native starch ($p>0.05$), but RDS, RAG and SDRI decreased significantly with HMT ($p<0.05$), mainly in HMT12 (~53%). Also, RS was improved in HMT5 (11%) and HMT12 (8%) indicating that

HMT reduced the digestible starch fraction. Dupuis, Liu, & Yada, (2014) reported that HMT allows amylose and amylopectin to reach a rubbery state that enables the molecular mobility needed for the establishment of interactions among them and the formation of double helices, thus increasing the general stability of the granule and the RS content. The RAG reduction (HMT12) means that there is less glucose available for absorption in the small intestine and gives an idea of the glycemic response (Englyst et al., 2000).

The RDS, RS, RAG and SDRI values were not significantly different to native starch when HMT-UST was applied ($p>0.05$). However, SDS increased (~30% at 2 h and 5 h) with respect to native starch ($p<0.05$), which represents a nutritional advantage. SDS is associated with positive health effects including improved glycemic control, reduction of postprandial circulated free fatty acids and reduction of oxidative stress (Zeng et al., 2015).

The digestible starch content (RDS+SDS) of all HMT-UST samples increased with respect to HMT samples. This could be explained by the effect of UST, which is more pronounced in the amorphous region of the starch, causing the disruption of the double helices, leading to an increase in digestibility (Monroy et al., 2018).

3.6 Pasting properties

The pasting properties of native and treated starches are shown in Table 3. The pasting behavior of native starch agrees with that previously reported for other cowpea varieties (Kim et al., 2018). The treated starches (HMT and HMT-UST) presented higher pasting temperatures than the native starch denoting that thermal treatments generate changes that prevent swelling of the granules. HMT strengthens the bonds and interactions between the adjacent amylopectin chains, improving the crystalline lamellae within the starch granules (Arns et al., 2015). These results are in accordance with the gelatinization temperatures obtained by DSC and indicate that thermostability was improved. PV is related to the ability

of the granule to swell freely before rupture and depends on the structural arrangements of amylose and amylopectin (Kaur & Singh, 2019). PV of all treated starch samples was significantly lower ($p<0.05$) than that of native starch, leading to substantial reductions: HMT2: 19%; HMT5: 61%; HMT12: 71%; HMT2-UST: 56%; HMT5-UST: 59%; HMT12-UST: 64%. HMT favors the increase of intermolecular bonds in the starch chains, limiting the hydration of the granule. This causes a decrease in swelling capacity and explains the decrease in starch PV (Arns et al., 2015). UST has also demonstrated the ability to modify starch intermolecular bonds, resulting in less compact granular rearrangements and weaker structures leading to a decrease of PV (Luo et al., 2008) or increasing the swelling power leading to an increase of PV, as reported by Jambrak et al. (2010) for corn starch. UST led to a significant decrease in the PV of the sample HMT2, an increase in the value of the sample HMT12 and left sample HMT5 unchanged; consequently, the samples, once sonicated, showed very similar PV values regardless of the duration of HMT. This demonstrates the greater effectiveness of the UST treatments and the reversibility of molecular movements when combining the two treatments.

BV of all treated starches was significantly lower ($p<0.05$) than that of native starch, indicating that the starch granules became stronger (mainly at HMT12 and HMT2-UST), making swelling and rupture more difficult, providing greater thermal and mechanical stability. HMT promotes bond strengthening by increasing the interactions between amylose and amylopectin molecules, forming a more stable structure (Arns et al., 2015). SV, which reflects amylose retrogradation, decreased in all treated samples except in HMT2, which showed the opposite trend (+35%), in parallel to what happened with FV value. HMT reduced the amylose leached from starch granules (Chung et al., 2009) decreasing its retrogradation capacity. However, it seems that very short heat treatments do not allow time

for this change to occur, and on the contrary, molecular changes in the early stages of the process may facilitate amylose leaching and retrogradation by increasing the viscosity of the cold paste. Some authors have reported that longer HMT times may favor the formation of amylose-lipid complexes, decreasing also amylose retrogradation (Arns et al., 2015). The dual treatments HMT5-UST and HMT12-UST did not show significant differences ($p > 0.05$) compared to their respective HMT, indicating that the UST did not cause substantial changes in the starch retrogradation of HMT samples when the treatment time was sufficiently high (HMT5 and HMT12). However, it had a significant effect when applied to the HMT2 sample, demonstrating the ability of UST to modulate the changes obtained by HMT.

4. Conclusion

HMT and HMT-UST induced changes in the physicochemical properties of cowpea starch. NMR evidenced that UST and short-term (2h) HMT decreased the branching degree of amylopectin by destroying α(1,6) bonds while HMT of longer duration (5 h and 12 h) seemed to reduce also α(1,4) bonds. HMT and HMT-UST increased the relative crystallinity of cowpea starch with non-change of X-Ray diffraction pattern and morphology of starch granule. All treatments improved the thermal stability and reduced the viscosity of the pasting profile of cowpea starch. HMT was the most effective treatment to modulate the pasting properties of cowpea starch by means of the control of the treatment time. HMT increased the RS fraction while HMT-UST improved SDS content. The data presented here show that the use of HMT and dual HMT-UST offer great potential for the modification of cowpea starch, increasing the opportunities for its use in different food applications.

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Table 1. Thermal properties of native and treated starches.

Sample	To (°C)	Tp_{gel} (°C)	Te (°C)	ΔT_{gel} (ºC)	ΔH_{gel} (J/g)	Tp_{ret} (°C)	ΔH_{ret} (J/g)
Native starch	69.87±0.14^{f}	76.18±0.07^{e}	82.18±0.66^{d}	12.30±0.59^{b}	12.75±0.47^{a}	52.91±0.78^{bc}	8.05±0.16^{d}
HMT2	71.73±0.08^{d}	77.97±0.09^{c}	85.99±0.59^{ab}	14.26±0.66^{a}	12.45±0.59^{ab}	52.28±1.22^{c}	8.69±0.40^{bc}
HMT5	73.45±0.04^{b}	78.96±0.01^{b}	85.87±0.14^{ab}	12.43±0.18^{b}	12.71±0.34^{a}	54.53±0.06^{a}	9.38±0.21^{a}
HMT12	75.78±0.13^{a}	80.41±0.06^{a}	86.52±0.62^{a}	10.74±0.49^{c}	12.70±0.25^{a}	53.99±0.59^{ab}	8.69±0.01^{bc}
HMT2-UST	71.19±0.01^{e}	77.33±0.06^{d}	85.12±0.26^{b}	13.93±0.27^{a}	12.14±0.18^{ab}	53.57±0.71^{abc}	9.25±0.36^{ab}
HMT5-UST	72.49±0.04^{c}	77.67±0.06^{d}	83.70±0.01^{c}	11.22±0.02^{c}	11.77±0.04^{b}	54.24±0.71^{ab}	8.99±0.21^{abc}
HMT12-UST	73.59±0.30^{b}	78.65±0.33^{b}	86.17±0.50^{a}	12.60±0.20^{b}	12.75±0.21^{a}	53.60±0.62^{abc}	8.50±0.38^{cd}

To, Tp_{gel}, Te: onset, peak and endset gelatinization temperatures, ΔT_{gel}: gelatinization temperature range (Te – To), ΔH_{gel}: enthalpy of gelatinization, Tp_{ret} and ΔH_{ret}: peak temperature and melting enthalpy of recrystallized amylopectin measured in the second scan (after 7 days of storage). Data are the mean ± standard deviation. Values with a letter in common in the same column are not significantly different ($p>0.05$). HMT2 (2 h), HMT5 (5 h), HMT12 (12 h), HMT2-UST, HMT5-UST, HMT12-UST. HMT: heat moisture treatment (100 °C, 20%); UST: ultrasound treatment (20KHz, 30 min, 2 s on - 2 s off).

Table 2. Starch fractions, TS, RAG and SDRI values of native and treated starches.

Samples	RDS (%)	SDS (%)	TDS (%)	RS (%)	TS (%)	RAG (%)	SDRI (%)
Native starch	8.2±1.4^{a}	18.6±1.5^{b}	26.8±0.9^{c}	60.6±1.5^{c}	88.01±1.0^{ab}	9.1±1.5^{a}	9.3±1.6^{a}
HMT2	6.4±0.5^{ab}	21.1±1.0^{ab}	27.5±0.9^{c}	62.1±0.2^{bc}	90.2±1.1^{a}	7.7±1.4^{ab}	7.7±1.4^{ab}
HMT5	4.9±0.3^{bc}	18.8±0.9^{b}	23.7±0.5^{b}	67.2±1.2^{a}	90.5±1.5^{a}	5.4± 0.3^{ab}	5.4±0.3^{bc}
HMT12	3.8±0.5^{c}	17.4±0.7^{b}	21.2±0.7^{a}	65.6±1.1^{ab}	88.7±2.7^{ab}	4.3± 0.5^{b}	4.3±0.5^{c}
HMT2-UST	7.6±0.3^{a}	24.5±0.9^{a}	32.1±0.5^{d}	59.6±1.2^{c}	90.9±2.7^{a}	8.5± 0.4^{a}	8.6± 0.5^{ab}
HMT5-UST	7.5±0.7^{a}	24.1±1.4^{a}	31.6±0.5^{d}	58.8±2.6^{c}	91.1±2.3^{a}	8.5± 0.9^{a}	8.3±0.6^{ab}
HMT12-UST	6.9±1.1^{ab}	18.7±1.5^{b}	25.6±0.3^{bc}	58.6±1.4^{c}	83.5±1.8^{b}	7.7± 1.7^{ab}	8.4±1.3^{ab}

RDS: rapidly digestible starch, SDS: slowly digestible starch, TDS: Total digestible starch: RDS+SDS; RS: resistant starch, TS: total starch, RAG: rapidly available glucose, and SDRI: starch digestion rate index. Data are on dry basis (except SDRI) and the mean ± standard deviation. Values with a letter in common in the same column are not significantly different ($p>0.05$). HMT2 (2 h), HMT5 (5 h), HMT12 (12 h), HMT2-UST, HMT5-UST, HMT12-UST. HMT: heat moisture treatment (100 °C, 20%); UST: ultrasound treatment (20KHz, 30 min, 2 s on - 2 s off).

Table 3. Pasting properties of native and treated starches.

Sample	PV (Pa·s)	TV (Pa·s)	BV (Pa·s)	FV (Pa·s)	SV (Pa·s)	PT (°C)
Native starch	6.18±0.01^{a}	3.84±0.01^{b}	2.35±0.02^{a}	6.55±0.15^{b}	2.71±0.15^{b}	85.23±0.28^{e}
HMT2	4.99±0.01^{b}	4.49±0.01^{a}	0.51±0.00^{b}	8.16±0.00^{a}	3.67±0.01^{a}	94.66±0.01^{a}
HMT5	2.41±0.02^{e}	2.19±0.01^{e}	0.22±0.01^{d}	3.28±0.00^{d}	1.08±0.01^{d}	89.51±0.45^{c}
HMT12	1.81±0.03^{g}	1.75±0.01^{g}	0.06±0.02^{e}	2.29±0.04^{f}	0.54±0.04^{e}	90.33±0.09^{b}
HMT2-UST	2.74±0.01^{c}	2.66±0.03^{c}	0.09±0.02^{e}	4.24±0.01^{c}	1.58±0.04^{c}	88.57±0.14^{d}
HMT5-UST	2.52±0.04^{d}	2.33±0.04^{d}	0.19±0.00^{d}	3.34±0.03^{d}	1.02±0.00^{d}	88.84±0.19^{d}
HMT12-UST	2.19±0.01^{f}	1.89±0.03^{f}	0.29±0.01^{c}	2.55±0.02^{e}	0.65±0.01^{e}	90.24±0.09^{b}

PV: peak viscosity, TV: trough viscosity, BV: breakdown viscosity, FV: final viscosity, SV: setback viscosity, PT: pasting temperature. Data are the mean ± standard deviation. Values with a letter in common in the same column are not significantly different ($p>0.05$). HMT2 (2 h), HMT5 (5 h), HMT12 (12 h), HMT2-UST, HMT5-UST, HMT12-UST. HMT: heat moisture treatment (100 °C, 20%); UST: ultrasound treatment (20KHz, 30 min, 2 s on - 2 s off).

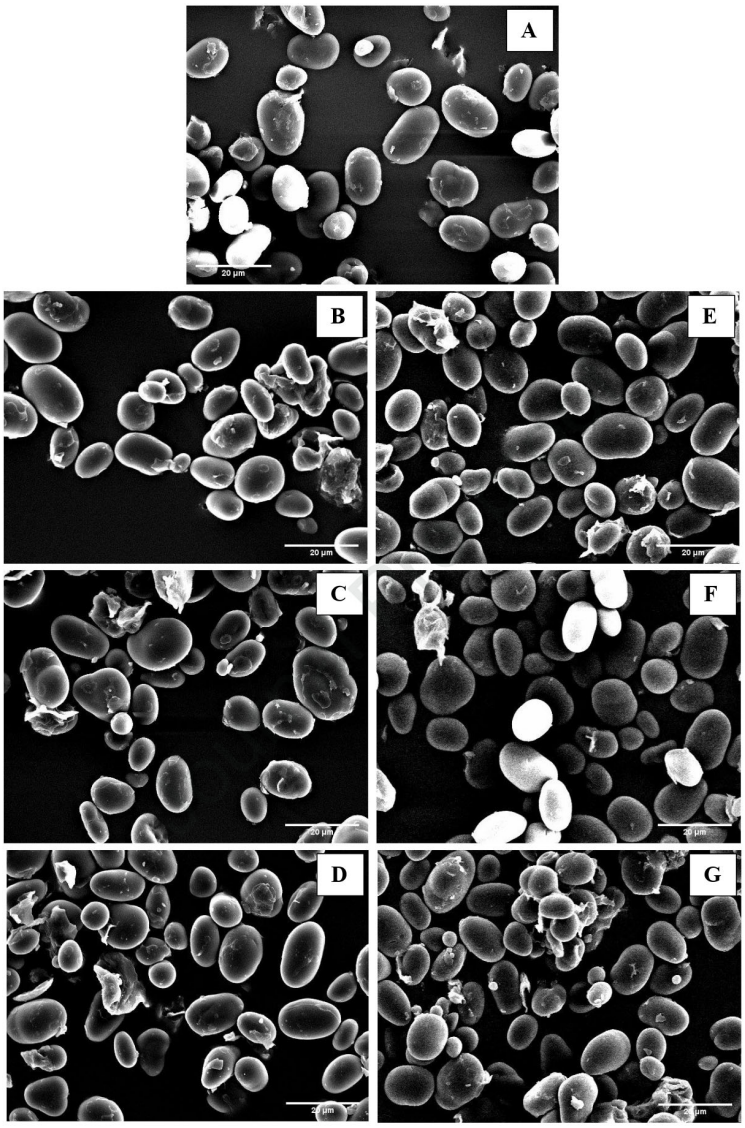

Figure 1. SEM pictures of starches at magnifications of 1000x. A) native starch, B) HMT2 (2 h), C) HMT5 (5 h), D) HMT12 (12 h), E) HMT2-UST, F) HMT5-UST, G) HMT12-UST. HMT: heat moisture treatment (100 °C, 20%); UST: ultrasound treatment (20KHz, 30 min, 2 s on - 2 s off).

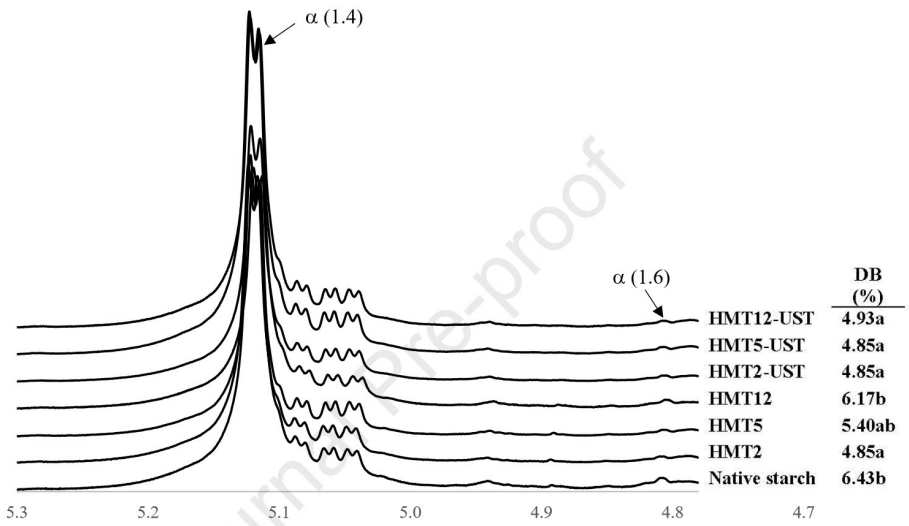

Figure 2. ^{1}H NMR spectra and degree of branching (%DB) of native and treated starches. The %DB values followed by a letter in common are not significantly different ($p>0.05$). HMT2 (2 h), HMT5 (5 h), HMT12 (12 h), HMT2-UST, HMT5-UST, HMT12-UST. HMT: heat moisture treatment (100 °C, 20%); UST: ultrasound treatment (20KHz, 30 min, 2 s on - 2 s off).

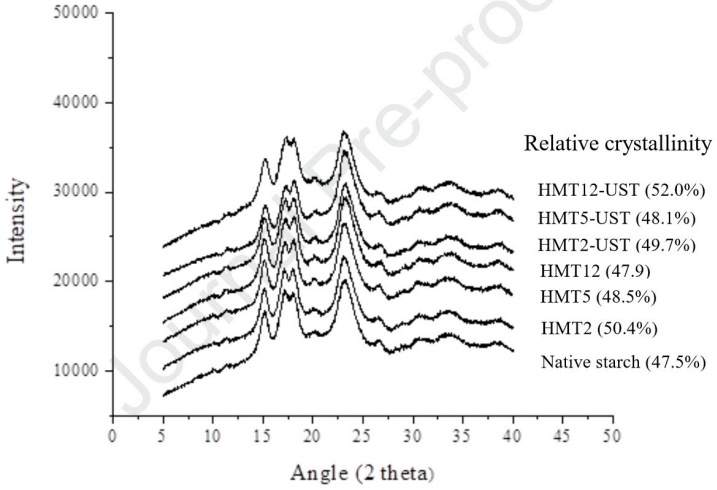

Figure 3. X-ray diffraction patterns of native and treated starches. HMT2 (2 h), HMT5 (5 h), HMT12 (12 h), HMT2-UST, HMT5-UST, HMT12-UST. HMT: heat moisture treatment (100 °C, 20%); UST: ultrasound treatment (20KHz, 30 min, 2 s on - 2 s off).

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Highlights

- Heat Moisture Treatment (HMT) and HMT + Ultrasound (HMT-UST) were applied
- HMT and HMT-UST modified the physicochemical properties of cowpea starches.
- Starch α (1,6) bonds were more sensible to HMT and HMT-UST than α (1,4) bonds.
- HMT and HMT-US improved the thermal stability and reduced the pasting viscosity
- HMT increased the RS fraction meanwhile HMT-UST improved SDS content.

Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.