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Characterization of red and white cocoyam (*Xanthosoma sagittifolium*) roots, flours and starches during heating by low field NMR

María Gudjónsdóttir[a], Abena Achiaa Boakye[b], Faustina Dufie Wireko-Manu[b], Ibok Oduro[b]
[a]University of Iceland, Faculty of Food Science and Nutrition, Vínlandsleid 14, 113 Reykjavík, Iceland. E-mail: mariagu@hi.is
[b]Kwame Nkrumah University of Science and Technology, Department of Food Science and Technology, UPO, PMB, Kumasi, Ghana.
Corresponding Author: abenaboakye13@gmail.com

Cocoyams are important root and tuber staples in West African communities, but limited information exists on their physicochemical and processing characteristics. In this study low field proton relaxation analysis was used to characterize the water distribution and gelatinization behaviour during cooking of red and white cocoyam roots, as well as water dispersions of their corresponding processed flours and purified starches. Up to four fast-interacting water populations were observed in the roots, identifying water associated with starch (T_{2a}≈1.5 ms), water interacting with the cell walls (T_{2b}=5-10 ms), water in the cytoplasm (T_{2c}=13-54 ms), and water in vacuoles/extracellular water (T_{2d}=51-246 ms). Two populations were observed in 10% (w/w) flour and starch dispersions. All relaxation parameters were sensitive towards swelling and gelatinization in the flours and starches, but T_{22} was more sensitive towards water expelled from the matrix at temperatures above gelatinization (approx. 80°C in the roots and flours, and 75°C in the starch). Shorter relaxation times observed in the white variety roots, and a higher proportion of more restrained water, indicated that the white root variety was slightly more sensitive towards forming a gel, and that it held a higher proportion of water after gelatinization. A higher proportion of more restrained water was also observed after retrogradation in the starch and flour suspensions from the white variety. The observed differences are believed to mainly relate to differences in the starch characteristics of the two varieties, including a higher amylose/amylopectin ratio in the white roots. Furthermore, the study showed that the roots have much wider potential than their current utilization.

Introduction

Xanthosoma sagittifolium, commonly referred to as *tannia* or cocoyam, is an important root in most West African communities. With over 10 million tonnes produced in 2012 and a proven potential to alleviate hunger and significantly contribute to food security, the crop remains an underexploited food resource. In Ghana, where root and tuber crops contribute to more than 40% of the agricultural GDP[1], is cocoyam the third most important root crop. Only two commercially cultivated varieties of the roots exist on the market[2], as well as three other released (but not yet commercialized) varieties. Endeavours made to better understand and predict the food properties of cocoyam varieties have largely focused on assessing the chemical, pasting and functional properties of flours and starches by use of traditional methods[3-8]. However, limitations of these studies have been recognized in predicting the overall effect of processing on the final quality of foods with regards to water distribution and transitions of the food material[9]. Thus, more sensitive methods are needed to explore the potentials of cocoyam in a quest to expand its utilization and contribute to food security measures.

Low Field Nuclear Magnetic Resonance (LF-NMR) relaxometry has become a commonly used tool in food research. This non-destructive analytical method has a unique advantage in determining the distribution and mobility of water and lipid protons of various food materials, in addition to the analysis of the dynamic changes that occur in the food matrix during processing. Amongst others, it has been used to predict the sensorial characteristics, cooking properties and dry matter content of temperate crops, such as potatoes (*Solanum tuberosum*)[10-12]. The use of LF-NMR in analytical studies of tropical crops for assessing their potential food uses is, however, limited.

The aim of this study was therefore to investigate the water distribution and transitions during heating of roots, starches and flours of the two commercially cultivated varieties (red and white varieties) of *Xanthosoma saggitifolium* in Ghana using LF- NMR relaxometry, in order to shed light on further utilization possibilities of this tropical root crop species.

Materials and methods

Raw materials and processing of flours and starches

Roots of *Xanthosoma sagittifolium* of the red and white varieties were obtained from the Birim North District in the Eastern region of Ghana. Roots were processed into flours and starches on the fourth and eighth day after harvest, respectively. For production of flours roots from each variety were peeled and sliced by hand using stainless steel knives. The pieces were washed and soaked in potable tap water for an hour before drying at 60°C in a hot-air oven dryer for 10 hours. After cooling the samples were milled once with a hammer mill, and subsequently twice in an attrition mill. The flours were finally sieved in order to produce flours with particle sizes below 425 µm (laboratory test sieve (ISO 3310-1:2000, BS 410-1:2000, UK).

For the preparation of starches roots of each variety were manually peeled and washed with potable tap water and subsequently mashed with a hammer mill. The mash was mixed with potable tap water in the ratio 1:2. The obtained slurry was washed with potable tap water through a double-layered cheese (muslin) cloth to remove cell debris. The filtrate was kept overnight in plastic containers for starch sedimentation. The supernatant was discarded and the sediment (starch) was washed 3-5 times with potable tap water to remove any traces of dirt. The washed starch was transferred unto drying trays and solar dried for 48 hours. The dried starch was then milled into fine powder using a Phillips home blender. The flour and starch samples were packaged in high density zip-lock bags until further use.

Physicochemical analysis

The water content was calculated by assessing weight changes before and after drying of 2 g of each root samples in on oven at 110°C for 24 hours. Ten roots were used from each variety for the physicochemical analyses.

The total starch was determined by the anthrone method[13]. A sample of 0.5 g was homogenised in hot 80% ethanol to remove sugars. The mixture was centrifuged and the supernatant discarded. The residue was washed repeatedly with hot 80% ethanol until the washings did not give any colour with an anthrone reagent. It was then dried over a water bath. 5.0 mL of water and 6.5 mL of 52% perchloric acid was added and extracted at 0°C for 20 min. The extract was centrifuged and the supernatant kept. The extraction was repeated using fresh perchloric acid. 0.2 mL of the supernatant was pipetted, then topped up with distilled water to 1 mL and heated for eight minutes. The absorbance was read at 630 nm. Standard glucose solutions were prepared for concentrations of 0.2, 0.4, 0.6, 0.8 and 1% and 4 mL of anthrone reagent was added to each solution. This was heated for eight minutes in a boiling water bath, cooled rapidly and the absorbance, read at 630 nm. The glucose content in the sample was determined from the standard curve and multiplied by a factor off 0.9 to obtain the starch content.

The amylose content was determined by a calorimetric method[14]. 1 ml ethanol and 10 ml of 1N NaOH were added to 100 mg of sample and kept overnight. The solution was topped up with 20 ml of distilled water and three drops of phenolphthalein were added and 0.1 N HCl added dropwise until the pink colour disappeared. 1 mL of iodine reagent was added and the volume topped up to 50 mL with distilled water. The absorbance was read at 590 nm. The protocol was repeated for varying concentrations (0.2 – 1%) of a standard amylose solution to develop a standard curve. 1 mL of iodine reagent was diluted to 50 mL with distilled water and used as a blank. In calculating the % amylose, it was estimated that the absorbance corresponded to 2.5 mL of the test solution. The amylopectin was determined as the difference between the total starch content and the amylose content.

LF-NMR analysis

The water distribution and characteristics of the roots, flours and starches were performed by transverse relaxation time analysis by low field NMR on a 23 MHz Maran benchtop analyzer (Resonance Instruments, Witney, UK) with an 18 mm temperature adjustable probe. Samples from the middle portion of the white and red root varieties (n=10 for each variety) were cut and weighed. The samples were placed in small sample tubes (45x15x0.6 mm) and covered with a plastic cap. These were then inserted into longer test tubes (180x17.75x0.6 mm) prior to their introduction to the magnet. Aqueous dispersions of the flours and starches with a concentration of 10% (w/w) were prepared by dissolving the flours and starches in distilled water. Approximately 3 mL samples were then pipetted into the small sample tubes mentioned earlier.

Measurements were performed at several temperatures in the range from 25 to 90°C and after overnight cooling at 25°C. A pre-set water bath was used to equilibrate the temperature of the samples for at least 35 min at each temperature prior to the NMR analysis. A Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with an interpulse spacing τ of 150 µs was used for the roots, but 500 µs for the flours and starches. Eight repeated scans were used, 2048 collected echoes (every other echo collected) per scan and 6 s repetition time between scans. The obtained relaxation data was fitted to a multi-exponential function using the Low-Field NMR Toolbox for Matlab[15] (The Mathworks Inc., Natric, MA, USA).

Results and discussion

Physicochemical results

Results from the physicochemical analysis of water, starch amylose and amylopectin content of the roots, flours and starches can be viewed in Table 1. No significant difference was observed in the water content between the red (55.4 ± 4.7%) and white (52.6 ± 3.3%) varieties of the roots. A slightly higher water content was though observed in the flour of the white variety (6.50 ± 0.10%) than in the red variety (5.46 ± 0.08%). No significant difference was observed in the water content of the two starch powders.

A significantly higher starch content was observed in the red variety for both the flours and produced starch powders. Analysis

Table 1. Physicochemical results of water content, starch content and amylose and amylopectin content in the roots, flours and starches from both the red and white varieties.

Sample group	Water content [% w/w]	Starch [% w/dry matter]	Amylose [% w/dry matter]	Amylopectin [% w/dry matter]
Roots – Red	55.4 ± 4.7			
Roots - White	52.6 ± 3.3			
Flour powder - Red	5.46 ± 0.08	58.65 ± 1.17		
Flour powder - White	6.50 ± 0.10	44.06 ± 0.11		
Starch powder - Red	12.06 ± 0.13	96.00 ± 0.15	37.65 ± 0.00	58.35 ± 0.07
Starch powder - White	11.71 ± 0.88	89.12 ± 0.03	52.38 ± 0.02	36.73 ± 0.02

of the amylose and amylopectin contents in the starch powders indicated that the red variety contained a high amylopectin content (amylose/amylopectin ratio of 0.65), while the white variety was high in amylose content (amylose/amylopectin ratio of 1.43).

LF-NMR results

The obtained LF-NMR spectra of the roots indicated the presence of up to four fast interacting water proton populations. These were identified as water associated with starch (T_{2a}≈1.5 ms), water interacting with the cell walls (T_{2b}=5-10 ms), water in the cytoplasm (T_{2c}=13-54 ms), and water in vacuoles/extracellular water (T_{2d}=51-246 ms), in fair agreement with the water distribution in potatoes[16]. However, heating of the roots led to fast proton exchange between the populations, which led to the merging and reappearance of populations during the heating process. Bi-exponential changes of the roots have earlier been described in detail and showed a gradual increase in the more rigid water population during heating[17]. This increase was related to swelling of the starch granules, as well as increased proton mobility due to the increased temperature. The proportion of restrained water was higher in the white variety during the heating process, indicating more swelling of the granules in the white variety than in the red. Furthermore, generally lower T_2 relaxation times were observed in the roots of the white variety roots, compared to the red variety roots, supporting that the water in the white variety was more effectively restrained than the water in the red roots.

When comparing the flours and starches from the two varieties all products showed bi-exponential behaviour. Protons with T_2 values around or below 10 ms, commonly assigned to CH protons of starch in crystalline or rigid amorphous structures[18], were not

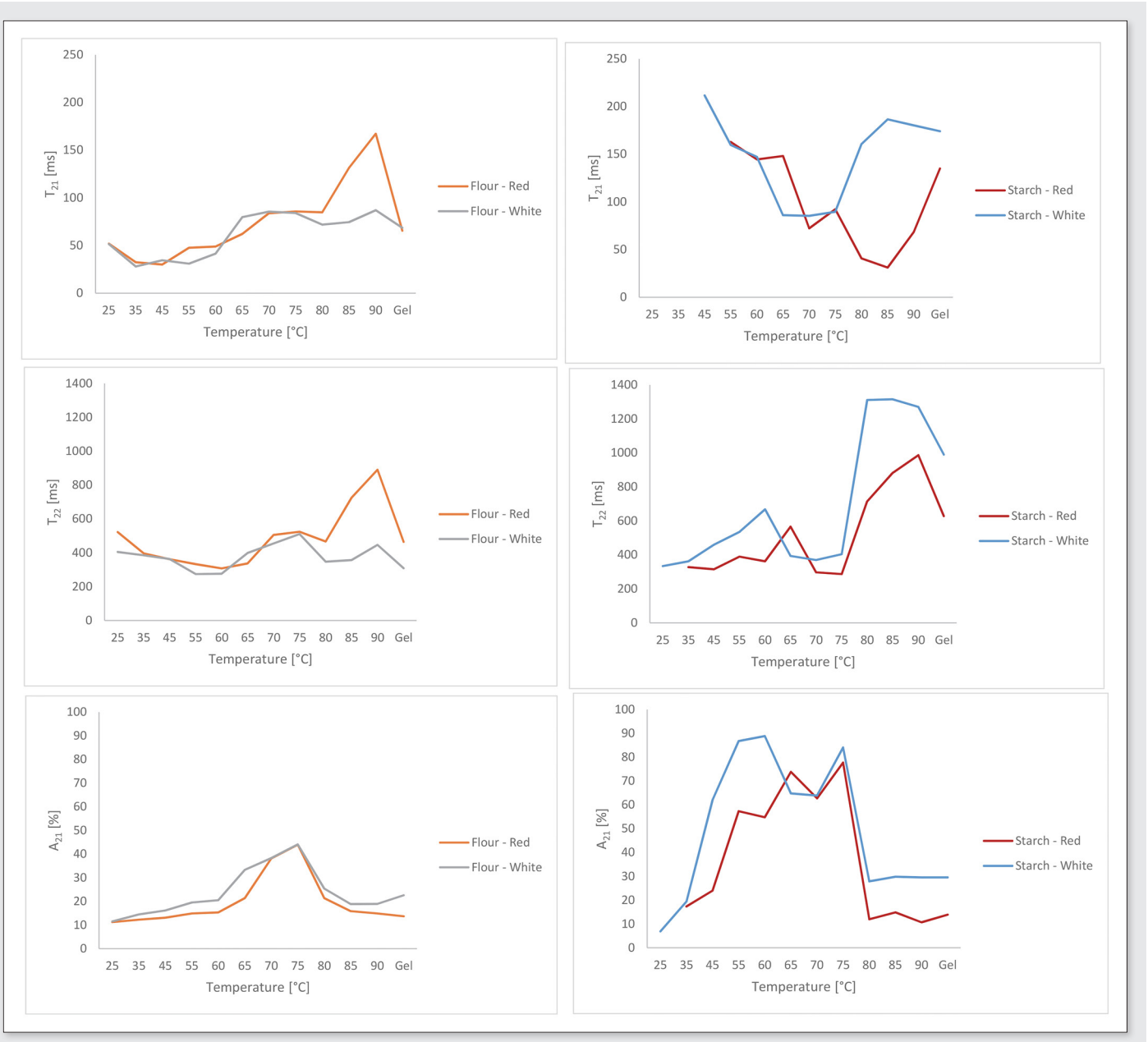

Figure 1. Carr-Purcell-Meiboom-Gill (CPMG) transverse relaxation times T_{21} (top) and T_{22} (middle) for flours (left) and starches (right) from the red and white cocoyam varieties, as well as the apparent more restricted population A_{21} (bottom).

detected with the current settings. However, their contact and interaction with water is very limited and therefore more focus on the less restricted water populations is appropriate. The gelatinization and retrogradation effects were generally more subtle in the flours than in the starches, possibly due to the impurities present in the flours. As the temperature increased, from ambient temperature up to approximately 60°C, a higher proportion of water was interacting with the flours due to starch swelling and leaching of amylose[16].

A peak in T_{22} was observed at 60°C and 65°C in the white and red starch solutions, respectively. This peak is believed to relate to the gelatinization of amylopectin crystal residues, which commonly occurs in the range from 60-70°C, dependent on the type of starch studied[16]. However, such clear transitions of the gelatinizing amylopectin were not observed in the flours, possibly due to the presence of other substances and impurities in the flours. Additional swelling of the starch granules was observed at higher temperatures until gelatinization initiation was observed, as indicated by a fast reduction in the apparent population of the rigid water protons A_{21}[17]. However, a sharp increase in T_{22} was observed simultaneous to the sudden release of water from the gel network to the less restricted population during the gelatinization. This was observed at 75°C in the starch suspensions, while gelatinization was observed at higher temperatures in the red (75-80°C) and white (80-85°C) flour suspensions.

Due to the dominance of the water in the more mobile population after heating, the changes caused in the matrix due to retrogradation during overnight cooling was best described by the slower relaxation time T_{22}. A significant decrease was observed in this parameter for all flours and starches during the overnight cooling, indicating the rearranging of the starch towards a more crystalline and rigid structure. The insignificant changes in the apparent water populations however indicated that no substantial changes in the water distribution were included in the retrogradation process. After the retrogradation process the white variety had a higher proportion of restricted water than the red variety, for both starches and flour suspension, as well as the raw roots. This indicates that the products from the white variety generally retain water more effectively than the products from the red variety of the cocoyam roots. However, the strength of these gels, as indicated by lower relaxation times, especially T_{22}, was higher in the red starch suspensions than in the white, while the opposite trend was observed between the flour suspensions. Generally, more energy would be required to break up bonds to gelatinize high amylose starches into its starch molecules, leading to the formation of more rigid gels. In the current case the purity and absolute content of starch in the flours and starch dispersions may have a larger impact on the gel strength than the amylose/amylopectin ratio.

Conclusions

A detailed analysis on the gelatinization characteristics of cocoyam roots from the white and red variety, and their subsequent flours and starches was performed with LF-NMR. The study indicated that the proportion of amylose and amylopectin had a significant effect on the gelatinization characteristics of the in both roots, flours and starches from the two varieties. Furthermore, the white variety was shown to be more prone to retain water after retrogradation of the gels, in agreement with its higher amylose content. However, the absolute starch content and its purity might have a greater effect on the final gel strength of the starch suspensions. This information is important for the cocoyam producers in Ghana, since it sheds further light on how these species can be further utilized.

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