

# Histological and biophysical changes of cassava roots during retting, a key step of fufu processing

Germaine-Alice Wakem,<sup>a,b</sup> Libert Brice Tonfack,<sup>a</sup> Emmanuel Youmbi,<sup>a</sup> Apollin Fotso-Kuate,<sup>c</sup> Cargele Masso,<sup>c</sup> Komi K. M. Fiaboe,<sup>c</sup> Rose Ndango,<sup>c</sup> Isaac Tizé,<sup>c</sup> Joel Grabulos,<sup>b,d</sup> Dominique Dufour,<sup>b</sup> Robert Ndjouenkeu<sup>e</sup> and Didier Mbéguie-A-Mbéguie<sup>b,d,f\*</sup>



## Abstract

**BACKGROUND:** Retting is a key step of cassava processing into widely consumed foods (fufu, chikwangue, miondo and bobolo) in sub-Saharan Africa. For some populations, retting ability is a major quality criterion that drives the adoption of new cassava varieties. Despite this importance, the physiological basis associated with this process remains poorly understood, and should lead to improved screening tools for breeding. Eight cassava varieties contrasting in retting ability properties were used in the present study. Roots and soaking water were sampled during retting and characterized at both histological and biochemical levels.

**RESULTS:** Histological data highlighted the degradation of root cell wall during retting. The average pH of soaking water decreased from 5.94 to 4.31 and the average simple sugars decreased from 0.18 to 0 g L<sup>-1</sup>, whereas the organic acids increased up to 5.61 g L<sup>-1</sup>. In roots tissue, simple sugars and organic acid contents decreased from 22.9 to 0 g kg<sup>-1</sup> and from 80 to 0 g kg<sup>-1</sup>, respectively. The total pectin content of roots among varieties at harvest was similar, and decreased during the retting process. Overall, there was a negative correlation between total pectins content and root softening, although this did not reach statistical significance.

**CONCLUSION:** Major histological and biochemical changes occurred during cassava root retting, with some of them associated with the process. Retting affected starch pasting properties more than starch content. Although this process is characterized by root softening and degradation of cell wall structure, the present study strongly suggested that pectin is not the only cell wall component involved in these changes.

© 2023 The Authors. *Journal of The Science of Food and Agriculture* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Supporting information may be found in the online version of this article.

**Keywords:** *Manihot esculenta*; retting process; root firmness; starch pasting properties

## INTRODUCTION

Cassava plays a major role in food security for the African population with an average annual consumption of 100 kg of roots per capita,<sup>1</sup> represented by a wide diversity of culinary recipes.<sup>2,3</sup> Up to 70% of cassava peasant production is processed,<sup>4</sup> with fermentation representing the major unit operation.<sup>5</sup> Retting is one of the major fermentation procedures, consisting of soaking cassava roots in water for a period of 3–7 days. This softens the cassava flesh (main expectation of the processors) for malleability into further products (bobolo, miondo, fufu, chikwangue etc.) and detoxifies the roots from its cyanogenic glucosides.<sup>6</sup> In addition, production of organic acids confers these products with a characteristic typology.<sup>7,8</sup>

The retting process has already been the subject of numerous studies focusing mainly on its kinetics and related factors<sup>9,10</sup> and on the biochemical and microbiological characterization of

\* Correspondence to: D Mbéguie-A-Mbéguie, CIRAD, UMR Qualisud, 73 Rue Jean Francois-Breton, 34398 Montpellier Cedex 5, France. E-mail: didier.mbeguie-a-mbeguie@cirad.fr

a Plant Physiology and Improvement Unit, Laboratory of Biotechnology and Environment, Department of Plant Biology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

b CIRAD, UMR Qualisud, Montpellier, France

c International Institute of Tropical Agriculture, Yaoundé, Cameroon

d Qualisud, Univ Montpellier, CIRAD, Univ d'Avignon, Institut Agro, IRD, Univ de La Réunion, Montpellier, France

e Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Université de Ngaoundéré (Cameroun), Ngaoundéré, Cameroon

f Université Nangui Abrogoua, Abidjan, Côte d'Ivoire

involved enzymes.<sup>6,10-15</sup> Optimization of the retting process parameters was also investigated using suitable starters and assessing their effect in the product<sup>12,13,16-19</sup> to optimize and standardize the retting process, as well as the quality of the end-products. Based both on its central position in cassava processing and on biochemical phenomena involved in the process, retting can influence the acceptability and the adoption of cassava varieties, in terms of their retting ability. Indeed, a 'high retting capacity' is one of the main quality traits expected by the end users from new cassava hybrids as it influences the organoleptic properties (color, aroma, texture) of the end products (fufu, miondo, min-tumba, etc.) and their overall acceptance.<sup>20</sup>

Retting ability is a complex quality trait of which elaboration is the result of a coercive action of several genetic and environmental factors. The improvement of such trait by breeding requires a better knowledge of the physiological bases that govern their components, as well as how the related mechanism are regulated. Indeed, the malleability efficiency of cassava flesh expected by processors on retting and the resulting effect of the latter on organoleptic properties of end-products are linked to histological modifications in cassava flesh, in that the structural disorganization of cells during soaking both facilitates the malleability and is assumed to enable multiple interactions and chemical reactions among the flesh components. Hence the interest in understanding the histological changes on retting and, moreover, the physiological base of retting process. This would provide biochemical and molecular indicators that can be used as early phenotyping and selecting tools of promising hybrids that meet consumer expectations.

The present study focuses on the physiological bases associated with retting in cassava roots, aiming to identify biophysical indicators related to the retting ability.

To this end, a selected group of cassava genotypes, contrasting in their retting ability, was used to undertake a comparative biochemical characterization and histological analysis.

## MATERIALS AND METHODS

### Source and retting treatment of plant material

A set of eight cassava genotypes including hybrids developed by the International Institute of Tropical Agriculture (IITA), with improved agronomic and nutritional performance, were selected. These genotypes comprised four with yellow-pulp (01/0040-27, 01/1797, I090593 and I071026; hereafter referred to as YP1, YP2, YP3 and YP4, respectively) and four with white-pulp (LMR, 92/0326, I090616 and local variety; hereafter referred to as WP1, WP2, WP3 and WP4) were used in the present study. The cassava genotypes were grown in the research station of the Institute of Agricultural Research for Development (IRAD) of Mbalmayo, Cameroon (N 03°31', E 11°30', altitude: 335 m), in partnership with IITA under the Agricultural Investments and Market Development Project (AIMDP) trials. Conventional cultural practices without fertilizers or herbicides<sup>21</sup> were followed. Cassava roots were harvested 14 months after planting.

For each genotype, approximately 30 kg of roots were harvested from an average of 10 plants randomly selected in the field. The roots were peeled, washed and divided into three batches of approximately 10 kg each. Each batch was soaked in an individual container containing tap water for 3 days in accordance with the traditional retting method used by the local populations.

The soaking water sample (approximately 2 mL) was filtered through a 45- $\mu$ m cellulose acetate membrane (Legallais, Montferrier, France) and stored at 4 °C until analysis by HPLC. For roots, two samples of approximately 100 and 250 g were collected every 24 h from each fermentation batch for microscopy and biochemical analysis, respectively. For microscopy analysis, the root sample was stored at 4 °C in a fixing buffer (glutaraldehyde 3%, phosphate buffer 0.1 M, pH 7.4) until used. The second root sample was freeze-dried, then ground with a hammer mill (Perten Laboratory Mill 3100; Perking Helmer, Villebon-sur-Yvette, France) and the resulting flours packaged in plastic bottles with screw caps and stored at -20 °C for further biochemical and functional analyses.

### Physical characterization of cassava retting process

#### Soaking water pH measurement

The pH of soaking water was directly measured at room temperature during retting using a portable pH meter (Multi 3630 IDS SET; Xylem Analytics, Nanterre, France).

#### Roots histology analysis

The preparation of the samples for histology analysis was carried out as described by Ngolong *et al.*<sup>8</sup> except that cassava flesh sections were embedded in paraffin and stained successively with Schiff reagent and methyl blue. The histology observations were made at magnification 200 $\times$  using an optical microscope (Scientico STM-50; Leitz, Wetzlar, Germany).

#### Softening measurement

Cassava root softness was assessed using a texturometer (TA-XTPPlus; Stablemicrosystems, Swantec, Gennevilliers, France) in accordance with the method described by Mbéguié *et al.*<sup>22</sup> The softer the root, the lower its firmness. Measurement was performed on root samples taken at 0, 24 and 48 and 72 h after beginning of the retting process. Retting rate was defined as the percentage loss of firmness calculated for a given sampling point. It was calculated according to:

$$rr = \frac{F_i - F_f}{F_i} \times 100$$

where *rr* is the retting rate, *F<sub>i</sub>* is the initial firmness value measured on fresh root and *F<sub>f</sub>* is the firmness of fermented root at the sampling point.

### Biochemical characterization of cassava retting process

#### Fermentable sugars, alcohol and organic acids

Fermentable sugars and organic acids contents of soaking water were quantified by analyzing the sample directly by HPLC according to the procedure described by Hor *et al.*<sup>23</sup> The equipment used was a Dionex Ultimate 3000 HPLC system (Thermo Fisher, Illkirch-Graffenstaden, France) equipped with an analytical auto-sampler (WPS-3000 TSL; Thermo Fisher), an Aminex column (Aminex HPX-87H 300  $\times$  4.6 mm; Bio-Rad, Marnes la Coquette, France) and an UltiMate 3000 diode array detector. For root flour, fermentable sugars and organic acids were extracted as described Mestres *et al.*<sup>24</sup> and analyzed by HPLC as described above.

#### Pectin measurement

The total pectins of flours were extracted and quantified in accordance with the method described by Mestre *et al.*<sup>25</sup>

### Starch content and amylose content

The determination of the starch content was carried out as described by Jourda *et al.*<sup>26</sup> according to the modified Holm enzymatic method.<sup>27</sup> Glucose was quantified by HPLC as described above. The amylose content of the cassava flour samples was determined by differential scanning calorimetry (DSC) using a DSC 8500 apparatus (Perkin-Elmer, Norwalk, CT, USA) in accordance with the method described by Mestres *et al.*<sup>28</sup>

### Pasting properties of cassava flours

A Rapid Visco Analyzer (RVA 4500; Perten Instrument, Newport, NSW, Australia) was used to assess the pasting properties of native and retted cassava flours in accordance with the method developed by Batey *et al.*<sup>29</sup> Peak (PV), breakdown (BD) and setback (SB) viscosities were the main parameters assessed.

### Statistical analysis

All analyses were performed at least in triplicate and the results expressed as the mean  $\pm$  SD. Analysis of variance of the means made it possible to compare the influence of the sources of variation (genotype and retting time) on the responses measured. Tukey's test was used to rank significantly different means ( $P = 0.5$ ), using XLSAT software 2021 (XLSAT, Paris, France).

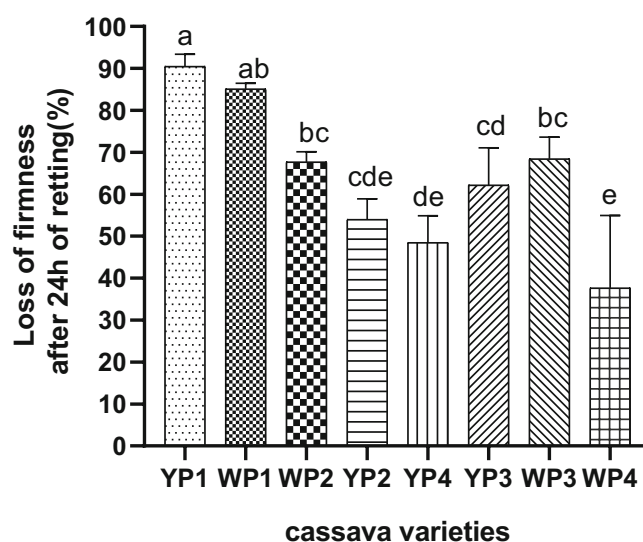
## RESULTS

### Physical characterization of contrasting cassava varieties after retting of roots

After 24 h of retting, the soaking water of all cassava varieties resulted in an acidic pH value of around 4.5 (see Supporting information, Fig. S1). Marked softening changes were observed at the same time on roots, considered as an indicator of their retting ability. According to the percentage of firmness loss after 24 h, YP1 and WP1 appeared as fast-retting rate varieties (90.46% and 85.08%, respectively), whereas varieties WP2, YP2, YP3, YP4 and WP3 were medium-retting rate (50–70%) and WP4 demonstrated a slow-retting rate (37.70%) (Fig. 1).

In an attempt to assess the putative relationship between roots softening and changes of root cell wall structure, histological analysis was performed and the results are shown in Fig. 2. At harvest stage and regardless of genotype, all cells except those from the xylem exhibited a consistent structure and contained the starch granules (red color). Xylem cells were larger with a thick wall surrounded by companion cells. At this stage, except WP4, which exhibited plasmolyzed cells (Fig. 2Hh), cells from all varieties had also a turgid appearance (Fig. 2A–G). After 72 h of retting, there was a loss of integrity of the root tissue and root cell wall degradation, leading to the observed loss of firmness (Fig. 2, right). This degradation was more pronounced in some varieties than in others. Indeed, the integrity of the cell structure was completely lost for YP1, YP4 and WP4, with a total disappearance of the cell walls and a disintegrated structure with starch granules as the main visible element. Genotypes WP3 and YP2 presented a cohesive cell structure with continuous cell walls, whereas WP1 presented a structure with cells that dissociated from each other with the intracellular starch granules, revealing clearly defined cell limits.

Because of the contrasting softening behavior observed for different varieties, YP1 and WP1 were considered as those which retted quickly and two others (WP2 and YP2) were selected from the group of varieties with medium softening speed. These four



**Figure 1.** Percentage loss of firmness of cassava roots of different genotype taken after 24 h of retting. Genotypes considered are hybrids with yellow flesh (YP1, YP2, YP3 and YP4), hybrids with white flesh (WP3 and WP2) and traditional varieties (WP1 and WP4).

genotypes were further used as contrasting models for advanced biochemical analysis.

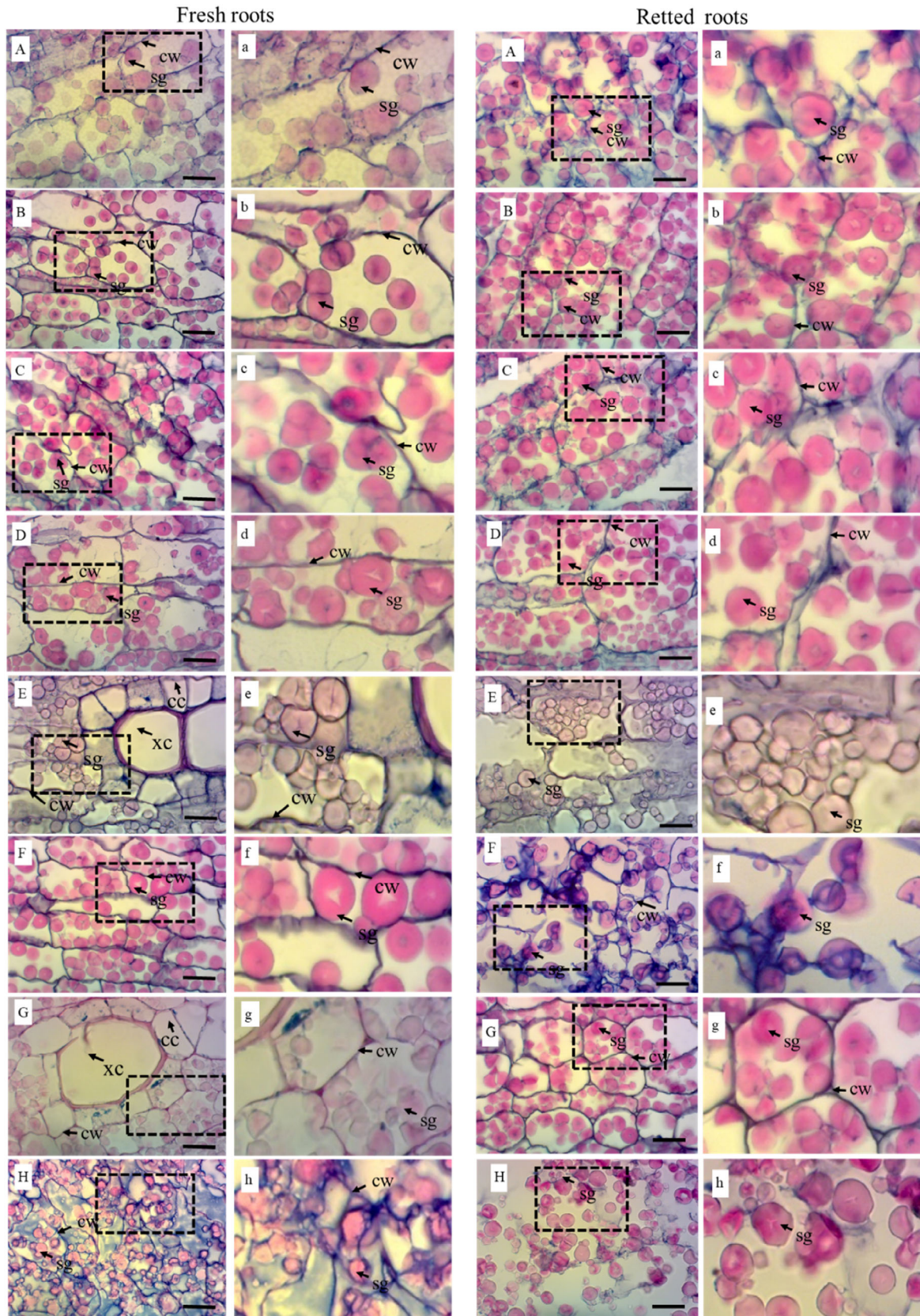
### Biochemical characterization of cassava retting process

#### Changes of sugar, alcohol and organic acids during the retting process

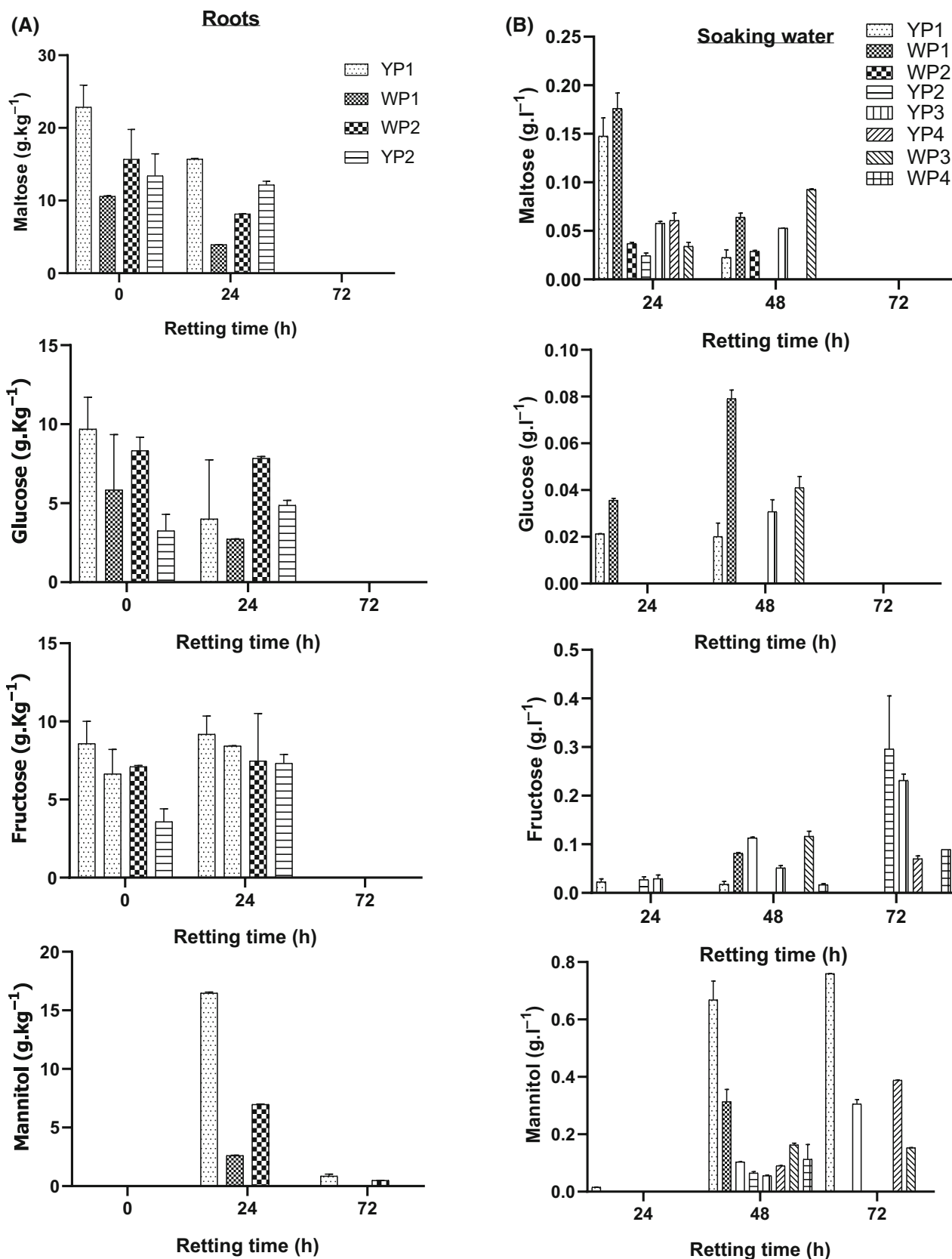
The kinetics of fermentable sugars in the soaking water and flours during retting is summarized in Fig. 3 and that of organic acids is shown in Fig. 4. Neither maltose, nor glucose was detected in the soaking water or in the flours after 72 h of retting. These sugars were more concentrated in the soaking water of genotypes YP1 and WP1 after 24 h of retting. Fructose and mannitol decreased in the flours and accumulated in the soaking water after 72 h of retting (Fig. 3). The main organic acids determined both in the soaking water and in the flours were lactate and acetate, with the former being more concentrated in the soaking water than any other compound analyzed. Unlike acetate, lactate was not detected in flours from unfermented roots (Fig. 4). The two acids accumulated differentially in soaking water and roots: lactate accumulated transiently in roots and continuously in soaking water. The acetate content in roots decreased during the retting, whereas it increased in soaking water. Butyrate appeared in the soaking water after 48 h of retting, regardless of the variety. Ethanol also evolved increasingly during retting. Butyrate and ethanol were not detected in the roots.

#### Totals pectin content

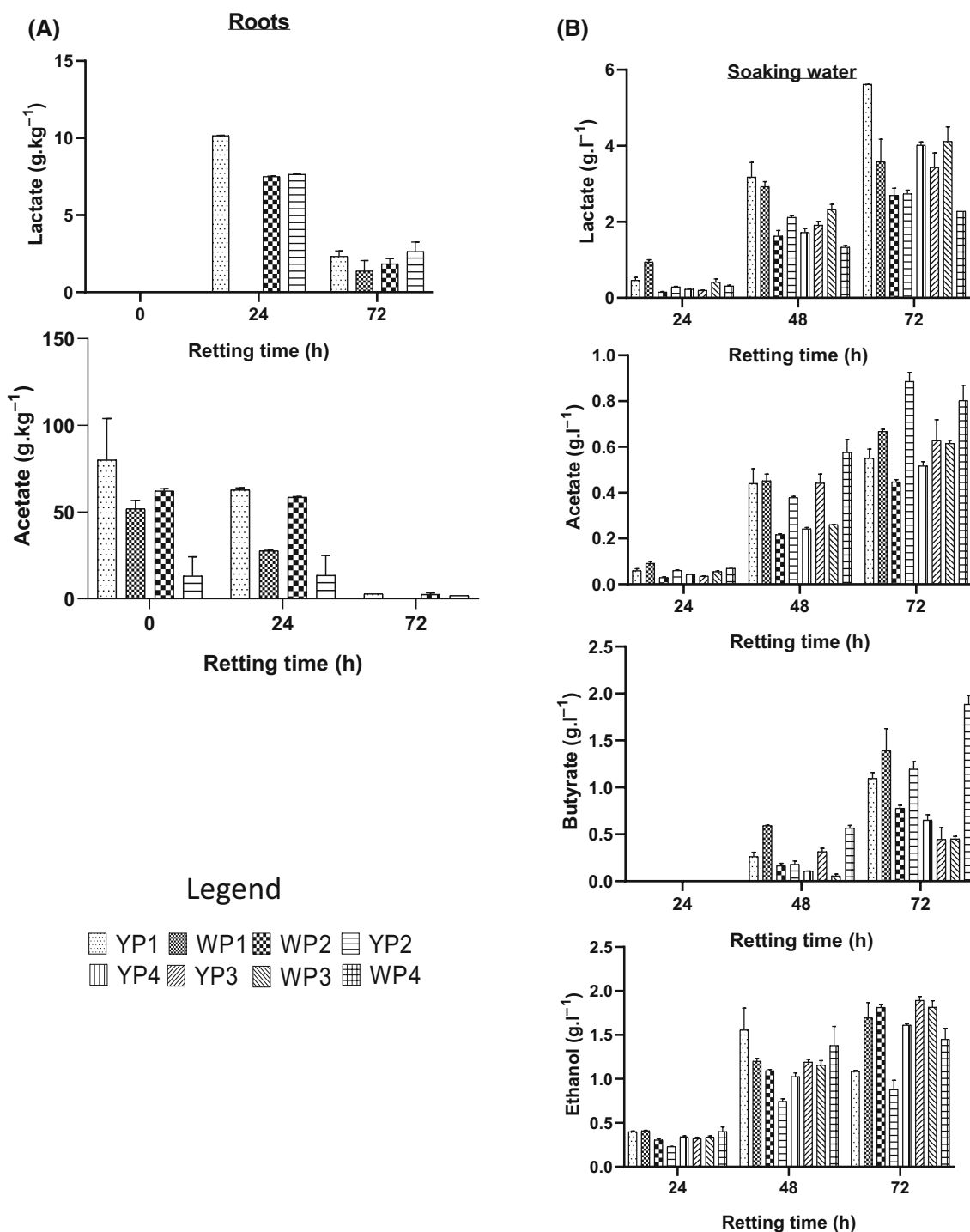
Evolution of the total pectins content is described in Table 1. The yields of total pectin in flours were significantly influenced by genotype (data not presented) and always decreased significantly ( $P < 0.05$ ) during retting. Flours from YP1 and WP2 had higher levels of total pectin compared to that from YP2. WP1 showed an atypically high increase in pectin content from 0 to 24 h, followed by the expected decrease through 72 h, whereas that of YP1 remained relatively unchanged during the retting process.



**Figure 2.** Light microscope histological sections of fresh and 72 h retted roots. cw, cell wall; cc, companion cell; xc, xylem cell; sg, starch granule. (A) YP1, (B) WP1, (C) WP2, (D) YP2, (E) YP4, (F) YP3, (G) WP3 and (H) WP4. (A–H) Fresh and retted roots sections, where (a) to (h) represent magnified portions of the regions indicated by dashed-line boxes in (A) to (H). Scale bar = 25  $\mu$ m.



**Figure 3.** Changes of sugars content in the roots (A) and soaking water (B) during retting. Sugar components were extracted twice from each root sample (two samples per data point), whereas, for soaking water, samples were analyzed directly. Each data point is the mean of the values obtained from four and six replicates of roots and soaking water samples, respectively. Vertical bars indicate the SD. When no bar is shown, the SD is lower than represented by the symbol.



**Figure 4.** Changes in organic acids and alcohol content in the roots (A) and soaking water (B) during retting. Sugar components were extracted twice from each sample root (two samples per data point), whereas, for soaking water, samples were analyzed directly. Each data point is the mean of the values obtained from four and six replicates of roots and soaking water samples, respectively. Vertical bars indicate the SD. When no bar is shown, the SD is lower than represented by the symbol.

#### Starch and amylose content of roots

The histological data suggested that retting affects the cell wall more than the physical structure of starch granules. Therefore, starch content and amylose contents were measured only on cassava roots taken at harvest time. The results presented in Table 2 show that the starch content varied significantly among genotypes, from 75.87% (WP2) to 80.33% (WP1),

whereas the amylose content ranged from 13.28% (WP1) to 15.79% (YP2).

#### Pasting properties of cassava flours

The pasting indicators, namely PV, BD and SB of flours from different cassava varieties taken at different retting stages (Fig. 5), displayed a wide genetic diversity, depending on retting duration

**Table 1.** Accumulation of total pectin content in flour during retting process of cassava roots from different genotype

Retting time	Total pectin (g kg <sup>-1</sup> dry matter)			
	01/0040-27 (YP1)	01/1797 (YP2)	92/0326 (WP2)	LMR (WP1)
0 h	6.54 ± 0.54 a	5.35 ± 0.36 a	6.07 ± 0.44 a	5.56 ± 0.46 a
24 h	6.08 ± 1.40 a	5.99 ± 0.08 a	5.97 ± 0.40 a	5.28 ± 0.46 a
72 h	5.29 ± 1.05 a	4.62 ± 0.38 b	5.72 ± 0.11 a	3.95 ± 0.75 b

Note: Each value corresponds to the mean ± SD of total pectin content. For each column, the means with the same lowercase letters are not statistically different at the 5% level (Tukey's test).

**Table 2.** Starch and amylose content in fresh cassava varieties

Varieties	Starch (%)	Amylose (%)
01/0040-27 (YP1)	77.47 ± 0.64 b	13.33 ± 0.49 b
LMR (WP1)	80.33 ± 1.13 a	13.28 ± 0.19 b
92/0326 (WP2)	75.87 ± 1.01 b	14.83 ± 1.27 ab
01/1797 (YP2)	77.47 ± 0.94 b	15.79 ± 0.88 a

Note: Each value corresponds to the mean ± SD of the compound concerned. For each column, the means with the same lowercase letters are not statistically different at the 5% level (Tukey's test).

and indicator considered. For gelatinization (Fig. 5A), retting did not affect significantly the swelling amplitude (represented by PV) of YP2 or YP1. WP1 tended to increase in PV, whereas that of WP2 tended to decrease after 24 h of retting. However, PV values for an extended retting period (72 h) tended to be similar to those of unretted roots, with the exception of WP2. The ability of gelatinized cassava starch to withstand high temperature (represented by BD) is genotype- and retting duration-dependent (Fig. 5B). In unretted cassava roots, WP2 displayed a high BD, characteristic of low resistance of its gelatinized starch to heat treatment at 90 °C. After 24 h of retting, the BD viscosity of gelatinized starch from WP1 and YP1 were higher (low resistance to heat). Their resistance to heat was improved (low BD values) after 72 h of retting with values close to those of unretted roots. After 72 h of retting, gelatinized starch of all cassava varieties converged towards relatively low BD values (i.e. high resistance to heat). Upon cooling to 50 °C (Fig. 5C), gelatinized starch from YP1 and WP1 varieties presented high SB values after 24 h of retting, characteristic of high susceptibility to retrogradation. However, when the retting time increases, all varieties displayed relatively low SB

values (low tendency to retrogradation), close to those of unretted roots for YP1 and WP1.

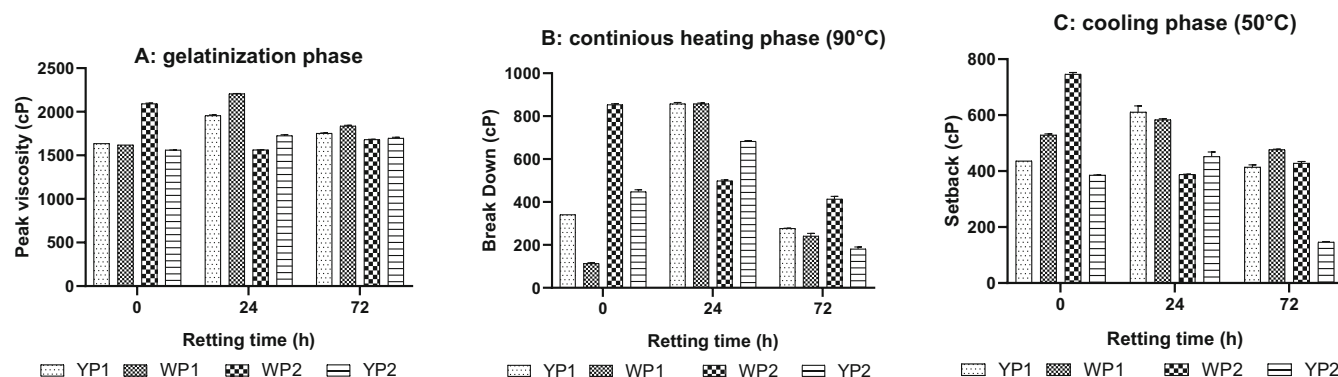
## DISCUSSION

In the process of fermented cassava products, one of the key steps is the production of cassava dough, obtained after the root retting. The quality and/or quantity of the dough impacts the quality of the ready-to-eat product (i.e. fufu, miondo, chikw-nague, muntumba, bobolo). Indeed, a cassava genotype with poor and/or low retting ability will give a low dough yield with poor quality. Such a genotype will be systematically rejected by end users because ready-to-eat products will not meet the expected sensory qualities (taste, texture in the mouth, aroma, etc.). With the prospect of increasing the rate of adoption of new hybrids from breeding program, an early and effective selection of hybrids that meet consumer expectations is a challenge that requires the availability of physiological indicators (biochemical and molecular) associated with the relevant quality trait of the consumer and usable as early selection filters for breeders.

In the present study aiming to understand the physiological bases associated with the retting process, we characterized the biochemical and histological changes during retting of different cassava genotypes.

### Physical characterization of retting process

The pH values of cassava soaking water obtained in the present study are in accordance with those previously reported.<sup>30</sup> This drop in pH was concomitant with the loss of root firmness, which correlated with the increase of organic acids that occurred in soaking water.<sup>30</sup>



**Figure 5.** Changes in pasting indicators including gelatinization (A), continuous heating (B) and cooling (C) phases of cassava flours from different genotypes during the retting process.

Histological analysis of cassava roots at harvest shows a structure similar to that already reported in cassava.<sup>6,8</sup> A change of root tissue structure during retting strongly indicates that the process mainly affected the cell wall, leading to root softening. However, based on integrity of starch granules, there was no apparent change in the starch macrostructure, even after 72 h of retting. In summary, retting appeared to affect the integrity of the cell walls without a marked effect on the starch granules. Probably, as suggested by Silva *et al.*,<sup>30</sup> 72 h of fermentation was insufficient to cause drastic changes in the granules of cassava starches.

### Biochemical characterization of the retting process

#### Kinetics of sugar, alcohol and organic acids

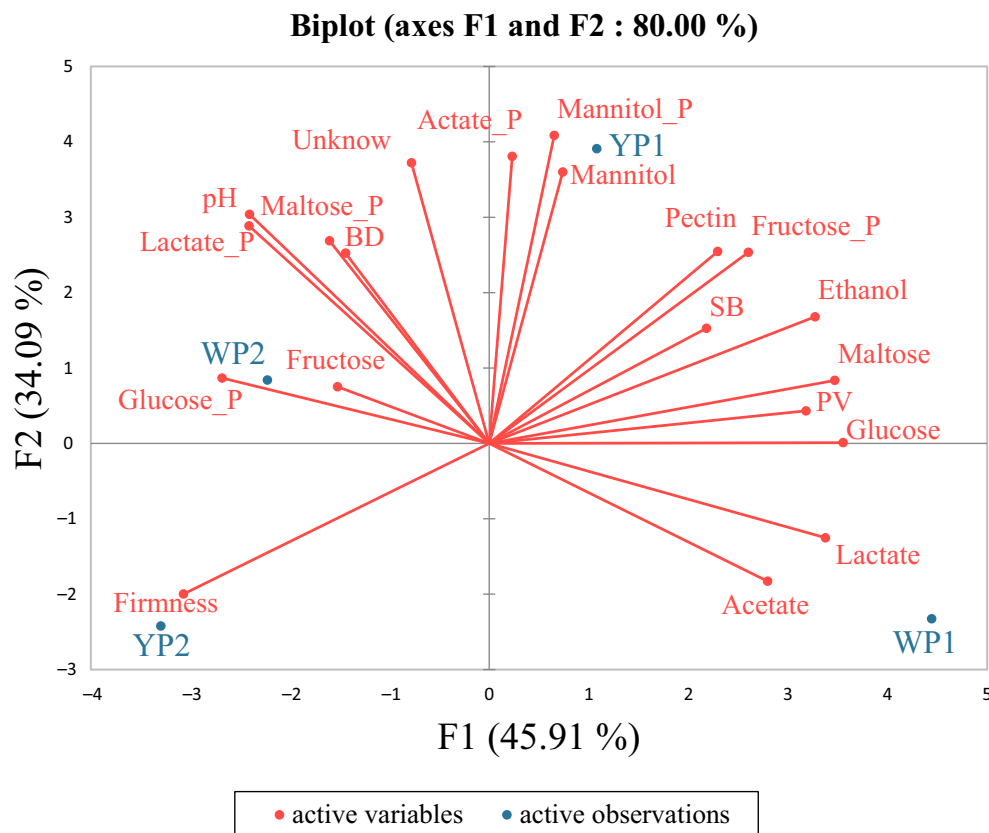
The high concentration of sugars (maltose, glucose) observed after 24 h in the soaking water of YP1 and WP1 roots was coupled with rapid softening observed in these varieties at this stage of retting. This resulted in a negative correlation ( $r = -0.95$ ) observed between firmness and maltose content (see Supporting information, Table S1). A negative correlation was also detected between softening and ethanol content ( $r = -0.96$ ), indicating that the loss of firmness was accompanied by the release of soluble sugars, which underwent anaerobic fermentation by different microorganisms in the soaking water, leading to a production of ethanol and lactate.<sup>31</sup> This fermentation also likely explains the total disappearance of fermentable sugars (maltose and glucose) in the flours and soaking water after 72 h of retting. The high concentration of lactate in soaking water and its relative abundance in all genotypes confirms the predominance of lactic acid bacteria activities during the retting process, as previously reported.<sup>13,32,33</sup>

Lactic acid fermentation was followed by that of acid-tolerant yeasts. Ethanol is therefore the second most concentrated metabolite in soaking water.<sup>10</sup>

Organic acids accumulated differentially in the soaking water and in the flour. Lactate content accumulated transiently, a pattern corroborating the one previously reported by Brauman *et al.*<sup>10</sup> However, in the present study, higher lactate levels were obtained. Acetate content of the flour decreased continuously during retting, probably as the result of leaching into soaking water. This result is in contrast to that of Brauman *et al.*,<sup>10</sup> who reported a transient accumulation of acetate content, with a decrease 72 h after the beginning of retting. Organic acids metabolism during retting is probably a function both of genotype and microbial population involved, considering that both the study by Brauman *et al.*<sup>10</sup> and the present study were conducted in different areas and with different genotypes. The late appearance of butyrate in the soaking water confirms the assertion that the microorganisms responsible for their production may be strict anaerobes such as *Clostridia*.<sup>34</sup> These organic acids improve the aroma, flavor, texture and shelf life, and further contribute to preserving the quality of the products by inactivating the *Enterobacteria* and other infectious pathogens that produce toxins of food origin.<sup>35</sup>

#### Total pectin content

Previous studies revealed that the softening observed during cassava retting is the result of the degradation of cell wall pectin compounds.<sup>8,33</sup> Overall, there was a negative correlation between total pectin content observed during retting, and softening of the



**Figure 6.** Mapping cassava genotypes based on their retting ability assessed through firmness and biochemical characteristics of flours and soaking water after 24 h of retting. The sugars and organic acids followed by the letter 'P' are those contained in the roots.



roots (see Supporting information, Table S1), which failed to reach statistical significance probably as a result of the small size of the dataset. Similarly, the degree of softening of roots from the different genotypes was not linked to the initial levels of pectin concentration. Additionally, in YP1 and WP1, which had already lost more than 85% of their firmness after 24 h of retting, the lowest total pectin content was reached after 72 h of retting.

The main changes in wall polysaccharide composition during retting concern specific pectin polymers such as galactan, homogalacturonan and arabinan.<sup>8</sup> Thus, root softening during retting would not only be the result of pectin degradation, but also the distribution of methyl groups on the homogalactan skeleton, the degree of methylation or other cell wall component such as hemicellulose. All of them are known to affect cell wall stiffness<sup>33</sup> and therefore these component should be considered for further studies.

#### Starch and amylose content of roots

In the present study, the level of starch and amylose content was different according to the genotypes. They were between  $75.87 \pm 1.01\%$  and  $80.33 \pm 1.13\%$  for starch, which is within the range of levels generally obtained in cassava root harvested after 12 months of growth in the field. The significant difference observed for starch at this stage between the varieties confirms the effect of genotype on the starch content of cassava as previously shown.<sup>36</sup> The level of amylose content ranged between  $13.28 \pm 0.19\%$  and  $15.79 \pm 0.88\%$  for amylose, which is lower than those previously reported.<sup>37,38</sup> One of the characteristics of cassava retting process is the cell wall degradation and root disintegration leading to the slight loss of material including starch released into the quenching water, which becomes cloudy and whitish. Regardless of the genotype, no significant difference in terms of starch and amylose content was observed during retting, suggesting that the material released into the soaking water has little impact on the starch and amylose content. This could be a result of the pasty properties of starch brought out by soaking in water. Our data also suggest that the retting process is not associated with a significant starch degradation, although recent studies reported the production by microorganisms of amylase.<sup>39</sup>

#### Pasting properties of cassava flours

The present study confirms the apparent stability of starch structure during retting, as observed on histological cross-sections of roots. The retting process affected mainly starch pasting properties rather than starch granule structure. Moreover, the above findings indicate that the pasting behavior of cassava starch is genotype-dependent. This finding was similar to those previously reported in several studies.<sup>36,40</sup> The pasting behaviour of cassava starch was also retting-duration dependent, as previously reported.<sup>40,41</sup> This pasting behaviour is probably linked to the rate of softening of the roots during retting. Indeed, an apparent correlation was observed between pasting behavior and the softening rate of cassava genotypes because the samples of each of the two selected groups (YP1/WP1) as fast retting speed and (WP2/YP2) as medium retting speed have comparable pasting behavior.

It is obvious that other cassava components and characteristics, such as polyphenols and pectin structure and composition, sugars, and starch structure and composition, may be involved in the pasting and cell wall behavior as recently suggested.<sup>42-44</sup> Therefore, these components need to be investigated in relation

with cell wall degradation and pasting property changes that occur during the retting process.

Mapping cassava genotypes based on their retting ability assessed using loss of firmness and biochemical characteristics of flours and soaking water after 24 h of retting clearly discriminate the four genotypes (Fig. 6). The first two principal components were responsible for 80.00% of total variance. The WP1 variety presented the lowest firmness and more concentrated organic acids in the soaking waters, which is a symbol of effective fermentation, and, above all, a high swelling rate of flour resulted. Accordingly, it can be considered as the best in terms of retting ability after 24 h of retting. The WP1 variety was opposed in the principal component 1 (45.91%) to YP2 in which fermentation was very weakly established after 24 h of retting with weak softening. The YP1 was driven by principal component 2 (34.09%) and characterized by the metabolites contained in the roots. This could reflect a more advanced fermentation in the roots than in the soaking water as a result of the high pH of the roots. Flour of WP2 variety (driven by principal component 3) was most resistant to prolonged heating and less susceptible to retrogradation after 24 h of retting, making it suitable for fufu production.

The data reported in the present study represent a substantial contribution towards understanding the physiological basis of the retting process through biochemical and histological studies. Histological and biochemical changes in the soaking water and in the roots were highlighted. The level of maltose decreased during retting in both soaking water and roots, whereas acetate content decreased in soaking water and increased in roots. This study also showed that (i) the retting process affects the functional properties of starch more than its content and that (ii) the loss of firmness shown by cell wall degradation is not well correlated with the evolution of total pectin content. This suggests the involvement of other cell wall compounds (e.g., hemicellulose, methylated forms of pectin and polyphenols) that warrant further research.

#### AUTHOR CONTRIBUTIONS

DMAM, RNdj, LBT and EY were responsible for conceptualization. GAW, RNdj and DMAM were responsible for data curation. GAW and RNdj were responsible for formal analysis. DMAM, DD, RN, AFK, KKMf and CM. were responsible for funding acquisition. DMAM, GAW, RNdj, IT, Rnda and JG were responsible for investigation. DMAM, GAW and RN were responsible for methodology. DMAM and RNdj were responsible for project administration. AFK, CM and KKMf were responsible for field trials and plant material resources. DMAM, RNdj and EY were responsible for supervision. GAW, RNdj and DMAM were responsible for writing the original draft. GAW, LBT, EY, KKMf, RNdj and DMAM were responsible for reviewing manuscript content and editing.

#### ACKNOWLEDGMENTS

In memoriam of our colleague and friend Geneviève Fliedel, PhD, Food technologist at CIRAD who suggested and contributed to this work and for the passion and enthusiasm that characterized her work as a researcher. We are grateful to Hernan Ceballos and Clair Hershey for reviewing and editing the manuscript, respectively. We thank Karine Alary and Léa Ollier for their technical assistance. We are also grateful to CRP-RTB cluster CC4.2 'cassava processing', PIDMA project funded by MINADER and World Bank and the grant opportunity INV-008567 (formerly OPP1178942): Breeding RTB Products for End User Preferences (RTBfoods) to the French

Agricultural Research Centre for International Development (CIRAD), Montpellier, France, by the Bill & Melinda Gates Foundation (BMGF) (<https://rtbfoods.cirad.fr>).

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

## REFERENCES

- 1 FAO, Étude diagnostique de la réduction des pertes après récolte de trois cultures (2018).
- 2 IRAD, Augmentation de la productivité du manioc et diffusion des semences améliorées. Projet C2D IRAD n°2 (2013). p. 1–44.
- 3 Ndjouenkeu R, in *Cassava in Central and Western Africa: Postharvest Constraints and Prospects for Research and Market Development*, ed. by Waisundara VY. InTechOpen Publisher, Cassava, pp. 199–217 (2018).
- 4 Bechoff A, Tomlins K, Fliedel G, Becerra Lopez-lavalle LA, Westby A, Hershey C et al., Cassava traits and end-user preference: relating traits to consumer liking, sensory perception, and genetics. *Crit Rev Food Sci Nutr* **58**:547–567 (2017). <https://doi.org/10.1080/10408398.2016.1202888>.
- 5 Westby A, Importance of fermentation in cassava processing. *Acta Horticulturae* **380**:249–255 (1994). <https://doi.org/10.17660/ActaHortic.1994.380.39>
- 6 Nkoudou NZ and Ngang Essia JJ, Cyanides reduction and pasting properties of cassava (*Manihot Esculenta* Crantz) flour as affected by fermentation process. *Food Nutr Sci* **8**:326–333 (2017).
- 7 Djoulde DR, *Mise au Point d'un Ferme Mixte destiné à la Bioconversion Des Tubercules de Manioc cyanogène*. Université de Ngaoundéré, Ngaoundéré, Cameroon pp. 210 (2005).
- 8 Ngolong NLG, Guillon F, Essia NJJ, Bonnin E, Bouchet B and Saulnier L, Modification of cell wall polysaccharides during retting of cassava roots. *Food Chem* **213**:402–409 (2016). <https://doi.org/10.1016/j.foodchem.2016.06.107>.
- 9 Ampe F, Kéléké S, Robert H, Brauman A, Keleke S and Robert W, in *The role and origin of pectin degrading enzymes during cassava retting*, ed. by Agbor Egbe T, Brauman A, Griffon D and Trèche S. Transformation Alimentaire du Manioc Cassava food processing. PARIS: OSTORM, pp. 331–344 (1995).
- 10 Brauman A, Machicout M, Treche S and Miambi E, Étude préliminaire d'une technique de rouissage des racines de manioc en sacétanche preliminary study of new retting technic in airtight plastic bag without water add. In: Agbor Egbed Brauman A, Griffon D and Trèche S (eds). *Transformation Alimentaire du Manioc Cassava Food Processing*. PARIS: OSTORM, pp. 531–546 (1995).
- 11 Brauman A, Keleke S, Malonga M, Mavougou O, Ampe F and Miambi E, Cassava lactic fermentation in Central Africa: microbiological and biochemical aspects. *Cassava Flour Starch Progrss Res Dev* (1996). 197–209.
- 12 Djoulde DR, Etoa F, Essia NJ and Mbofung CM, Fermentation du manioc cyanogène par une culture mixte le *Lactobacillus plantarum* et *Rhizopus oryzae*. *Microb Hyg Ali* **15**:9–13 (2003).
- 13 Djoulde DR, Etoa F-X, Essia NJ-J and Mbofung CMF, Screening des microorganismes à potentialités fermentaires pour le manioc. *Tropicultura* **23**:11–18 (2005).
- 14 Umeh SO and Odibo FJC, Amylase activity as affected by different retting methods of cassava tubers. *Int J Agric Policy Res* **2**:267–272 (2014).
- 15 Ampe F, Brauman A, Treche S and Agossou A, Cassava retting: optimisation of a traditional fermentation by an experimental research methodology. *J Sci Food Agric* **65**:355–361 (1994).
- 16 Djoulde DR, *Mise au Point d'un Ferment Mixte destiné à la Bioconversion Des Tubercules de Manioc cyanogène* (2004).
- 17 Djoulde DR, Essia NJJ and Etoa F, Amélioration du rouissage du manioc par utilisation d'un starter microbien de trois souches [amelioration of cassava retting by a stater culture of three strains]. *Int J Innov Sci Res* **14**:268–277 (2015).
- 18 Ickofa J, Kayath CA and Gadet MD, First development of a biotechnological ferment based on a consortium of the genus bacillus for the optimization of the fermentation process of cassava tubers. *Adv Microbiol* **10**:563–574 (2020).
- 19 Nkoudou ZEN, Kamdem SS, Agnia HB, Mengo FE and Essia Ngang Justin J, Assessment of cassava (*Manihot esculenta* Crantz) fermentation and detoxification using previously cassava-fermented chips. *Br J Appl Sci Technol* **13**:1–10 (2016).
- 20 Chijioke U, Madu T, Okoye B, Ogunka AP, Ejechi M, Ofoeze M et al., Quality attributes of fufu in south-East Nigeria: guide for cassava breeders. *Int J Food Sci Technol* **56**:1247–1257 (2021).
- 21 Tizé I, Fotso AK, Nukenine EN, Masso C, Ngome FA, Suh C et al., New cassava germplasm for food and nutritional security in Central Africa. *Sci Rep* **11**:7394 (2021). <https://doi.org/10.1038/s41598-021-86958-w>.
- 22 Mbéguié A, Mbéguié D, Dahdouh L, Julien R, Mbéguié A, Mbéguié D et al., Softening measurement of Cassava: Development of a method to evaluate the retting process (2019).
- 23 Hor S, Léchaudel M, Mith H and Bugaud C, Fruit density: a reliable indicator of sensory quality for mango. *Sci Hortic (Amsterdam)* **272**: 109548 (2020). <https://doi.org/10.1016/j.scienta.2020.109548>.
- 24 Mestres C, SOP for Sugars by HPLC (2019).
- 25 Mestres C, Ollier L and Domingo R, SOP for the Determination of Galacturonic Content (2021).
- 26 Jourda C, Cardi C, Gibert O, Toro AG, Ricci J, Mbéguié-A-Mbéguié D et al., Lineage-specific evolutionary histories and regulation of major starch metabolism genes during banana ripening. *Front Plant Sci* **7**: 1–21 (2016).
- 27 Holm J, Björck I, Drews A and Asp NG, A Rapid method for the analysis of starch. *Starch-Stärke* **38**:224–226 (1986).
- 28 Christian M, Léa O, Romain D, Otegbayo Bolanle Omolara, Rolland-Sabaté Agnès and Dutheil de la Rochère Aliénor, SOP for the determination of galacturonic content, in *Biophysical Characterization of Quality Traits, WP2*. RTBfoods Project-CIRAD, Montpellier, p. 12 (2022) <https://doi.org/10.18167/agritrop/00605>.
- 29 Batey IL, Curtin BM and Moore SA, Optimization of rapid-visco analyser test conditions for predicting Asian noodle quality. *Cereal Chem* **74**: 497–501 (1997).
- 30 Paixão E, Silva GD, Bento JAC, Oliveira AR, Garcia MC, Soares Júnior MS et al., Pasting and thermal properties of fermented cassava (*Manihot esculenta* Crantz). *J Food Sci Technol*. **58**:1441–1448 (2021).
- 31 Brauman A, Kéléké S, Malonga M, Miambi E and Ampe F, Microbiological and biochemical characterization of cassava retting, a traditional lactic acid fermentation for fufu (cassava flour) production. *Appl Environ Microbiol* **62**:2854–2858 (1996).
- 32 Oyedeji O, Ogunbanwo ST and Onilude AA, Predominant lactic acid bacteria involved in the traditional fermentation of fufu and ogi, two Nigerian fermented food products. *Food Nutr Sci* **04**:40–46 (2013).
- 33 Obi CN and Agu UB, Enzymatic potentials of microorganisms associated with cassava retting. *J Adv Microbiol* **17**:1–9 (2019).
- 34 Brauman A, Kéléké S, Mavougou O, Ampe F and Miambi E, Etude cinétique du rouissage traditionnel des racines de manioc en Afrique centrale (Congo) = Kinetic studies of cassava retting in Central Africa (Congo). *Transform Aliment du Manioc* **1995**: 287–305 (2015).
- 35 Oyeyinka SA, Ayinla SO, Sanusi CT, Akintayo OA, Oyedeji AB, Oladipo JO et al., Chemical and physicochemical properties of fermented flour from refrigerated cassava root and sensory properties of its cooked paste. *J Food Process Preserv* **44**:1–9 (2020).
- 36 Sakdaronnarong C, Sraphet S, Srisawad N, Chantasod R, Jonglertjunya W and Triwitayakorn K, Rheological characteristics and genotype correlation of cassava root for very high gravity ethanol production: the influence of cassava varieties and harvest times. *Biotechnol Appl Biochem* **67**:105–116 (2020).
- 37 Nuwamanya E, Baguma Y, Emmambux N, Taylor J and Patrick R, Physicochemical and functional characteristics of cassava starch in Ugandan varieties and their progenies. *J Plant Breed Crop Sci* **2**:1–11 (2010) Available from: <http://www.academicjournals.org/jpbcs>.
- 38 Alvarado MP, Grosmaire L, Dufour D, Toroa AG, Sánchez T, Calle F et al., Combined effect of fermentation, sun-drying and genotype on

- breadmaking ability of sour cassava starch. *Carbohydr Polym* **98**: 1137–1146 (2013).
- 39 Umeh SO and Odibo FJC, Amylase activity during retting of cassava tubers for wet fufu mash production. *Sci-Afric J Sci Issues Res Essays* **2**:132–137 (2014).
- 40 Oyeyinka SA, Adeloye AA, Smith SA, Adesina BO and Akinwande FF, Physicochemical properties of flour and starch from two cassava varieties. *Agrosearch* **19**:28 (2019).
- 41 Oyeyinka SA, Adeloye AA, Olaomo OO and Kayitesi E, Effect of fermentation time on physicochemical properties of starch extracted from cassava root. *Food Biosci* **33**:100485 (2020). <https://doi.org/10.1016/j.fbio.2019.100485>.
- 42 Luo SJ, Chen RY, Huang L, Liang RH, Liu CM and Chen J, Investigation on the influence of pectin structures on the pasting properties of rice starch by multiple regression. *Food Hydrocoll* **63**:580–584 (2017). <https://doi.org/10.1016/j.foodhyd.2016.10.016>.
- 43 Musah Bawa NK, Agbenorhevi JM, Kpodo F and Sampson GO, Pasting properties of starch-okra pectin mixed system. *CYTA–J Food* **18**:742–746 (2020). <https://doi.org/10.1080/19476337.2020.1838616>.
- 44 Wu Y, Chen Z, Li X and Li M, Effect of tea polyphenols on the retrogradation of rice starch. *Food Res Int* **42**:221–225 (2009). <https://doi.org/10.1016/j.foodres.2008.11.001>.