
Article:
Gomez, Leonardo Dario orcid.org/0000-0001-6382-9447 (2024) Cell wall polysaccharides determine cooking quality in cassava roots. Plants, People, Planet. ISSN: 2572-2611

https://doi.org/10.1002/ppp3.10558

Received: 20 November 2023 | Revised: 28 April 2024 | Accepted: 21 June 2024

DOI: 10.1002/ppp3.10558

Plants People Planet PPP Open Access

RESEARCH ARTICLE

Cell wall polysaccharides determine cooking quality in cassava roots

Sukhita Sathitnaitham[1] | Hernan Ceballos[2] | Passorn Wonnapinij[1,3,4] | Ekaphan Kraichak[5] | Supanut Utthiya[1] | Anongpat Suttangkakul[1,3,4] | Leonardo D. Gomez[6] | Piya Kittipadakul[7,8] | Nongnuch Siriwong[9] | Pasajee Kongsil[7,8] | Supachai Vuttipongchaikij[1,3,4]

[1]Department of Genetics, Faculty of Science, Kasetsart University, Chatuchak, Bangkok, Thailand

[2]International Center for Tropical Agriculture (CIAT), Cali, Colombia

[3]Center of Advanced Studies for Tropical Natural Resources, Kasetsart University, Chatuchak, Bangkok, Thailand

[4]Omics Center for Agriculture, Bioresources, Food and Health, Kasetsart University (OmiKU), Bangkok, Thailand

[5]Department of Botany, Faculty of Science, Kasetsart University, Chatuchak, Bangkok, Thailand

[6]CNAP, Department of Biology, University of York, Heslington, York, UK

[7]Department of Agronomy, Faculty of Agriculture, Kasetsart University, Chatuchak, Bangkok, Thailand

[8]Center for Advanced Studies of Agriculture and Food (CASAF), Kasetsart University, Chatuchak, Bangkok, Thailand

[9]Department of Home Economics, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand

Correspondence

Supachai Vuttipongchaikij, Department of Genetics, Faculty of Science, Kasetsart University, 50 Ngam Wong Wan Road, Chatuchak, Bangkok 10900, Thailand.
Email: supachai.v@ku.th

Pasajee Kongsil, Department of Agronomy, Faculty of Agriculture, Kasetsart University, 50 Ngam Wong Wan Road, Chatuchak, Bangkok 10900, Thailand.
Email: pasajee.k@ku.th

Social Impact Statement

Cassava is a vital food source for millions worldwide, crucial for food security and economic stability. This study analyzed cell wall polysaccharides in cassava roots to understand their impact on cooking properties. We found these polysaccharides influence the textural attributes of cassava roots, essential for both cooking and consumption. The research highlights the need to further identify and analyze cassava cell wall components. By improving our understanding of these components, we can improve food security, affordability, and resilience in diverse regions, ultimately contributing to global food security and better aligning with consumer preferences.

Summary

- The textural attributes of cassava roots significantly influence preferences in cooking and consumption as a food source, yet the specific components dictating these properties remain unclear. We aimed to identify the factors shaping the cooking properties of cassava roots.
- We conducted a compositional analysis of 22 traits in a diverse F1 biparental population derived from soft- and hard-boiling progenitors. The traits encompassed cooking qualities, starch properties, and cell wall composition.
- Specific cell wall components including cellulose, xylan, un-esterified, and methyl-esterified homogalacturonan (HG) demonstrated a correlation with cooking quality attributes: sensory assessments related to texture (SAT) and water absorption during boiling (WAB). Correlation and regression analyses revealed that these wall components collectively contribute to 20% of SAT variability and 14% of WAB variability. SAT appeared to be influenced by methyl-esterified and un-esterified HGs, xylan, and cellulose, impacting the tensile strength of the cell wall. Conversely, WAB appeared to be associated with methyl-esterified HG, potentially altering water absorption properties. Although genome-wide association studies (GWAS) were unable to identify significant SNPs for SAT and WAB,

Funding information
National Research Council of Thailand, Grant/Award Number: NRCT5-RSA63002-02; The Office of the Ministry of Higher Education, Science, Research and Innovation; Thailand Science Research and Innovation through the Kasetsart University Reinventing University Program 2021; Foreign, Commonwealth and Development Office; Kasetsart University Research and Development Institute, Grant/Award Number: FF(KU)13.64; UK's Foreign, Commonwealth and Development Office (FCDO) and Bill and Melinda Gates Foundation, Grant/Award Number: INV-007637; National Research Council of Thailand, Grant/Award Number: NRCT-N42A650286

notable associations emerged for cellulose, xylan, and un-esterified- and methyl-esterified HG. Candidate genes associated with these SNPs point towards diverse cell wall-related proteins, transcription factors, sugar metabolism-related genes, and glycosyl hydrolases.

- This study provides insights into the relationship between cassava root compositions and cooking characteristics and the role of wall components in determining the cooking quality of edible cassava. This information represents a substantial contribution towards the development of protocols for selecting varieties with texture preferences.

KEYWORDS
cell wall, crop; storage root, GWAS, plant breeding, texture assessment, water absorption

1 | INTRODUCTION

Cassava (*M. esculenta* Crantz) and the other 97 Manihot species originated in the Americas (Rogers & Appan, 1973). The prevailing hypothesis is that cultivated cassava originated and was domesticated in South America (Allem, 2002; Watling et al., 2018). However, the botanical origin of cassava is still unclear, and its ancestry remains uncertain (Léotard et al., 2009). Large repositories of genetic diversity with thousands of landraces and improved clones of cassava and wild relatives are maintained in germplasm collections (Hershey, 2008) at the International Center for Tropical Agriculture in Colombia, Empresa Brasileira de Pesquisa Agropecuaria in Brazil, International Institute of Tropical Agriculture in Nigeria, and at the Central Tuber Crops Research Institute of India. Cassava is a major food staple cultivated over 32 million hectares (80% in Africa, 13% in South America, 5% in Asia) with over 330 million tons of root production with the global export of all types of cassava root and products totally 16 million tons in the value of 5.5 billion dollars in 2022 (FAO, 2022). Cassava is not only an important feedstock for various industries such as starch, animal feed, and bioethanol but also plays a significant role in human nutrition, contributing to the food security of millions of people, particularly in Africa.

The primary focus for breeders worldwide has been the development of cassava varieties capable of effectively supporting value chains and addressing food security challenges. Achieving a high, stable, and reliable yield has been of paramount importance, often overshadowing considerations for consumer-preferred traits and post-harvest aspects in cassava cultivation for human consumption (Dufour et al., 2024; Eriksson et al., 2018; Thiele et al., 2021). As breeders and food technologists turned their attention to improving the cooking quality of cassava, they discovered a significant lack of understanding regarding the inheritance of quality traits. One of the objectives of the RTBfoods project (https://rtbfoods.cirad.fr/) is to address these gaps by identifying relevant varietal traits and preferences for boiled and steamed cassava roots. The softness of the processed root and non-bitter taste are the key quality parameters that need to be addressed (Dufour et al., 2024; Iragaba et al., 2021). Additionally, the project aims to develop high-throughput phenotyping protocols to streamline the selection of genotypes that align with consumer preferences. While ample literature exists concerning bitterness in cassava and associated assessment protocols, there is a notable dearth of knowledge regarding the textural attributes linked to the softness of processed roots.

Key textural attributes of cassava roots, including mealiness, fiber content, adhesiveness, softness, taste, aroma, and color, along with shorter cooking times, are key determinants of user preferences (Eggleston & Asiedu, 1994; Favaro et al., 2008; Talma et al., 2013). The cooking quality of cassava roots is also significantly influenced by genotype-environment interactions (Franck et al., 2011; Ngeve, 2003; Uchendu et al., 2022). Recent developments in phenotyping and genotyping tools have facilitated the selection of cassava varieties with improved cooking quality (Iragaba et al., 2019; Meghar et al., 2023; Mestres et al., 2023; Tran et al., 2021). However, the precise components in cassava roots that dictate cooking quality, particularly the mealiness property, remain elusive.

Mealiness, a term used to describe cassava roots that become soft and chewable when boiled (Ngeve, 2003), is the primary factor shaping consumer preferences (Iragaba et al., 2021). Several investigations have explored potential correlations between root structure, composition, and cooking quality to better understand the factors influencing the mealiness texture of boiled cassava roots. Notably, the texture (hard or soft), swelling properties during boiling, and attributes associated with cooking time are believed to be linked to root mealiness (Favaro et al., 2008; Menoli & Beleia, 2007; Tran et al., 2021), warranting further exploration. While some evidence suggests that larger starch granules may contribute to mealiness (Safo-Kantanka & Owusu-Nipah, 1992), the influence of starch composition on culinary attributes remains inconclusive (Padonou et al., 2005). Despite recent advancements in phenotyping and genotyping tools, the specific components responsible for cassava root mealiness continue to be a subject of ongoing investigation.

Several studies have provided compelling evidence suggesting that starch is not the sole determinant of cooking properties in root

crops, including cassava, sweet potato, yam, and potatoes. It is increasingly recognized that plant cell wall components play a crucial role in shaping the cooking quality of these crops (Mestres et al., 2023). Recent advancements in genetics, particularly Genome-Wide Association Studies (GWAS), which analyze genetic variations across the entire genome, have identified significant single nucleotide polymorphisms (SNPs) associated with mealiness and other textural attributes in boiled cassava, and these SNPs were found to be linked to genes related to cell wall components (Uchendu et al., 2021). GWAS involves scanning the genomes of many individuals to find genetic variations associated with a particular trait. While this association is interesting, there are starch traits and essential cell wall components that have received limited attention. Besides, these studies involve a limited number of cassava varieties, and, therefore, further research is required to provide a comprehensive understanding of the complex interplay between root traits and cooking quality.

This study aimed to understand the underlying factors influencing the cooking properties of cassava storage roots through a comprehensive analysis of their composition. We carried out a detailed examination of the root cellular structure, with a primary focus on parenchyma cells responsible for storing starch granules. We conducted a thorough examination of sensory assessments related to texture (SAT) and water absorption during boiling (WAB) to represent cooking qualities within an F1 biparental population derived from soft- and hard-boiling varieties. We analyzed six starch traits and 13 cell wall components, including cellulose and matrix polysaccharides. Employing mathematical modeling, we further delineated the potential impact of these cell wall components on cooking properties. Moreover, GWAS were conducted in search of genetic associations related to various cell wall components, providing insights into the genetic basis of the cooking quality traits, and offering a path for genetic improvements.

2 | MATERIALS AND METHODS

2.1 | Plant materials and cultivation conditions

HNT × C33 biparental F1 hybrids were generated by crossing. HNT (Hanatee) is a Thai traditional cassava landrace with excellent cooking quality (Sraphet et al., 2011). HNT and its progenies were shown to have a soft texture and higher pasting viscosity values than those of cassava commercial varieties with firm texture (Chaengsee et al., 2020). C33 is a genotype developed at CIAT (International Center for Tropical Agriculture), which is heterozygous for the resistance to Cassava Mosaic Disease and has a very poor cooking quality (Thuy et al., 2021). This biparental full-sib family is an ideal material to study the biochemical and genetic variation of cooking quality within a common genetic background. F1 seeds were germinated, and the resulting seedlings were grown in the field for field evaluation in seedling trials.

2.2 | Assessing cooking time and quality

Around 110 genotypes produced enough sizeable roots to assess dry matter content (DMC), cooking time, and quality. DMC was estimated by the standard gravimetric method (Kawano et al., 1987). Root sections (≈400 g) were weighed, boiled for 30 min, and then weighed again. The weight gains (expressed as a percentage of the initial weight) due to water absorption during boiling (WAB) is a proxy parameter for estimating cooking time (Tran et al., 2021). A sensorial assessment of texture (SAT) of boiled samples (e.g., softness) was carried out by the forking method by two people on five boiled root samples. A fork was introduced into the root section for a sensory assessment of the texture (e.g., softness) of the boiled samples using a 1–5 scale (1 = very hard; 5 = very soft).

2.3 | Sample preparation from storage roots

Storage roots were cut into thick cross sections and stored at −80°C prior to lyophilization. Some of the fresh tuberous roots were kept in a refrigerator at 4°C for microscopy. Freeze-dried slices of storage roots (without cortex) were ground using an ultra centrifugal mill ZM 200 (Retsch) through a 0.5 mm sieve. The powder was stored in a container with silica gel until use. Figure 1 provides a general depiction of how root and leaf samples were used for the different analyses described in this article. Following this procedure, a sample of the root was used for weighing, WAB, and SAT immediately after harvesting. Samples of the remaining roots were freeze-dried (designated as biomass) and used for five starch-related assays and a starch granule measurement. Subsequently, the biomass was ground and treated with amylase and amylo-glucosidase to obtain de-starched materials for cell wall composition analyses. The cell wall analyses included ELISA-based quantification of cell wall matrix polysaccharides using cell wall-specific antibodies and sugar assays for neutral sugars and uronic acids in CDTA, TFA, and cellulose fractions.

2.4 | Microscopy

Fresh and cooked cassava roots (HNT, C33, 9/15, and 9/12 F1 lines) were free-hand cut into cross sections using razor blades. The sections were stained with 0.05% toluidine blue for 1 min and then washed in distilled water to remove excess dye. Imaging was performed using a Leica THUNDER imager 3D tissue equipped with LAS X software. For vessel counting, merged images consisting of 64 tiles were photographed at 10x magnification from three cross-sectional areas of two tuberous roots per genotype. The number of vessels that were stained green-blue was manually counted in six pictures per genotype using ImageJ. For wall thickness determination, freeze-dried slices of cassava storage roots were observed at 1,500x magnification using a scanning electron microscope (SEM) (Tabletop Microscopes TM4000II, Hitachi).

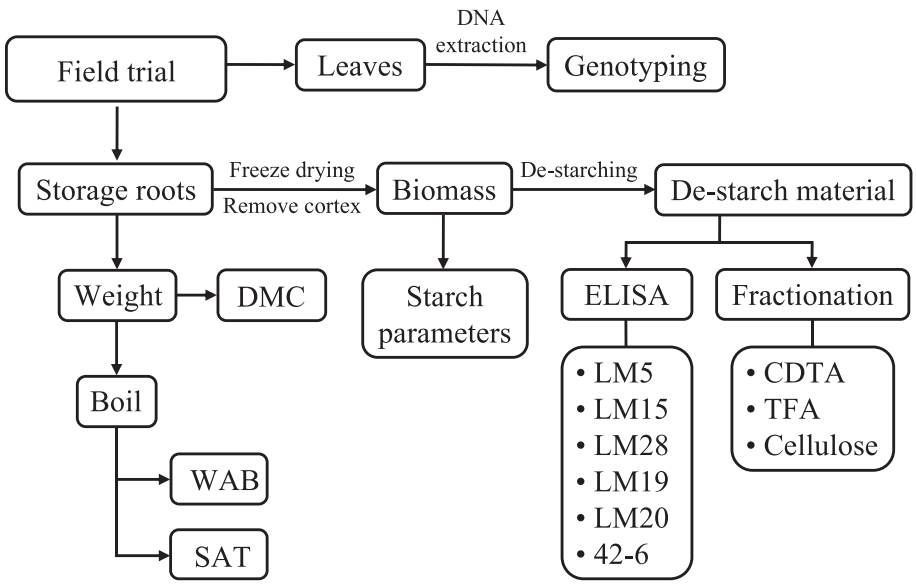

FIGURE 1 A schematic of analysis workflow for cassava storage roots and leaves. CDTA; trans-1,2-Diaminocyclohexane-N,N,N′,N′-tetraacetic acid, DMC; dry matter content, ELISA; the enzyme-linked immunosorbent assay, SAT; sensorial assessment of texture, TFA; Trifluoroacetic acid, WAB; water absorption during boiling.

2.5 | Determination of starch granule sizes

Twenty grams of biomass samples were suspended in 1.95 ml of a 1:5 dilution of 0.1 N iodine solution, and 10 µl of the sample suspension was loaded onto a counting slide. Starch granule sizes in diameter were then automatically analyzed using a TC20 automated cell counter (Bio-Rad).

2.6 | Determination of starch content

Resistant, digestible, and total starch contents in cassava flour samples were determined using a Megazyme-resistant starch assay kit (rapid format) (K-RAPRS). In brief, biomass samples (20 mg) were treated with pancreatic α-amylase/amyloglucosidase at 37°C for 4 h to hydrolyze digestible starch. After 95% (v/v) ethanol was added to stop the reaction, the digestible starch supernatant and the resistant starch pellet were separated by centrifugation. The pellet was washed in 50% (v/v) ethanol, which was combined with the initial supernatant, and then resuspended in 1.7 M NaOH to dissolve resistant starch prior to neutralization with acetate buffer. The starch in the digestible starch supernatant and the resistant starch pellet was hydrolyzed with amyloglucosidase to glucose, which reacted with glucose oxidase/peroxidase reagent. Absorbance was read at 492 nm.

2.7 | Determination of amylose and amylopectin contents

Amylose and amylopectin contents were determined using a Megazyme amylose/amylopectin assay kit (K-AMYL). Briefly, biomass samples (20 mg) were fully dispersed by heating in dimethyl sulfoxide (DMSO) and then precipitated in ethanol to remove lipids. The precipitated sample was dissolved in an acetate/salt solution, and the solution was further assayed for two separate aliquots. Lectin concanavalin A (ConA) was added to the aliquot to precipitate amylopectin, which was then removed by centrifugation. Amylose in the supernatant and total starch in the pellet were separately hydrolyzed with α-amylase/amyloglucosidase to glucose, which was calorimetrically detected by glucose oxidase/peroxidase reagent. Absorbance was measured at 492 nm. Amylose content (%) was calculated by multiplying the ratio of absorbance of the supernatant to that of the total starch aliquot by 66.8. Amylopectin content (%) was obtained by subtracting amylose content from 100.

2.8 | De-starching of cassava biomass

Biomass samples (5 g) were suspended in 20 ml of 0.1 M sodium acetate buffer pH 5.0 containing 5 mM $CaCl_2$, which was mixed with 50 µl of α-amylase from *Bacillus licheniformis* (A3403; Sigma-Aldrich). The mixture was incubated at 80°C in a shaking water bath for 18 h. After cooling to 40°C, 100 µl of amyloglucosidase solution (280 U/ml) (E-AMGDF; Megazyme) was added to the mixture, followed by incubation at 60°C in a shaking water bath for 18 h. The sample was cooled to room temperature, mixed with absolute ethanol to reach a total volume of 40 ml, and placed at 4°C for 1 h. After centrifugation at 3,000 *g* for 3 min, the supernatant was decanted. The pellet was washed twice with 20 ml of 80% ethanol, air-dried overnight, freeze-dried, and then ground using a tube mill control (IKA). De-starched samples are referred to as de-starched material in percentage of biomass.

2.9 | ELISA of cell wall polysaccharides

De-starched materials (10 mg) were separately extracted with 500 µl of 50 mM CDTA pH 7.0 and 500 µl of 2 M NaOH for 18 h. The CDTA extract was diluted with 500 µl of ultrapure water, and the NaOH

extract was neutralized with 500 µl of 2 M HCl. After centrifugation at 13,800 *g* for 10 min, the supernatants were collected and diluted prior to ELISA. ELISA was carried out following the previously described method (Sathitnaitham et al., 2021). Eight glycan-directed monoclonal antibodies (mAbs) were used: LM5 (β-1,4-D-galactans; Jones et al., 1997) LM6 (α-1,5-L-arabinan; Torode et al., 2018), LM12 (ferulated polysaccharides; Pedersen et al., 2012), LM15 (XyGs; Marcus et al., 2008), LM19 (unesterified HGs; Verhertbruggen et al., 2009), LM20 (methyl-esterified HGs; Verhertbruggen et al., 2009), LM28 (GXs; Cornuault et al., 2015) and 42–6 antibody (borate-rhamnogalacturonan II [RG-II] and RG-II monomers; Zhou et al., 2018). In the detection step, 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution was freshly prepared by adding 50 µl of 10 mg ml^{-1} TMB (860,336; Sigma-Aldrich) in DMSO and 50 µl of 0.3% H_2O_2 to 5 ml of 0.1 M sodium acetate buffer pH 5.0 for one plate. After the reaction was stopped by adding 0.5 N sulfuric acid, absorbance was read at 450 and 620 nm using a microplate reader (Infinite F50; Tecan). Commercial polysaccharides were used as standards for relative quantification: galactan (ex. potato) (Megazyme) for LM5, tamarind XG (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) for LM15, xylan (Beechwood) (P-XYLNBE; Megazyme) for LM28, PGA from citrus pectin (P-PGACT; Megazyme) for LM19 and apple pectin (70%–75% esterification; 76,282, Sigma-Aldrich) for LM20. The standards were dissolved in ultrapure water at a concentration of 1 mg ml^{-1}, followed by incubation at 60°C for 3 h. The solutions were diluted to a range of concentrations (19.5 ng ml^{-1} to 2.5 µg ml^{-1}), and 50 µl of the solution was included in each ELISA plate in triplicates in parallel with the sample. Polysaccharide quantities (ng) were calculated using 4PL standard curves generated by R programming (DR4PL) and divided by the starting weight of biomass to obtain µg of polysaccharide amounts per mg of biomass. Noting that there were no available RG-II standards, and results from 42 to 6 antibodies are expressed in absorbance reads. For the calculation of degrees of methylation (%) HG, amounts of methyl-esterified HG per mg of biomass were divided by amounts of un-esterified and methyl-esterified HG per mg of biomass and then multiplied by 100.

2.10 | Determination of sugar content

De-starched materials (10 mg) were extracted with 500 µl of 50 mM CDTA pH 7.0 for 18 h. The CDTA extract supernatant was collected, combined with 1 ml of pellet-washing ultrapure water, and then dried down in a SpeedVac concentrator prior to precipitation with cold absolute ethanol (80% ethanol in the final solution). The pellet was washed twice with 80% ethanol and, along with the CDTA-insoluble material, dried in the concentrator. Subsequently, the samples were hydrolyzed with 2 M trifluoroacetic acid (TFA) at 100°C for 4 h and dried in the concentrator. Isopropanol was added and dried twice to remove the remaining TFA. The respective pellets were resuspended in 500 µl of ultrapure water, followed by centrifugation at 13800 *g* for 10 min. The supernatants were collected, and neutral sugars and uronic acids were quantified by the anthrone-sulfuric acid assay and the m-hydroxydiphenyl method, respectively, as described in Sathitnaitham et al. (2021). For determination of cellulose content, TFA-insoluble residues of CDTA-insoluble material were washed with ultrapure water and acetone and then air-dried overnight. The residue was hydrolyzed with 72% (w/w) sulfuric acid at room temperature for 4 h, and ultrapure water was added to obtain 3.2% sulfuric acid before incubation at 120°C for 4 h. After centrifugation at 13800 *g* for 10 min, the supernatant was diluted and used for glucose quantification by the anthrone-sulfuric acid assay as above.

2.11 | SNP genotyping and analysis

Approximately 5 mm diameter leaf disc samples were collected from cassava plants in the field and freeze-dried in a 96-deepwell format. The samples were sent to Intertek (Australia) for DNA extraction and Diversity Arrays Technology for DArTseq genotyping (cassava DArTseqLD Pap 1.0). Libraries were prepared following DArTseq™ complexity reduction method (Kilian et al., 2012). SNP marker scoring was obtained using DArTsoft14, an in-house marker scoring pipeline. The sequenced reads were assigned to accessions using unique barcode sequences and aligned to the *Manihot esculenta* reference genome, version 6.1. The SNPs were called using the TASSEL 5.0 GBS pipeline version 2 (Glaubitz et al., 2014). A total of 13,160 bi-allelic SNP markers were detected across 18 chromosomes, plastid and unplaced contigs of *M. esculenta* v6 (GCF_001659605.1). All 184 samples with 13–49% missing rate were included in the analysis. For each sample, missing genotypes were imputed based on the mean value using rrBLUP (Endelman, 2011). After the filtering and data quality control process, a total of 12,567 SNPs distributed across the 18 cassava nuclear chromosomes were retained and used for population structure, genomic kinship estimation, and GWAS analysis. The observed heterozygosity and polymorphic information criteria (PIC) were calculated only for polymorphic sites located throughout 18 chromosomes using a formula proposed by Botstein et al. (1980).

2.12 | Data analysis

All data analysis was performed in R packages (R Core team, 2013). Pearson's correlation analysis was performed by Hmisc and corrplot packages (Harrell, 2023; Wei et al., 2017). Multiple regression analysis was performed using the MASS package (Ripley et al., 2013). A coefficient plot was performed using the dotwhisker package (Solt & Hu, 2015). PCA was performed using FactoMineR and factoextra packages (Kassambara & Mundt, 2020; Lê et al., 2008). For association studies, the population structure was evaluated based on the PCA using function prcomp of stat package and plot using functions in ggfortify (Tang et al., 2016) and ggplot (Wickham & Wickham, 2009) packages. The kinship matrix was calculated from the SNP markers using function A.mat in the rrBLUP package (Endelman, 2011). The genome-wide association analysis (GWAS) was

performed for each trait based on the mixed linear mocel (MLM), fixed and random effects model for circulating probability unification (FarmCPU) and Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) model using GAPIT package (Wang & Zhang, 2021). The linkage disequilibrium (LD) values (r^2) and physical distance (bp) were calculated using PopLDdecay (Zhang et al., 2019). The r^2 and physical distance (bp) were plotted and the LOESS method was applied to summarize the relationship between these values using ggplot package. The genomic region potentially linked to the statistically associated SNPs was identified using the GALLO package (Fonseca et al., 2020).

3 | RESULTS

3.1 | Comparative analysis of storage root tissues in soft-boiling and hard-boiling cassava varieties

To elucidate the structural basis underlying the distinct softening and mealiness attributes in eating varieties, we conducted an in-depth examination of root cell structure before and after boiling. We utilized root samples from both soft-boiling varieties (HNT and 9/15 F1 line) and hard-boiling counterparts (C33 and 9/12 F1 line). While an earlier report suggested a potential connection between eating varieties and post-boiling disrupted parenchyma cells in storage root tissues or altered root cell structures (Figueiredo et al., 2021), our investigations did not corroborate this finding across the four varieties/lines (Figure 2). Notably, the storage root tissues predominantly consist of xylem parenchyma cells housing starch granules, alongside a scattering of vessel cells that contribute to the fibrous texture of cassava flesh. At microscopic resolution, these parenchyma cells appear quite similar in terms of size and thickness, with notable differences in the abundance of vessel cells among the varieties/lines (Figure 2a,b). Yet, our assessment of the number of vessel cells per unit area did not show any significant difference between soft- and hard-boiling varieties (Student T test $P > 0.05$). This indicated that the general cell structure and abundance of thickened vascular cells do not discernibly influence the softening and mealiness properties of cassava roots. We subsequently focused on the intrinsic properties of root cells themselves, hypothesizing that structural characteristics allowing for post-boiling cell separation and enabling water penetration into root cells could govern the softening and mealiness properties. We determined the thickness of parenchyma cell walls of freeze-dried samples using SEM for the soft- and hard-boiling varieties. However, no significant disparity in the thickness of parenchyma cell walls between the two sets of varieties was observed (Figure 2c,d). Furthermore, no significant difference was observed in the size of parenchyma cells between soft- and hard-boiling varieties (Figure 2e). Taken together, the softening and mealiness properties inherent to cassava storage roots cannot be attributed to the tissue characteristics observed, indicating that other characteristics of the root cells are responsible for these properties.

3.2 | Biochemical composition analysis of cassava storage roots in a biparental population

We generated a biparental population between the soft-boiling HNT and hard-boiling C33 varieties to identify the factors behind the softening and mealiness properties of cassava storage roots. Approximately, 200 F1 seeds from this cross were germinated and grown in the field, and their storage roots were analyzed after harvesting. As expected, there was a wide variation in WAB ranging from 1.48 to 19.71%, and in SAT ranging from 1.8 to 4.9 (Figure 3 and Supporting Information Table S1). Average SAT for HNT (4.53 ± 0.13) was higher than for C33 (3.40 ± 0.31), and WAB for HNT was considerably higher (14.45 ± 2.11) than in C33 (2.30 ± 0.49). SAT data exposed some inconsistencies, common in sensory assessments, as indicated by the moderate correlation between the scores of the two panelists ($r = 0.65$). These inconsistencies, partly due to the limited experience of the panelist, may explain the moderate differences in the average SAT values for the two progenitors. Noting that while the genetic variability within our biparental population is expected to be lower than in the set of the selected 36 contrasting genotypes used by Tran et al. (2021), our WAB range is similar to previously reported values (less than 5% to 20%).

A comprehensive analysis of 20 additional traits was performed to identify factors that could explain the observed variability in cooking quality (WAB and SAT). Figure 3 and Supporting Information Table S1 present data ranges obtained from the F1 population and their progenitors. This data indicates that the softening and mealiness properties accessed by SAT and WAB tests are of a quantitative nature and may be associated with several unknown parameters within the storage roots. The DMC was distributed in a narrow range within the population, and it was similar between HNT and C33. For starch traits, the data from the F1 population was distributed in ranges across the six traits, and, interestingly, the values for these starch traits for HNT and C33 were very close to one another and situated in the midranges of the F1 data. Based on the HNT and C33 data, there are no clear differences in starch profiles between the soft- and hard-boiling genotypes. The amount of de-starched material in the population ranged between 7% and 41% of the total biomass, in which HNT and C33 had 8.73% and 11.4%, respectively.

To validate the antibodies to determine cell wall composition, we performed preliminary ELISA using various cell wall antibodies including LM5 (galactan), LM6 (arabinan), LM15 (xyloglucan), LM19 (un-esterified HGA), LM20 (methyl-esterified HGA), LM21 (galactomannan), LM28 (xylan), LM12 (ferulic acid and feruloylated xylan) and 42–6 (RG-II) with a number of commercial cassava varieties. As a result, LM5, LM15, LM19, LM20, LM28, and 42–6 antibodies showed variability in the amount of the polysaccharides recognized, indicating that they are suitable for assessing the F1 population. Other antibodies that produced signals too low for quantification were excluded from the analysis. (Supporting Information Table S2).

Analysis of the F1 population by ELISA showed that, in storage roots, there were relatively high amounts of un-esterified HG (12.18–205.38 μg mg^{-1} biomass) and a smaller amount of methyl-esterified HG (1.21–6.22 μg mg^{-1} biomass). The amounts of xylan, galactan,

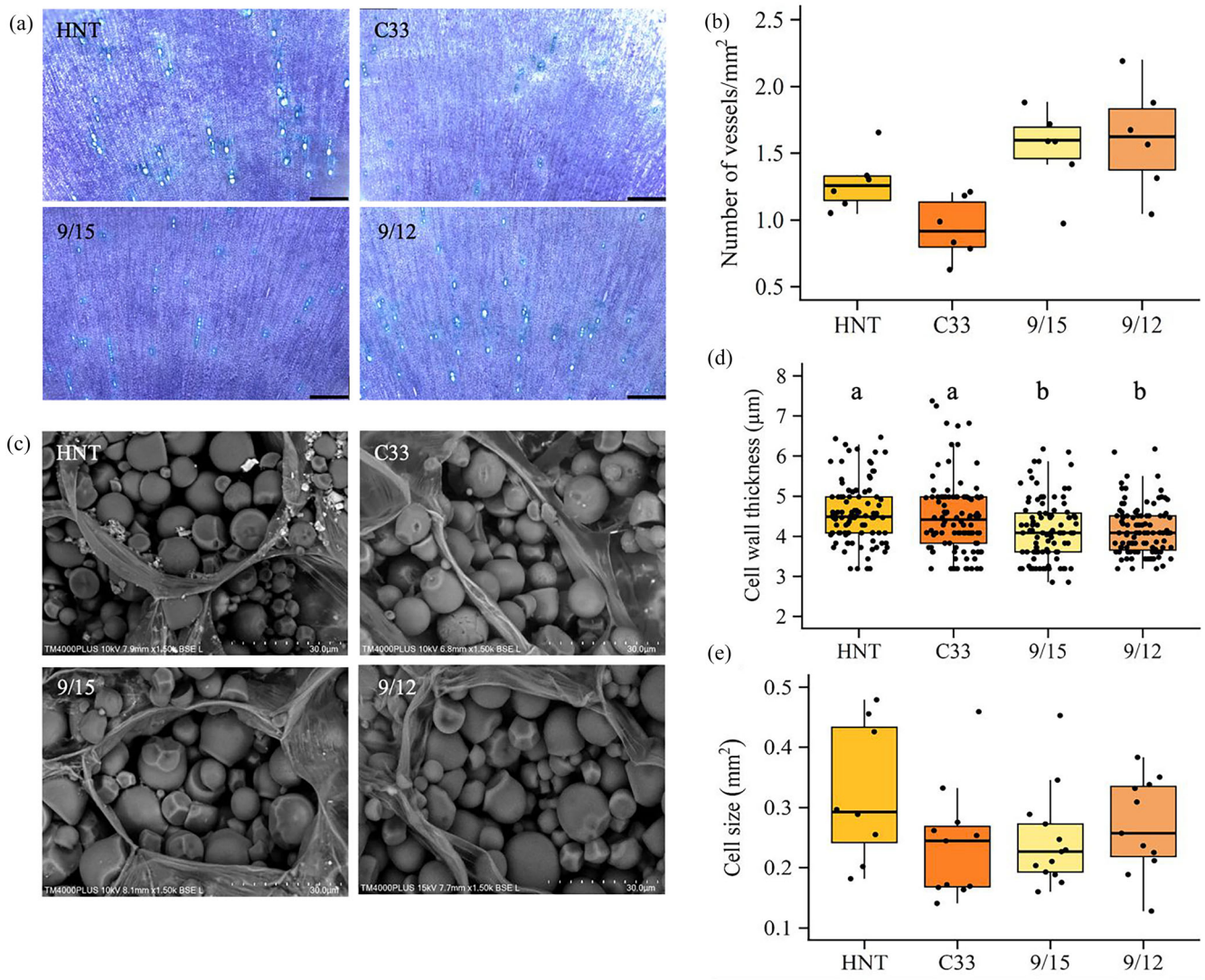

FIGURE 2 Analysis of tissue and cell structure in soft-boiling (HNT and 9/15 F1 line) and hard-boiling (C33 and 9/12 F1 line) cassava roots. (a) Merged images (64 tiles at 10x magnification) showing xylem vessels spread through starch-accumulated parenchyma cells in storage root cross-sections stained with 0.05% toluidine blue (scale bars = 1 mm). (b) Box plots of vessel density in the storage roots are calculated as the number of vessels per 1 mm^2 (n = 6). The number of vessels was manually counted from six merged imaged (64 tiles) taken from three cross-sectional areas of two tuberous roots per genotype. (c) Scanning electron microscope (SEM) images of freeze-dried cassava storage roots (un-boiled) showing parenchyma cells packed with starch granules (1,500x magnification). (d) Box plots of parenchyma cell wall thickness measured using ImageJ, obtained from 20 positions per image and five cross sections per genotype (n = 100). The same letters indicate no statistical significance (Tukey's test, $P < 0.05$). (e) Box plots of the size of parenchyma cells measured using ImageJ (n ≥ 8).

and xyloglucans are mostly similar to one another. Furthermore, we quantified cell wall polysaccharides based on neutral sugars and uronic acids of CDTA and TFA fractions and found that the CDTA fraction as a representative of pectin polymers had similar amounts of neutral and uronic sugars (2.13–11.01 and 2.21–15.42 μg mg^{-1} biomass, respectively), while the TFA fraction representing hemicelluloses had a higher proportion of neutral sugars (11.81–62.38 μg mg^{-1} biomass) than uronic sugars (3.02–20.91 μg mg^{-1} biomass). On the other hand, cellulose in the storage roots ranged from 10.73–117.12 μg mg^{-1} biomass. Interestingly, the values of these cell wall traits in HNT and C33 progenitors appeared to be similar to one another and located in the midranges of those from the F1 population, except the methyl-esterified HG and the degree of methylation of HG, where the two varieties are situated at opposite ends of the F1 data range. Based on the data from HNT and C33, this study suggests that the degree of methylation of pectin HG is likely to be involved in the determination of the soft and mealiness properties in cassava storage roots.

3.3 | Correlation analysis of cooking properties, starch, and cell wall compositions in the F1 population

To understand the interplay between the cooking quality parameters and the composition of the roots, we performed a correlation analysis of the 22 traits determined in 98 samples of our F1 biparental

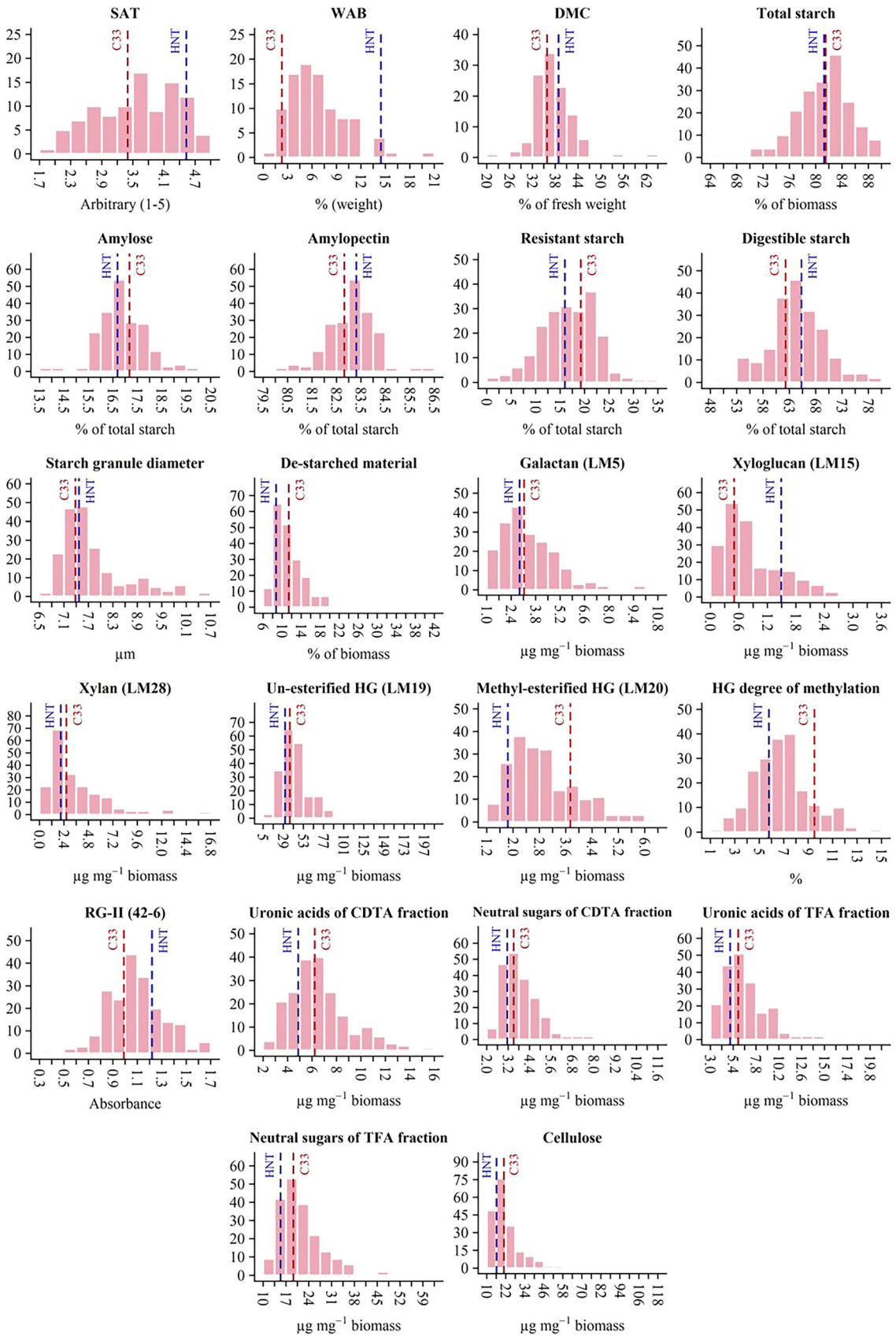

FIGURE 3 Legend on next page.

population (Figure 4). We found that the SAT and WAB, which are based on tensile strength and water absorption respectively, were positively correlated (r = 0.48), validating the methodology to measure the softening and mealiness properties. Despite our efforts to analyze starch traits, we did not observe any significant correlation between these traits with either SAT or WAB, indicating that starch characteristics are not involved in the softening and mealiness properties of cassava roots in our population. Surprisingly, we found negative correlations between SAT versus xylan (−0.32), un-esterified HG (−0.30), and cellulose (−0.34) and between WAB versus methyl-esterified HG (−0.25) and cellulose (−0.23). Furthermore, SAT was correlated with both uronic and neutral sugars of the TFA fraction (−0.25 and −0.27, respectively), and WAB was correlated with the neutral sugars of the TFA fraction (−0.25). Although the levels of correlation of these traits were moderate (0.23–0.34), they demonstrate that these cell wall components can have an impact on cooking quality in this population. This finding indicates that the softening properties based on the SAT test are associated with lower amounts of xylan, un-esterified HG, and cellulose, whereas those based on WAB are associated with lower amounts of methyl-esterified HG and cellulose.

Besides the cooking properties, the total starch was correlated to the resistant and digestible starch (0.43 and 0.33, respectively), but negatively correlated to de-starched materials (−0.53). Indeed, amylose was positively correlated with the resistant starch (0.28) and negatively correlated with digestible starch (−0.18), and these relationships, as expected, are inverse with amylopectin. Furthermore, the starch granule size also plays a role in the starch recalcitrance, as the size is positively correlated with resistant starch (0.22). The de-starched material is negatively correlated to the resistant starch (−0.35), meaning that the highly resistant starch associates with less de-starched materials or those with higher amounts of starch. For cell wall composition, de-starched materials are highly correlated with most of the cell wall parameters, except xyloglucan and degree of methylation, and negatively correlated with RG-II, suggesting that the de-starched materials were enriched in cell wall components. De-starched materials are highly correlated to the amount of cellulose, indicating that cellulose is the key determinant for the cell wall material in cassava roots. Taken together, these results indicate that SAT and WAB (representing cooking quality) are associated with the cell wall components including cellulose, xylan, and both un-esterified and methyl-esterified HG.

3.4 | Impact of cell wall components on cassava cooking quality

A multiple regression analysis was conducted to investigate the influence of key cell wall components on cassava cooking quality, specifically SAT and WAB. Our analysis focused on the four critical parameters based on their correlation with SAT and WAB, namely cellulose, xylan, un-esterified HG, and methyl-esterified HG. We also included adjustments for the interplay between un-esterified and methyl-esterified HG within the same molecule, calculated as a percent methylation of HGA. The test models and the best-fitted regression, selected based on their Akaike Information Criterion (AIC) values, are presented in Supporting Information Table S3. Our results revealed that the best models could explain 17.96–20.37% of the variation in SAT and 12.34–14.45% of the variation in WAB. Detailed statistical analysis of these selected regression models is provided in Supporting Information Figures S1 and S2. ANOVA tests showed that the selected best regression models were not significantly different from the full testing models ($P > 0.05$), indicating that the best-reduced model did not perform significantly worse than the full model. While the multiplication terms remain challenging to interpret, the main terms are discernible as indicated in coefficient plots (Figure 5). For SAT, un-esterified HG, methyl-esterified HG, and degree of methylation are more efficient in determining both SAT1 and SAT2 models than xylan and cellulose. Conversely, for WAB, cellulose exhibits a larger contribution to both WAB1 and WAB2 models compared to the other four terms.

Furthermore, we performed a principal component analysis (PCA) to gain insights into the individual effects of these cell wall parameters on SAT and WAB (Figure 6). Remarkably, SAT and WAB gave different patterns in the PCA analysis. For SAT, the vectors indicated that cellulose, xylan, and un-esterified HG exhibited opposite directions to the SAT vector, showing a strong negative relationship with SAT-related cooking properties. In contrast, the vector for methyl-esterified HG suggested a positive effect on SAT, albeit less pronounced compared to the aforementioned three parameters. In the case of WAB, the vectors suggested that methyl-esterified HG had a strong negative effect on water absorption during boiling, as indicated by the opposite direction, while cellulose, xylan, and un-esterified HG had comparatively less influence on WAB. Taken together, our results suggest that approximately 20% of SAT variability and 14% of WAB variability can be attributed to cellulose, xylan, and un-esterified and methyl-esterified HG. Notably, un-esterified HG, methyl-esterified HG, and the degree of methylation are primary factors affecting SAT, while cellulose emerges as the primary factor for WAB. Pearson's correlation and PCA reveals that xylan, cellulose, and un-esterified HG exert negative effects on SAT, while methyl-esterified HG positively affects SAT but negatively affects WAB. Importantly, our data indicates the presence of other, as yet unidentified parameters, contributing significantly to cooking properties, potentially explaining up to 80% of SAT variability and 86% of WAB variability. These findings underscore the complex nature of cassava cooking quality and highlight the need for further exploration to uncover the remaining factors.

FIGURE 3 Data distribution of 22 traits obtained from the cassava F1 population. X-axis presents data units, and the Y-axis presents data frequency. Lines indicate the data for HNT and C33 progenitors within the distribution data. CDTA; trans-1,2-Diaminocyclohexane-N,N,N′,N′-tetraacetic acid, DMC; dry matter content, ELISA; the enzyme-linked immunosorbent assay, RG-II; Rhamnogalacturonan-II. SAT; sensory assessment of texture, TFA; Trifluoroacetic acid, WAB; water absorption.

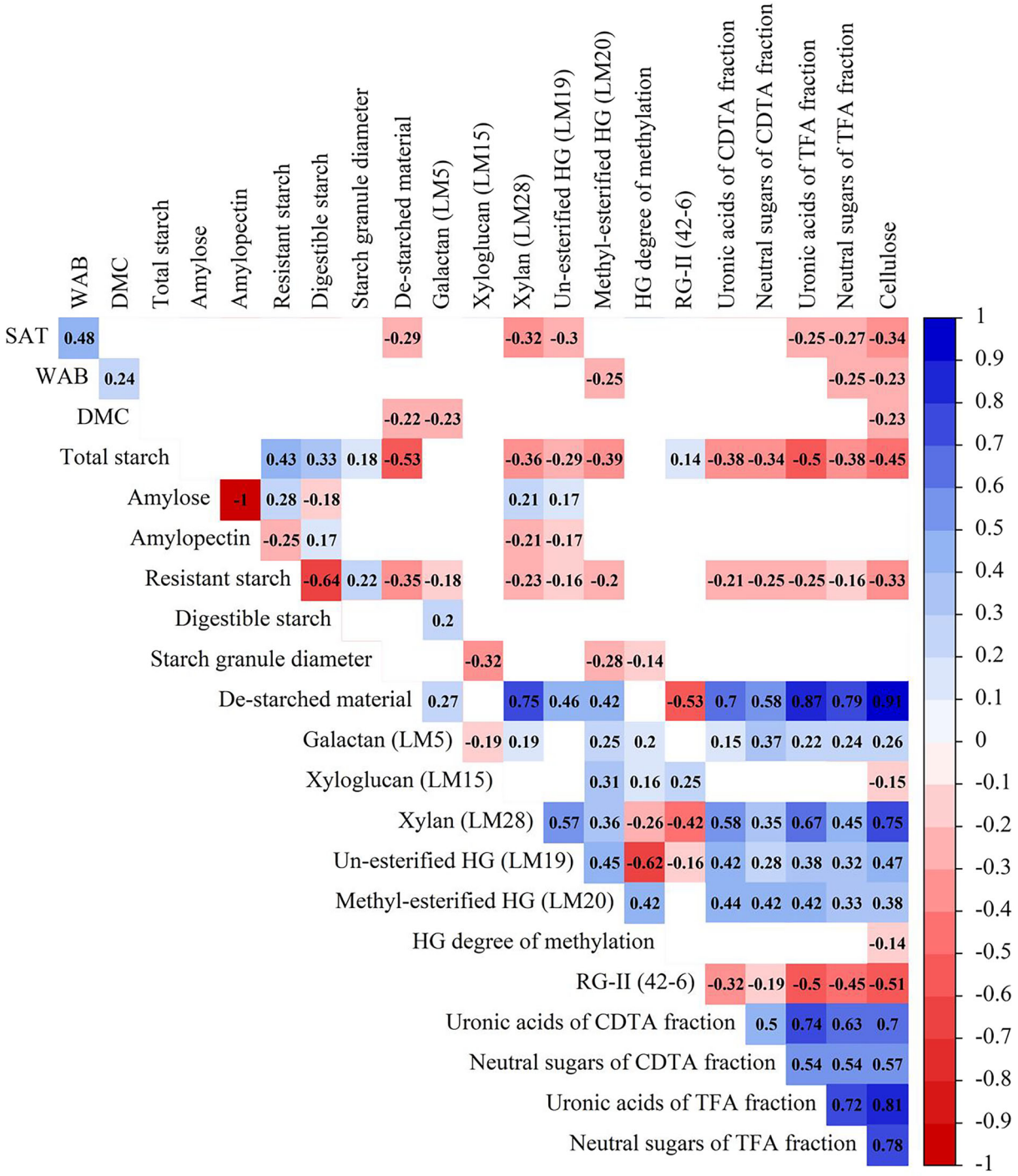

FIGURE 4 Correlation analysis of cassava cooking quality. A scatter plot between SAT and WAB illustrates the segregated F1 population (n = 98). Data points of the parental lines (HNT and C33) are indicated. Pearson's correlation matrix of 22 traits of cassava storage roots including cooking properties, starch properties, and cell wall compositions from the F1 population. Only correlation coefficients with statistical significance are shown (P < 0.05). CDTA; trans-1,2-Diaminocyclohexane-N,N,N′,N′-tetraacetic acid, DMC; dry matter content, ELISA; the enzyme-linked immunosorbent assay, RG-II; Rhamnogalacturonan-II, SAT; sensory assessment of texture, TFA; Trifluoroacetic acid, WAB; water absorption.

3.5 | GWAS of cell wall traits

To uncover insights into the cooking quality with regard to SAT and WAB, as well as their association with cell wall components including cellulose, xylan, un-esterified HG, and methyl-esterified HG, we conducted GWAS utilizing a comprehensive set of genome-wide SNP markers obtained through genotyping-by-sequencing. This analysis was conducted on 184 individuals after filtering for ungenotyped SNPs and SNPs located on unplaced contigs. We identified a total of 12,567 SNP markers within the nuclear genome, with a mean SNP density of 1 SNP per 17.97 kb across the 18 chromosomes of the cassava genome sequence v6.1 (Supporting Information Figure S3). The

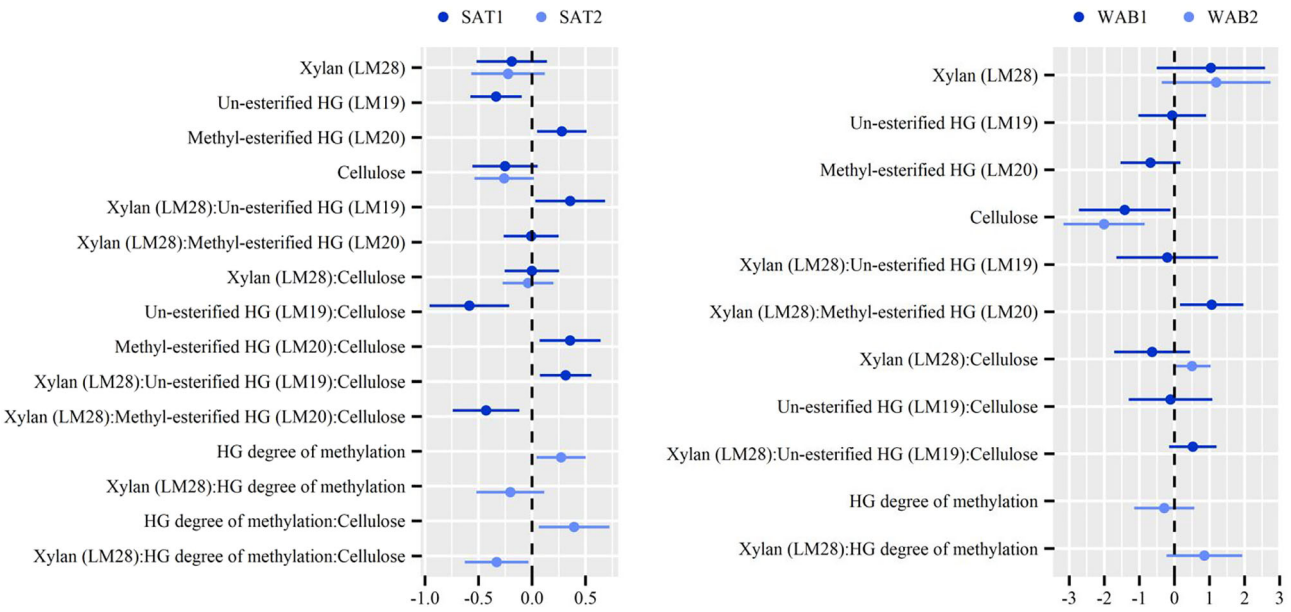

FIGURE 5 Coefficient plots of the best models for SAT and WAB traits of the cassava storage roots. Each point represents the estimate of the coefficient of that term with the 95% confidence interval. The vertical dashed line indicates the value of the coefficient at zero. HG; homogalacturonan, SAT; sensory assessment of texture, WAB; water absorption.

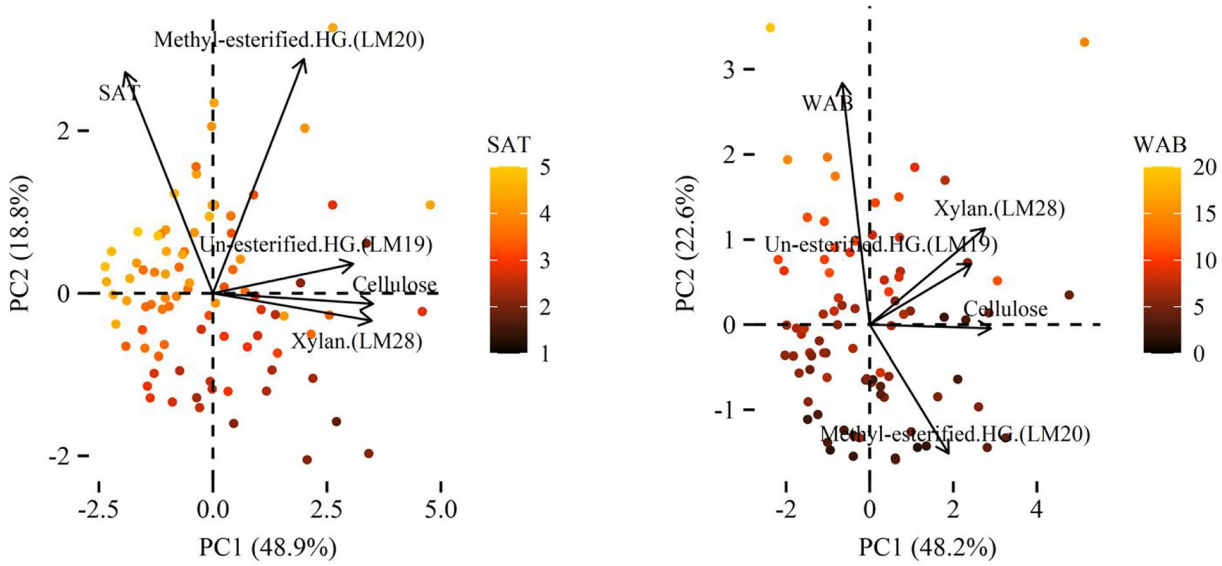

FIGURE 6 PCA analysis of cassava storage roots' properties, including SAT and WAB and their correlated cell wall parameters. Color scales of SAT and WAB for individual F1 lines in PCA are presented. HG; homogalacturonan, SAT; sensory assessment of texture, WAB; water absorption.

lowest and highest number of SNPs per chromosome were 496 on chromosome 7 and 1,313 on chromosome 1. The mean minor allele frequency was 0.11, with minimum and maximum frequencies of 0 and 0.5. The PIC values, indicating SNP diversity, ranged from 0.38 to 0.99 with an average of 0.68, underscoring the reliability of our GWAS. Heterozygosity estimates, both observed and expected, ranged from 0.00007 to 0.01 and 0.005 to 0.5, respectively, across the genome. To account for population structure, we utilized PCA, though we did not detect any subpopulations in our reference population, as our population was generated from a biparental cross. Linkage disequilibrium (LD) was evaluated for each pair of SNP markers, and a midpoint r^2 value of 0.15 was chosen as the linkage decay point, roughly corresponding to 60 kb.

Our GWAS analysis was carried out using three distinct methods: MLM, FarmCPU, and BLINK, with a focus on SAT, WAB, and correlated cell wall attributes. Unfortunately, we obtained matching phenotype–genotype only for 77 and 75 individuals for SAT and WAB, respectively, and did not identify any significant SNPs for

these cooking traits. This may be attributed to the limited statistical power resulting from the sample size. However, our study revealed significant SNPs associated with the four cell wall traits: cellulose, xylan, un-esterified HG, and methyl-esterified HG (15, 7, 13, and 1 SNPs, respectively) (Figure 7). A detailed summary of the identified significant SNPs is provided in Supporting Information Table S4. These significant SNPs, as identified by the MLM method, accounted for approximately 13–37% of the phenotypic variances, consistently exhibiting either positive or negative effects on the traits. Moreover, our investigation showed two shared significant SNPs between cellulose and xylan traits. In this particular, two genomic regions represented by SNP_9,574 and SNP_12,624 on chromosomes 13 and 18, exhibited negative and positive effects, respectively, on cellulose and xylan, underpinning their multifaceted influence on these traits. The SNPs related to HG did not overlap with other polysaccharides.

To explore genes surrounding SNP positions, we examined the 60 kb upstream and downstream regions of the identified SNPs. All associated gene loci, along with annotations, are detailed in Supporting Information Dataset S1. Candidate genes were selected based on annotations for their potential functions related to cell wall traits (Table 1). Cellulose content in roots was found to be associated with genes encoding various cell wall-related proteins, including cell wall integrity and stress response component 1, cell wall invertase, and Expansin A8. Rho GTPase-activating protein 5, which regulates vesicle packages related to cell wall biosynthesis (Lauster et al., 2022), also showed associations with cellulose. Notably, WRPK and NAC transcription factors were identified in association with xylan, cellulose, and un-esterified HG. While a number of *WRKY* and *NAC* gene families have been shown to play roles in plant cell wall biosynthesis, further detailed characterization of these specific genes is needed to confirm their involvement in the traits related to cell wall components. Intriguingly, genes related to sugar metabolism were identified, including cell wall invertase and UDP-glucose 4-epimerase 5 (catalyze the conversion of UDP-galactose to UDP-glucose) for cellulose trait, and D-xylose-proton symporter-like 2 for methyl-esterified HG trait. Glycosyl hydrolases were associated, including polygalacturonate for xylan and rhamnogalacturonate lyase B for un-esterified HG, suggesting their role as polysaccharide-modifying enzymes during wall

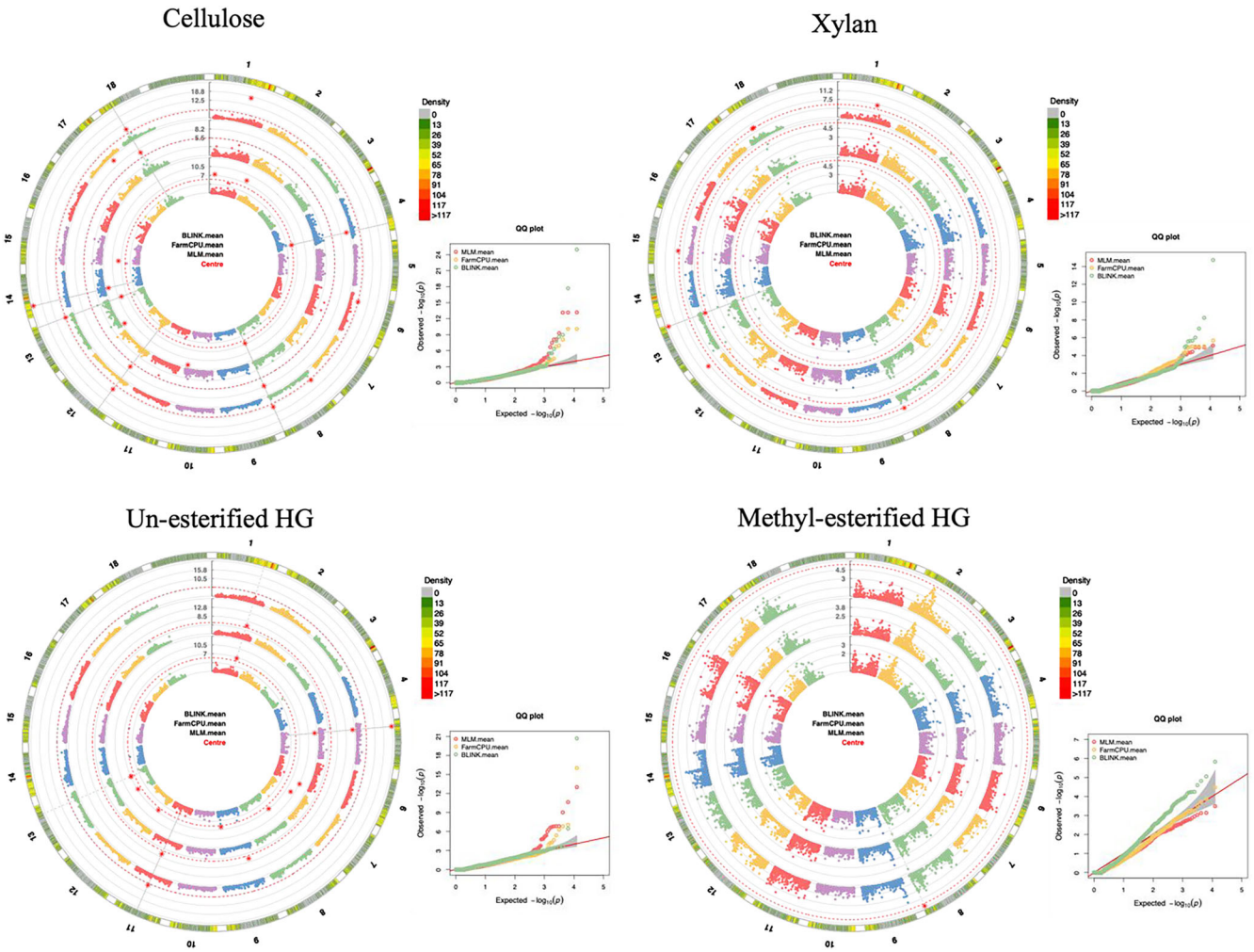

FIGURE 7 Circular Manhattan plots present SNPs associated with four cell wall components analyzed from 184 F1 lines. SNP locations on chromosomes and P values are plotted on the x-axis and y-axis, respectively. The dotted red line presents the Bonferroni correction at $P < 0.05$ the quantile-quantile (QQ) plots for observed and expected P values are presented based on MLM, FarmCPU, and BLINK methods.

TABLE 1 Selected candidate genes located within 120 kb of significant SNPs associated with cellulose, xylan, un-esterified, and methyl-esterified homogalacturonans (HGs) in cassava, identified through genome-wide association studies (GWAS) aimed at understanding the genetic basis of cell wall composition.

Trait	SNP.ID	Chr	Position (bp)	Manes ID candidates	Description
Cellulose	3,476	4	24,267,025	MANES_04G108000	Cell wall integrity and stress response component 1
Cellulose	5,846	8	2,382,714	MANES_08G027300, MANES_08G027200, MANES_08G026700	Beta-fructofuranosidase (cell wall invertase), Beta-fructofuranosidase (cell wall invertase), Rho GTPase-activating protein 5
Cellulose	6,348	8	33,102,354	MANES_08G170600	WRKY transcription factor 70
Cellulose	6,351	8	33,224,659	MANES_08G172000	Expansin-A8
Cellulose	1,353	2	871,030	MANES_02G009300	UDP-glucose 4-epimerase 5
Cellulose	7,629	10	24,506,348	MANES_10G132800	WRKY transcription factor 9
Cellulose	9,948	14	6,148,152	MANES_14G075800	WRKY transcription factor 72A
Xylan	9,017	12	31,366,360	MANES_12G156100	Probable polygalacturonase
Xylan	10,849	15	8,339,152	MANES_15G110400	WRKY transcription factor 50
Xylan Cellulose	9,574	13	27,023,046	MANES_13G141600	NAC domain-containing protein 73
Xylan Cellulose	12,624	18	7,357,832	MANES_18G084335	Wall-associated receptor kinase 3
Un-esterified HG	806	1	27,368,982	MANES_01G170600	Laccase-2
Un-esterified HG	5,141	6	25,603,721	MANES_06G152300	SPIRAL1-like 5
Un-esterified HG	8,728	12	7,323,821	MANES_12G072800	WRKY transcription factor 31
Un-esterified HG	9,198	13	5,301,200	MANES_13G049900	Galacturonosyltransferase 8
Un-esterified HG	9,403	13	22,479,336	MANES_13G102800	Rhamnogalacturonate lyase B
Methyl-esterified HG	6,319	8	32,431,982	MANES_08G162600	D-xylose-proton symporter-like 2

biosynthesis. The identification of Galacturonosyltransferase 8, a primary enzyme for HG biosynthesis, suggested a direct link to HG content in the roots. Furthermore, genes encoding Laccase-2 and SPIRAL1-like 5 were associated with un-esterified HG. While Laccase is involved in lignin biosynthesis (Dixon & Barros, 2019) and SPIRAL1 is related to actin (Sedbrook et al., 2004), establishing a direct relationship with un-esterified HG requires further investigation. These findings underscore the intricate genetic landscape governing cell wall traits in cassava roots, revealing potential candidate genes with roles in cell wall biosynthesis and modification. Further research will validate the specific functions of these candidate genes in shaping cassava root properties related to cooking quality.

4 | DISCUSSION

In this study, we investigated the cellular and molecular composition that determines the cooking quality of cassava storage roots. Previous reports have highlighted the substantial variation in cooking quality among different cassava varieties (Sajeev et al., 2008; Tran et al., 2021; Uchendu et al., 2022). While the impact of overall DMC and major components, such as starch and amylose, is evident for yams, plantains, and sweet potatoes, establishing a direct physicochemical characteristic of starch linked consistently to the texture and mealiness of boiled cassava has remained elusive (Asaoka et al., 1991; Charoenkul et al., 2006; Padonou et al., 2005; Kouadio et al., 2013). Additionally, there were no discernible differences in cell arrangement or cell condition between mealy and non-mealy cassava varieties (Safo-Kantanka & Owusu-Nipah, 1992). Our work, which examines cooking quality within a biparental population using SAT and WAB, aligns with previous findings, failing to establish correlations between cell structure, starch parameters, and cassava cooking quality. Despite an extensive examination of starch-related characteristics, including amylose and amylopectin content, resistant and digestible starch, starch granule sizes, and DMC, no definitive links with cooking properties emerged, as demonstrated in our and previous studies (Kouadio et al., 2013). These results emphasize the involvement of other factors within cassava storage roots in determining cooking quality. The segregation pattern of SAT and WAB within the F1 population also strongly suggests that cooking quality is a quantitative trait influenced by multiple attributes.

Pectin, the primary component of the middle lamella, is widely recognized for its role in cell–cell adhesion and, hence, for the crispness or mealiness of vegetables and fruits (Parker et al., 2001). Our results support this proposition in the same line as Favaro et al. (2008), who observed significantly higher uronic acid levels in cell

walls extracted from hard-cooking cassava varieties than in soft-cooking counterparts. Moreover, specific pectin fractions appear to correlate with vegetable and fruit crispness and mealiness, with harder cassava roots and/or ripe fruits exhibiting elevated chelate-insoluble pectin levels and reduced chelate-soluble pectin levels (Favaro et al., 2008; Xin et al., 2010; Zhang et al., 2008). Additionally, cooking rate, cell disruption level, and firmness of cassava roots are directly linked to divalent cations that promote pectin gelation and crosslinking (Eggleston & Asiedu, 1994; Favaro et al., 2008; Mestres et al., 2023). These findings underscore the pivotal role of pectins, especially un-esterified HG, in shaping cassava cooking quality. Nevertheless, other pectin components like RG-I and RG-II may also influence cooking properties, and their role warrants further exploration. Uchendu et al. (2021) conducted a GWAS study of root mealiness and other texture-associated traits in cassava, identifying 80 SNPs significantly associated with various attributes, including root mealiness, fiber, adhesiveness, taste, aroma, color, and firmness. Notably, the gene Manes.13G026900 (Plant invertase/pectin methylesterase inhibitor), known for its role in cell adhesion and the mealiness or crispness of vegetables and fruits, was associated with the cassava mealiness trait. This finding is particularly intriguing given the acknowledged role of pectin in influencing cell adhesion, mealiness, and crispness of vegetables and fruits (Jarvis et al., 2003; Parker et al., 2001). Moreover, various studies have reported evidence of PME activity in cooked potato texture (Ross et al., 2010; Ross, Morris, et al., 2011; Ross, Wright, et al., 2011).

The role of cell wall components, particularly pectins and their interaction with divalent cations, in determining the cooking quality of cassava roots has garnered significant attention (Eggleston & Asiedu, 1994; Favaro et al., 2008). Mestres et al. (2023) indirectly demonstrated the involvement of pectins in determining cooking quality by showing that cassava roots boiled in calcium chloride solutions exhibited reduced water absorption, as seen across several genotypes with varying cooking quality. The increased Ca^{2+} concentration was anticipated to strengthen the pectin network through crosslinking between Ca^{2+} and the carboxyl groups of un-esterified HG (Braccini & Pérez, 2001; Grant et al., 1973), resulting in reduced water absorption and firmer texture. A similar trend has been observed in potatoes (Murayama et al., 2019). Although most previous work on pectin in cassava was conducted by quantifying uronic acid or GalA content following acid hydrolysis (Franck et al., 2011), this method did not provide insights into methylation and its influence on crosslinking potential. Our use of ELISA with LM19 and LM20, which specifically recognize un-esterified and methyl-esterified HG, respectively, has shed light on the relationship between pectin and cooking properties. The correlation analysis, mathematical modeling, and PCA of cell wall components and cooking quality (SAT and WAB) presented here, revealed that both methyl-esterified and un-esterified HG, as well as xylan and cellulose, contribute to cooking quality. For SAT, higher levels of un-esterified HG may enhance the rigidity of the cell wall structure through calcium crosslinks, resulting in a firmer texture. Conversely, increased methyl-esterified HG content reduces the potential for calcium bridges to form, yielding a softer texture.

Additionally, xylan and cellulose provide structural support to the parenchyma wall, affecting the root's texture. In the case of WAB, increased methyl-esterified HG reduces pectin's water-absorbing capacity, while other polymers, including un-esterified HG, xylan, and cellulose, play less significant roles in water absorption. Thus, we observe two distinct scenarios concerning methyl-esterified HG: higher degrees of methylation result in both reduced water absorption and softer texture, while lower degrees of methylation lead to increased water absorption but greater firmness. Higher degrees of pectin methylation contribute to a gradual decrease in the affinity for Ca^{2+} (Thakur et al., 1997; Tibbits et al., 1998). Consequently, the balance of methyl-esterified HG is crucial for achieving an optimal texture based on SAT and WAB, hence enhancing cooking quality.

Studies on potatoes have revealed that cell wall components play a crucial role in determining tuber textural properties (Nonaka, 1980). Mealy potato varieties, for example, exhibit higher levels of pectin branching, methylation, and acetylation compared to non-mealy varieties (van Marle et al., 1997). Additionally, tubers with elevated total pectin methylesterase (PME) activity tend to have reduced pectin methylation in their cell walls. This reduction leads to increased peak force, indicating a higher abundance of charged HG, which enhances Ca^{2+} crosslinking and tensile strength (Ross et al., 2010). Expression profiling of potato varieties with different textures has also identified genes associated with cell wall modification, including those encoding pectin acetylesterase, xyloglucan endotransglycosylase, and PME (Ducreux et al., 2008). Moreover, the matrix polysaccharide, particularly RG-I, has been proposed to play a role in transmitting stresses to load-bearing cellulose microfibrils (Ulvskov et al., 2005). The hydration properties of potatoes have been linked to the length and characteristics of arabinan and galactan side chains within RG-I (Larsen et al., 2011). Reduced galactan and arabinan negatively impact hydration due to the lower hydrophilic properties of the rhamnose-galacturonic acid (Rha-GalA) backbone in RG-I. While the effect of pectin methylation levels in potatoes aligns with that of cassava, these findings from potato studies may not be directly applicable to cassava. Despite the presence of galactan and xyloglucan in cassava cell walls, as demonstrated in this work and by Staack et al. (2019), we did not find a correlation between galactan or xyloglucan and the cooking properties of cassava roots (SAT and WAB). Furthermore, their analysis using LM6 and LM12 indicated no presence of arabinan and ferulated galactan and is consistent with our analysis, indicating that these polymers are present in minimal amounts and are unlikely to be linked to cooking quality. Nonetheless, these insights from potato research provide valuable support for the hypothesis that cell wall components, with a specific focus in this study on xylan, pectin, and cellulose, influence the textural properties and cooking quality of cassava roots.

Regression models revealed that up to 20% and 14% of the variance in SAT and WAB, respectively, could be explained by specific cell wall components, namely cellulose, xylan, and HG. PCA provided additional insights, indicating strong negative associations between cellulose, xylan, and un-esterified HG with SAT, while methyl-esterified HG exerted a pronounced negative effect on

WAB. However, cellulose, xylan, and un-esterified HG exhibited lesser influence on WAB. Notably, the models suggested that the remaining 80% and 86% of variation in SAT and WAB, respectively, might be influenced by unknown components. Contrary to the common focus on lignins, Silva et al. (2022) demonstrated, that carbohydrates (ratio of sucrose, glucose, and starch) played a pivotal role in distinguishing various cooking genotypes, while lignins (syringes/guaiacyl ratio) from the cell wall did not differentiate cooking performances. In our work, quantification of pectin crosslinking through the borate-RG-II complex via the 42–6 antibody exhibited no correlation with cooking quality. Furthermore, crystalline cellulose analysis in samples with varying cooking textures did not reveal any correlation with soft- and hard-boiling varieties (Supporting Information Figure S4). Whereas analysis of total dietary fiber (soluble and insoluble) of selected hard- and soft-boiled cassava lines showed negative correlations of SAT and WAB versus insoluble dietary fiber and total dietary fiber ($P < 0.05$) (Supporting Information Figure S5), and this supports our finding considering the cell wall polysaccharides as the major component of dietary fiber. We also performed analysis of starch gelatinization using Rapid Visco Analyzer (RVA), and there was no correlation ($P > 0.05$) between SAT and pasting properties of samples with varying cooking textures (Supporting Information Figure S6). Although we found positive correlations ($P < 0.05$) between WAB versus minimum viscosity (0.62), final viscosity (0.61), and setback (0.58), these associations can be attributed to the characteristics of the starch granule and other components such as crude fiber, which results in a more resistant breakdown for those that absorb a larger amount of water during boiling. Noting that cassava lines with a high water-absorption capacity are likely to experience increased retrogradation as a result of their strong correlation with high final viscosity and setback value. Other factors related to cell wall that we have not explored in this study include crosslinking through cell wall proteins including AGPs and a tyrosine-lysine rich protein (TLRP), which was identified by a QTL for potato cooking type (Kloosterman et al., 2010). Nevertheless, the softening of tubers and roots during cooking may result from physicochemical changes in cell wall materials, including reduced hydrostatic pressure and solubilization of pectic materials (Sajeev et al., 2008), although these aspects were beyond the scope of our assessment.

Previous GWAS studies on a cassava diversity panel (Uchendu et al., 2021) found associations between cell wall-related genes and mealiness (pectin methylesterase inhibitor) and firmness (wall-associated kinase family protein). In our study, while direct associations with SAT and WAB were not identified, we discovered numerous genes associated with cell wall components correlated with cooking traits. These genes encompassed various aspects of cell wall biology, including sugar metabolism, cell wall biosynthesis, and cell wall-modifying proteins. Although our findings indirectly align with previous research on the relationship between cell wall genes and cooking quality, further investigation into the genes identified could reveal the role of the cell wall in controlling the cooking quality of cassava roots. This could potentially lead to the validation of these genes as valuable selection markers in breeding programs that satisfy consumer preferences.

In conclusion, our comprehensive analysis of soft and hard-boiling cassava varieties revealed no significant differences in the root tissue structure. The examination of 22 traits, encompassing cooking qualities, starch properties, and cell wall composition, highlighted specific cell wall components, cellulose, xylan, and HGs, as correlated with cooking properties. SAT is influenced by methyl-esterified and un-esterified HGs, xylan, and cellulose, impacting the tensile strength of the cell wall. On the other hand, WAB is regulated by methyl-esterified HG, potentially altering water absorption properties. Additionally, GWAS sheds light on genes governing these cell wall components. Although no significant SNPs were found for SAT and WAB, the correlation between these cooking parameters and cell wall parameters supports that the SNPs found for cell wall components will also regulate cooking quality. This study provides insights into the complex relationship between cassava root physiology and cooking characteristics, underscoring the role of cell wall components in determining the cooking quality of edible cassava. The trait-related genes should be further validated as the markers for the selection of soft-texture cassava breeding lines in the early breeding program of cassava for consumers.

AUTHOR CONTRIBUTIONS

SV, PKo, and HC conceived the project and designed experiments. SS, PKo, SV, HC, PW, SU, AS, PKi, NS, and LG performed the experiments and analyzed the data. SS, PW, and EK performed statistical and mathematical analysis. SS, PKo, PWo, and SV prepared Figures and Tables and wrote the manuscript. HC and LG provided critical edits and revised the manuscript. All authors have approved the final version.

ACKNOWLEDGMENTS

This work was supported by The Office of the Ministry of Higher Education, Science, Research and Innovation; and the Thailand Science Research and Innovation through the Kasetsart University Reinventing University Program 2021, and Kasetsart University Research and Development Institute (KURDI). The authors thank the UK's Foreign, Commonwealth and Development Office (FCDO) and the Bill and Melinda Gates Foundation (Grant INV-007637, http://www.gatesfoundation.org) for their financial support for SNP genotyping. SV was supported by the National Research Council of Thailand: NRCT5-RSA63002-02. PWo was supported by NRCT-N42A650286. We thank Masaru Kobayashi, Kyoto University, for RG-II (42-6) antibody. We thank Pornsawan Klayklung (COAX Group Corporation LTD) for SEM support.

CONFLICTS OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All data generated and used in this study are available upon request or as Supporting Information for this article.

ORCID

Hernan Ceballos https://orcid.org/0000-0002-8744-7918
Passorn Wonnapinij https://orcid.org/0000-0002-6224-0275
Ekaphan Kraichak https://orcid.org/0000-0002-8437-2180
Anongpat Suttangkakul https://orcid.org/0000-0001-5512-683X
Leonardo D. Gomez https://orcid.org/0000-0001-6382-9447
Piya Kittipadakul https://orcid.org/0000-0001-6798-5324
Nongnuch Siriwong https://orcid.org/0009-0003-6225-8963
Pasajee Kongsil https://orcid.org/0000-0002-1154-4340
Supachai Vuttipongchaikij https://orcid.org/0000-0002-1194-3552

REFERENCES

Allem, A. C. (2002). The origins and taxonomy of cassava. In R. J. Hillocks, J. M. Tresh, & A. C. Bellotti (Eds.), *Cassava: Biology, production and utilization* (pp. 1–16). CABI Publishing.

Asaoka, M., Blanshard, J. M. V., & Rickard, J. E. (1991). Seasonal effects on the physico-chemical properties of starch from four cultivars of cassava. *Starch-Stärke*, *43*(12), 455–459. https://doi.org/10.1002/star.19910431202

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, *32*(3), 314–331.

Braccini, I., & Pérez, S. (2001). Molecular basis of Ca2+−induced gelation in alginates and pectins: The egg-box model revisited. *Biomacromolecules*, *2*(4), 1089–1096. https://doi.org/10.1021/bm010008g

Chaengsee, P., Kongsil, P., Siriwong, N., Kittipadakul, P., Piyachomkwan, K., & Petchpoung, K. (2020). Potential yield and cyanogenic glucoside content of cassava root and pasting properties of starch and flour from cassava Hanatee var. and breeding lines grown under rain-fed condition. *Agriculture and Natural Resources*, *54*(3), 237–244. https://doi.org/10.34044/j.anres.2020.54.3.02

Charoenkul, N., Uttapap, D., Pathipanawat, W., & Takeda, Y. (2006). Molecular structure of starches from cassava varieties having different cooked root textures. *Starch-Stärke*, *58*(9), 443–452. https://doi.org/10.1002/star.200600515

Cornuault, V., Buffetto, F., Rydahl, M. G., Marcus, S. E., Torode, T. A., Xue, J., Crépeau, M. J., Faria-Blanc, N., Willats, W. G., Dupree, P., & Ralet, M. C. (2015). Monoclonal antibodies indicate low-abundance links between heteroxylan and other glycans of plant cell walls. *Planta*, *242*, 1321–1334. https://doi.org/10.1007/s00425-015-2375-4

Dixon, R. A., & Barros, J. (2019). Lignin biosynthesis: Old roads revisited and new roads explored. *Open Biology*, *9*(12), 190215. https://doi.org/10.1098/rsob.190215

Ducreux, L. J., Morris, W. L., Prosser, I. M., Morris, J. A., Beale, M. H., Wright, F., Shepherd, T., Bryan, G. J., Hedley, P. E., & Taylor, M. A. (2008). Expression profiling of potato germplasm differentiated in quality traits leads to the identification of candidate flavour and texture genes. *Journal of Experimental Botany*, *59*(15), 4–4231. https://doi.org/10.1093/jxb/ern264

Dufour, D., Ceballos, H., Hershey, C., Hamaker, B., & Lorenzen, J. (2024). Tropical roots, tubers and bananas: New breeding tools and methods to meet consumer preferences. *Journal of the Science of Food and Agriculture*, *104*(8), 4481–4484. https://doi.org/10.1002/jsfa.13064

Eggleston, G., & Asiedu, R. (1994). Effects of boiling on the texture of cassava clones: A comparison of compressive strength, intercellular adhesion and physicochemical composition of the tuberous roots. *Tropical Science*, *34*, 259–273.

Endelman, J. B. (2011). Ridge regression and other kernels for genomic selection with R package rrBLUP. *The Plant Genome*, *4*(3), 250–255. https://doi.org/10.3835/plantgenome2011.08.0024

Eriksson, D., Akoroda, M., Azmach, G., Labuschagne, M., Mahungu, N., & Ortiz, R. (2018). Measuring the impact of plant breeding on sub-Saharan African staple crops. *Outlook on Agriculture*, *47*(3), 163–180. https://doi.org/10.1177/0030727018800723

FAO. (2022). *Crops and livestock products in production and trade sections*. FAOSTAT. Food and Agriculture Organization of the United Nations. Retrieved April 8, 2024, from https://www.fao.org/faostat/en/#data

Favaro, S. P., Beléia, A., Junior, N. D. S. F., & Waldron, K. W. (2008). The roles of cell wall polymers and intracellular components in the thermal softening of cassava roots. *Food Chemistry*, *108*(1), 220–227. https://doi.org/10.1016/j.foodchem.2007.10.070

Figueiredo, P. G., Djabou, A. S. M., Fernandes, A. M., de Aragão, S. C., Oliveira, M. T., & Araújo, R. A. (2021). Histological description of fresh, cooked and frozen cassava flesh. *International Journal of Advanced Engineering Research and Science*, *8*(9), 120–124. https://doi.org/10.22161/ijaers.89.13

Fonseca, P. A., Suarez-Vega, A., Marras, G., & Cánovas, Á. (2020). GALLO: An R package for genomic annotation and integration of multiple data sources in livestock for positional candidate loci. *GigaScience*, *9*(12), giaa149. https://doi.org/10.1093/gigascience/giaa149

Franck, H., Christian, M., Noël, A., Brigitte, P., Joseph, H. D., Cornet, D., & Mathurin, N. C. (2011). Effects of cultivar and harvesting conditions (age, season) on the texture and taste of boiled cassava roots. *Food Chemistry*, *126*(1), 127–133. https://doi.org/10.1016/j.foodchem.2010.10.088

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., & Buckler, E. S. (2014). TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. *PLoS ONE*, *9*(2), e90346. https://doi.org/10.1371/journal.pone.0090346

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J., & Thom, D. (1973). Biological interactions between polysaccharides and divalent cations: The egg-box model. *FEBS Letters*, *32*(1), 195–198. https://doi.org/10.1016/0014-5793(73)80770-7

Harrell, J.F., 2023. Hmisc: Harrell miscellaneous, sl: R package version 4.8-0.

Hershey, C.H. (2008). A global conservation strategy for cassava (*Manihot esculenta*) and wild Manihot species. Available at: https://cgspace.cgiar.org/handle/10568/83105

Iragaba, P., Hamba, S., Nuwamanya, E., Kanaabi, M., Nanyonjo, R. A., Mpamire, D., Muhumuza, N., Khakasa, E., Tufan, H. A., & Kawuki, R. S. (2021). Identification of cassava quality attributes preferred by Ugandan users along the food chain. *International Journal of Food Science and Technology*, *56*, 1184–1192. https://doi.org/10.1111/ijfs.14878

Iragaba, P., Nuwamanya, E., Wembabazi, E., Baguma, Y., Dufour, D., Earle, E. D., Kerr, R. B., Tufan, H. A., Gore, M. A., & Kawuki, R. S. (2019). Estimates for heritability and consumer-validation of a penetrometer method for phenotyping softness of cooked cassava roots. *African Crop Science Journal*, *27*(2), 147–163. https://doi.org/10.4314/acsj.v27i2.3

Jarvis, M. C., Briggs, S. P. H., & Knox, J. P. (2003). Intercellular adhesion and cell separation in plants. *Plant, Cell & Environment*, *26*(7), 977–989. https://doi.org/10.1046/j.1365-3040.2003.01034.x

Jones, L., Seymour, G. B., & Knox, J. P. (1997). Localization of pectic galactan in tomato cell walls using a monoclonal antibody specific to (1 [−>] 4)-β-D-Galactan. *Plant Physiology*, *113*(4), 1405–1412. https://doi.org/10.1104/pp.113.4.1405

Kassambara, A. and Mundt, F., 2020. Factoextra: Extract and visualize the results of multivariate data analyses. [R package].

Kawano, K., Fukuda, W. M. G., & Cenpukdee, U. (1987). Genetic and environmental effects on dry matter content of cassava root 1. *Crop Science*, *27*(1), 69–74. https://doi.org/10.2135/cropsci1987.0011183X002700010018x

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., Caig, V., Heller-Uszynska, K., Jaccoud, D., Hopper, C., & Aschenbrenner-Kilian, M. (2012). Diversity arrays technology: A generic genome

profiling technology on open platforms. *Data Production and Analysis in Population Genomics: Methods and Protocols*, *888*, 67–89. https://doi.org/10.1007/978-1-61779-870-2_5

Kloosterman, B., Oortwijn, M., Uitdewilligen, J., America, T., de Vos, R., Visser, R. G., & Bachem, C. W. (2010). From QTL to candidate gene: Genetical genomics of simple and complex traits in potato using a pooling strategy. *BMC Genomics*, *11*, 1–16. https://doi.org/10.1186/1471-2164-11-158

Kouadio, O. K., N'dri, D. Y., Nindjin, C., Marti, A., Casiraghi, M. C., Faoro, F., Erba, D., Bonfoh, B., & Amani, N. G. G. (2013). Effect of resistant starch on the cooking quality of yam (Dioscorea spp.) and cassava (Manihot esculenta) based paste products. *International Journal of Food Sciences and Nutrition*, *64*(4), 484–493. https://doi.org/10.3109/09637486.2012.749839

Larsen, F. H., Byg, I., Damager, I., Diaz, J., Engelsen, S. B., & Ulvskov, P. (2011). Residue specific hydration of primary cell wall potato pectin identified by solid-state 13C single-pulse MAS and CP/MAS NMR spectroscopy. *Biomacromolecules*, *12*(5), 1844–1850. https://doi.org/10.1021/bm2001928

Lauster, T., Stoeckle, D., Gabor, K., Haller, T., Krieger, N., Lotz, P., Mayakrishnan, R., Späth, E., Zimmermann, S., Livanos, P., & Mueller, S. (2022). Arabidopsis pavement cell shape formation involves spatially confined ROPGAP regulators. *Current Biology*, *32*(3), 532–544. https://doi.org/10.1016/j.cub.2021.12.042

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software*, *25*, 1–18. https://doi.org/10.18637/jss.v025.i01

Léotard, G., Duputié, A., Kjellberg, F., Douzery, E. J., Debain, C., de Granville, J. J., & McKey, D. (2009). Phylogeography and the origin of cassava: New insights from the northern rim of the Amazonian basin. *Molecular Phylogenetics and Evolution*, *53*, 329–334. https://doi.org/10.1016/j.ympev.2009.05.003

Marcus, S. E., Verhertbruggen, Y., Hervé, C., Ordaz-Ortiz, J. J., Farkas, V., Pedersen, H. L., Willats, W. G., & Knox, J. P. (2008). Pectic homogalacturonan masks abundant sets of xyloglucan epitopes in plant cell walls. *BMC Plant Biology*, *8*, 60. https://doi.org/10.1186/1471-2229-8-60

Meghar, K., Tran, T., Delgado, L.F., Ospina, M.A., Moreno, J.L., Luna, J., Londoño, L., Dufour, D. and Davrieux, F., 2023. Hyperspectral imaging for the determination of relevant cooking quality traits of boiled cassava. *Journal of the Science of Food and Agriculture*, *104*, 4782–4792. https://doi.org/10.1002/jsfa.12654

Menoli, A. V., & Beleia, A. (2007). Starch and pectin solubilization and texture modification during pre-cooking and cooking of cassava root (Manihot esculenta Crantz). *LWT-Food Science and Technology*, *40*(4), 744–747. https://doi.org/10.1016/j.lwt.2005.12.011

Mestres, C., Taylor, M., McDougall, G., Arufe, S., Tran, T., Nuwamanya, E., Dufour, D., Nakitto, M., Meghar, K., Rinaldo, D. and Ollier, L., 2023. Contrasting effects of polysaccharide components on the cooking properties of roots, tubers and bananas. *Journal of the Science of Food and Agriculture*, *104*, 4652–4661. https://doi.org/10.1002/jsfa.12914

Murayama, D., Koaze, H., Ikeda, S., Palta, J. P., Kasuga, J., Pelpolage, S. W., Yamauchi, H., & Tani, M. (2019). In-season calcium fertilizer application increases potato cell wall calcium and firmness of french fries. *American Journal of Potato Research*, *96*, 472–486. https://doi.org/10.1007/s12230-019-09736-5

Ngeve, J. M. (2003). Cassava root yields and culinary qualities as affected by harvest age and test environment. *Journal of the Science of Food and Agriculture*, *83*(4), 249–257. https://doi.org/10.1002/jsfa.1307

Nonaka, M. (1980). The textural quality of cooked potatoes. I. The relationship of cooking time to the separation and rupture of potato cells. *American Potato Journal*, *57*, 141–149. https://doi.org/10.1007/BF02853865

Padonou, W., Mestres, C., & Nago, M. C. (2005). The quality of boiled cassava roots: Instrumental characterization and relationship with physicochemical properties and sensorial properties. *Food Chemistry*, *89*(2), 261–270. https://doi.org/10.1016/j.foodchem.2004.02.033

Parker, C. C., Parker, M. L., Smith, A. C., & Waldron, K. W. (2001). Pectin distribution at the surface of potato parenchyma cells in relation to cell– cell adhesion. *Journal of Agricultural and Food Chemistry*, *49*(9), 4364–4371. https://doi.org/10.1021/jf0104228

Pedersen, H. L., Fangel, J. U., McCleary, B., Ruzanski, C., Rydahl, M. G., Ralet, M. C., Farkas, V., von Schantz, L., Marcus, S. E., Andersen, M. C., & Field, R. (2012). Versatile high resolution oligosaccharide microarrays for plant glycobiology and cell wall research. *Journal of Biological Chemistry*, *287*(47), 39429–39438. https://doi.org/10.1074/jbc.M112.396598

R Core Team. (2013). R: A language and environment for statistical computing. R foundation for statistical computing. Vienna, Austria, *3*(0), 37.

Ripley, B., Venables, B., Bates, D. M., Hornik, K., Gebhardt, A., Firth, D., & Ripley, M. B. (2013). Package 'mass'. *Cran r*, *538*, 113–120.

Rogers, D. J., & Appan, S. G. (1973). *Manihot, Manihotoides (Euphorbiaceae)*. Flora Neotropica. Monograph No. 13. Hafner Press.

Ross, H. A., McDougall, G. J., Vincent, J. F., Stewart, D., Verrall, S., & Taylor, M. A. (2010). Discerning intra-tuber differences in textural properties in cooked Solanum tuberosum group Tuberosum and group Phureja tubers. *Journal of the Science of Food and Agriculture*, *90*(9), 1527–1532. https://doi.org/10.1002/jsfa.3979

Ross, H. A., Morris, W. L., Ducreux, L. J., Hancock, R. D., Verrall, S. R., Morris, J. A., Tucker, G. A., Stewart, D., Hedley, P. E., McDougall, G. J., & Taylor, M. A. (2011). Pectin engineering to modify product quality in potato. *Plant Biotechnology Journal*, *9*(8), 848–856. https://doi.org/10.1111/j.1467-7652.2011.00591.x

Ross, H. A., Wright, K. M., McDougall, G. J., Roberts, A. G., Chapman, S. N., Morris, W. L., Hancock, R. D., Stewart, D., Tucker, G. A., James, E. K., & Taylor, M. A. (2011). Potato tuber pectin structure is influenced by pectin methyl esterase activity and impacts on cooked potato texture. *Journal of Experimental Botany*, *62*(1), 371–381. https://doi.org/10.1093/jxb/erq280

Safo-Kantanka, O., & Owusu-Nipah, J. (1992). Cassava varietal screening for cooking quality: Relationship between dry matter, starch content, mealiness and certain microscopic observations of the raw and cooked tuber. *Journal of the Science of Food and Agriculture*, *60*(1), 99–104. https://doi.org/10.1002/jsfa.2740600116

Sajeev, M. S., Sreekumar, J., Moorthy, S. N., Suja, G., & Shanavas, S. (2008). Texture analysis of raw and cooked tubers of short-duration lines of cassava by multivariate and fractional conversion techniques. *Journal of the Science of Food and Agriculture*, *88*(4), 569–580. https://doi.org/10.1002/jsfa.3055

Sathitnaitham, S., Suttangkakul, A., Wonnapinij, P., McQueen-Mason, S. J., & Vuttipongchaikij, S. (2021). Gel-permeation chromatography–enzyme-linked immunosorbent assay method for systematic mass distribution profiling of plant cell wall matrix polysaccharides. *The Plant Journal*, *106*(6), 1776–1790. https://doi.org/10.1111/tpj.15255

Sedbrook, J. C., Ehrhardt, D. W., Fisher, S. E., Scheible, W. R., & Somerville, C. R. (2004). The Arabidopsis SKU6/SPIRAL1 gene encodes a plus end–localized microtubule-interacting protein involved in directional cell expansion. *The Plant Cell*, *16*(6), 1506–1520. https://doi.org/10.1105/tpc.020644

Silva, L. M. A., Alves Filho, E. G., Martins, R. M., Oliveira, W. J., Vidal, C. S., de Oliveira, L. A., & de Brito, E. S. (2022). NMR-based Metabolomic approach for evaluation of the harvesting time and cooking characteristics of different cassava genotypes. *Food*, *11*(11), 1651. https://doi.org/10.3390/foods11111651

Solt, F., & Hu, Y. (2015). dotwhisker: Dot-and-whisker plots of regression results. Available at the. In Comprehensive R Archive Network (CRAN).

Sraphet, S., Boonchanawiwat, A., Thanyasiriwat, T., Boonseng, O., Tabata, S., Sasamoto, S., Shirasawa, K., Isobe, S., Lightfoot, D. A.,

Tangphatsornruang, S., & Triwitayakorn, K. (2011). SSR and EST-SSR-based genetic linkage map of cassava (Manihot esculenta Crantz). *Theoretical and Applied Genetics*, *122*, 1161–1170. https://doi.org/10.1007/s00122-010-1520-5

Staack, L., Della Pia, E. A., Jørgensen, B., Pettersson, D., & Rangel Pedersen, N. (2019). Cassava cell wall characterization and degradation by a multicomponent NSP-targeting enzyme (NSPase). *Scientific Reports*, *9*(1), 10150. https://doi.org/10.1038/s41598-019-46341-2

Talma, S. V., Almeida, S. B., Lima, R. M. P., Vieira, H. D., & Bebert, P. A. (2013). Cooking time and texture of cassava roots. *Brazilian Journal of Food Technology*, *16*, 133–138. https://doi.org/10.1590/S1981-67232013005000016

Tang, Y., Horikoshi, M., & Li, W. (2016). Ggfortify: Unified interface to visualize statistical results of popular R packages. *R Journal*, *8*(2), 474. https://doi.org/10.32614/RJ-2016-060

Thakur, B. R., Singh, R. K., Handa, A. K., & Rao, M. A. (1997). Chemistry and uses of pectin—A review. *Critical Reviews in Food Science & Nutrition*, *37*(1), 47–73. https://doi.org/10.1080/10408399709527767

Thiele, G., Dufour, D., Vernier, P., Mwanga, R. O., Parker, M. L., Schulte Geldermann, E., Teeken, B., Wossen, T., Gotor, E., Kikulwe, E., & Tufan, H. (2021). A review of varietal change in roots, tubers and bananas: Consumer preferences and other drivers of adoption and implications for breeding. *International Journal of Food Science & Technology*, *56*(3), 1076–1092. https://doi.org/10.1111/ijfs.14684

Thuy, C. T. L., Lopez-Lavalle, L. A. B., Vu, N. A., Hy, N. H., Nhan, P. T., Ceballos, H., Newby, J., Tung, N. B., Hien, N. T., Tuan, L. N., & Hung, N. (2021). Identifying new resistance to cassava mosaic disease and validating markers for the CMD2 locus. *Agriculture*, *11*(9), 829. https://doi.org/10.3390/agriculture11090829

Tibbits, C. W., MacDougall, A. J., & Ring, S. G. (1998). Calcium binding and swelling behaviour of a high methoxyl pectin gel. *Carbohydrate Research*, *310*(1–2), 101–107. https://doi.org/10.1016/S0008-6215(98)00172-4

Torode, T. A., O'Neill, R., Marcus, S. E., Cornuault, V., Pose, S., Lauder, R. P., Kračun, S. K., Rydahl, M. G., Andersen, M. C., Willats, W. G., & Braybrook, S. A. (2018). Branched pectic galactan in phloem-sieve-element cell walls: Implications for cell mechanics. *Plant Physiology*, *176*(2), 1547–1558. https://doi.org/10.1104/pp.17.01568

Tran, T., Zhang, X., Ceballos, H., Moreno, J. L., Luna, J., Escobar, A., Morante, N., Belalcazar, J., Becerra, L. A., & Dufour, D. (2021). Correlation of cooking time with water absorption and changes in relative density during boiling of cassava roots. *International Journal of Food Science & Technology*, *56*(3), 1193–1205. https://doi.org/10.1111/ijfs.14769

Uchendu, K., Njoku, D. N., Ikeogu, U. N., Dzidzienyo, D., Tongoona, P., Offei, S., & Egesi, C. (2022). Genotype-by-environment interaction and stability of root mealiness and other organoleptic properties of boiled cassava roots. *Scientific Reports*, *12*(1), 20909. https://doi.org/10.1038/s41598-022-25172-8

Uchendu, K., Njoku, D. N., Paterne, A., Rabbi, I. Y., Dzidzienyo, D., Tongoona, P., Offei, S., & Egesi, C. (2021). Genome-wide association study of root mealiness and other texture-associated traits in cassava. *Frontiers in Plant Science*, *12*, 770434. https://doi.org/10.3389/fpls.2021.770434

Ulvskov, P., Wium, H., Bruce, D., Jørgensen, B., Qvist, K. B., Skjøt, M., Hepworth, D., Borkhardt, B., & Sørensen, S. O. (2005). Biophysical consequences of remodeling the neutral side chains of rhamnogalacturonan I in tubers of transgenic potatoes. *Planta*, *220*, 609–620. https://doi.org/10.1007/s00425-004-1373-8

van Marle, J. T., Recourt, K., van Dijk, C., Schols, H. A., & Voragen, A. G. (1997). Structural features of cell walls from potato (Solanum tuberosum L.) cultivars Irene and Nicola. *Journal of Agricultural and Food Chemistry*, *45*(5), 1686–1693. https://doi.org/10.1021/jf9607627

Verhertbruggen, Y., Marcus, S. E., Haeger, A., Ordaz-Ortiz, J. J., & Knox, J. P. (2009). An extended set of monoclonal antibodies to pectic homogalacturonan. *Carbohydrate Research*, *344*(14), 1858–1862. https://doi.org/10.1016/j.carres.2008.11.010

Wang, J., & Zhang, Z. (2021). GAPIT version 3: Boosting power and accuracy for genomic association and prediction. *Genomics, Proteomics & Bioinformatics*, *19*(4), 629–640. https://doi.org/10.1016/j.gpb.2021.08.005

Watling, J., Shock, M. P., Mongeló, G. Z., Almeida, F. O., Kater, T., De Oliveira, P. E., & Neves, E. G. (2018). Direct archaeological evidence for southwestern Amazonia as an early plant domestication and food production Centre. *PLoS ONE*, *13*(7), e0199868. https://doi.org/10.1371/journal.pone.0199868

Wei, T., Simko, V.R., Levy, M., Xie, Y., Jin, Y. and Zemla, J. (2017). Package "corrplot". *Statistician*, *56*(316), e24.

Wickham, H., & Wickham, H. (2009). Getting started with qplot. In *ggplot2: Elegant graphics for data analysis* (pp. 9–26). Springer Cham.

Xin, Y., Chen, F., Yang, H., Zhang, P., Deng, Y., & Yang, B. (2010). Morphology, profile and role of chelate-soluble pectin on tomato properties during ripening. *Food Chemistry*, *121*(2), 372–380. https://doi.org/10.1016/j.foodchem.2009.12.038

Zhang, L., Chen, F., An, H., Yang, H., Sun, X., Guo, X., & Li, L. (2008). Physicochemical properties, firmness, and nanostructures of sodium carbonate-soluble pectin of 2 Chinese cherry cultivars at 2 ripening stages. *Journal of Food Science*, *73*(6), N17–N22. https://doi.org/10.1111/j.1750-3841.2008.00799.x

Zhang, C., Dong, S. S., Xu, J. Y., He, W. M., & Yang, T. L. (2019). PopLDdecay: A fast and effective tool for linkage disequilibrium decay analysis based on variant call format files. *Bioinformatics*, *35*(10), 1786–1788. https://doi.org/10.1093/bioinformatics/bty875

Zhou, Y., Kobayashi, M., Awano, T., Matoh, T., & Takabe, K. (2018). A new monoclonal antibody against rhamnogalacturonan II and its application to immunocytochemical detection of rhamnogalacturonan II in Arabidopsis roots. *Bioscience, Biotechnology, and Biochemistry*, *82*(10), 1780–1789. https://doi.org/10.1080/09168451.2018.1485479

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sathitnaitham, S., Ceballos, H., Wonnapinij, P., Kraichak, E., Utthiya, S., Suttangkakul, A., Gomez, L. D., Kittipadakul, P., Siriwong, N., Kongsil, P., & Vuttipongchaikij, S. (2024). Cell wall polysaccharides determine cooking quality in cassava roots. *Plants, People, Planet*, 1–18. https://doi.org/10.1002/ppp3.10558