Scientific Progress Report

Biophysical Characterization of Quality Traits - Scientific Progress Report for Period 4 (Jan-Dec 2021)

Montpellier, France, 15/03/2022

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https://rtbfoods.cirad.fr

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Ethics: The activities, which led to the production of this manual, were assessed and approved by the CIRAD Ethics Committee (H2020 ethics self-assessment procedure). When relevant, samples were prepared according to good hygiene and manufacturing practices. When external participants were involved in an activity, they were priorly informed about the objective of the activity and explained that their participation was entirely voluntary, that they could stop the interview at any point and that their responses would be anonymous and securely stored by the research team for research purposes. Written consent (signature) was systematically sought from sensory panelists and from consumers participating in activities.

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SOPs Processing & Cooking Patterns: Thierry TRAN
SOPs Biochemical Composition: Christian MESTRES; Ring Tests: Dominique DUFOUR
Proofs of Concept Starch & Parietal Compounds: Christian MESTRES
Proofs of Concept & SOPs Texture: Layal DAHDOUH & Oluwatoyin AYETIGBO
SOPs Sensory: Christophe BUGAUD

ABSTRACT

During Period 4, RTBfoods partners involved in WP2 implemented the standard operating protocols (SOPs) developed in previous periods to characterize the target product profiles: boiled roots and tubers (cassava, Yam, sweet potato, plantain), paste (Fufu, pounded Yam, matooke) and granulated products (gari-eba, attiéké).

For **sensory characterizations** by trained panels, 10 SOPs are now available: boiled cassava (2), gari-eba, attiéké, Fufu, boiled Yam, pounded Yam, boiled plantain, matooke, and boiled sweet potato. Key sensory attributes common to several products include firmness/hardness, stickiness, colour, smoothness, moldability and sweetness / sourness, fibrousness, moisture/mealiness, stretchability and bitterness. Several of these sensory attributes were correlated with instrumental texture parameters, in particular sensory firmness (or hardness or softness) assessed either in the mouth or by hand can be reliably predicted by the hardness parameters measured by penetrometry, TPA, and texture-extrusion. For the first time, mealiness of boiled cassava was correlated with a parameter of the texture-extrusion test, as well as with water absorption. Mouldability and stretchability were correlated with TPA measurements especially on destructured products (Gari/Eba and pounded Yam).

For **textural characterizations**, 7 SOPs were developed and validated: boiled cassava (2), matooke, boiled Yam, pounded Yam, gari-eba, Fufu. These were implemented for several product profiles and several genotypes of various RTBs (typically 20 to 50 genotypes per product profile). Multivariate statistical analyses (e.g. PCA) of the resulting datasets allowed the classification of RTB genotypes into good, intermediate and poor quality, thus demonstrating the ability of postharvest quality measurements to contribute to varietal selection for breeding. Several correlations between textural parameters and sensory attributes were also identified (as discussed above), which also confirmed that instrumental texture is a valid approach to predict how consumers perceive RTB products.

SOPs for several **kitchen tests** were also finalized, which bring additional information on the functional quality traits of RTB products in complement to texture measurements, including water absorption, cooking time, swelling of gari upon addition of hot water, and pounding time of paste products (pounded Yam, Fufu). Processing ability criteria were also investigated such as ease of peeling, ease of sieving the dewatered cassava mash, retting ability (softening, foaming, turbidity, pH). In the case of boiled cassava, the water absorption protocol in particular offers relatively short analysis time (30 minutes/sample) and is well correlated with sensory mealiness, cooking time and textural parameters, thus qualifying as medium-throughput phenotyping method for screening cassava breeding populations. In 2021, more than 4000 genotypes were characterized by water absorption, including from Nextgen and HarvestPlus populations. This development constitutes an important contribution of RTBfoods to improving cassava breeding and selection operations of other projects, and demonstrates the potential of the RTBfoods approach to develop breeding selection tools for a variety of post-harvest quality traits.

Finally, **biochemical proof of concept studies** provided further evidence of the role of pectins in determining textural characteristics of RTB products, in particular non-destructured such as boiled or steamed. Starch and amylose contents also contributed to texture of boiled sweet potato and boiled Yam.

Key Words: biochemistry, sensory panel, textural evaluation, quality traits, RTBs, laboratory standard operating procedures

1 PRODUCT PROFILES & WP2 TEAMS ACROSS COUNTRIES

Please update table below (if necessary) with information provided by Partner Focal Points:

Crop	Product Profile	Product Champion	Partner Institutes responsible for WP2 activities	Institute Focal Point	WP2 Team Composition	
					Product Profile WP2 Correspondent	Names of Operational Staff for WP2 Analyses (texture, sensory, biochemistry, etc.) & Data Management
Cassava	**PP1- Boiled Cassava**	Robert Kawuki (NaCRRi-Uganda)	NaCRRI-Uganda [+ NARL for Sensory Analyses]	Robert Kawuki	Ephraim Nuwamanya	Ephraim Nuwamanya, Enoch Wembabazi
			UAC-FSA	Noel Akissoe	Noel Akissoe	Laurent Adinsi, Imayath Djibril Moussa, Laurenda Honfozo
			CIAT-Colombia	Thierry Tran	Thierry Tran	Luis Londoño, Jhon Larry Moreno, Maria Alejandra Ospina, Jorge Luna, Cristian Duarte, Luis Fernando Delgado, John Belalcazar
			CIRAD-Montpellier	Dominique Pallet	Christian Mestres	Christian Mestres, Layal Dahdouh, Julien Ricci, Léa Ollier, Didier Mbéguié-Mbéguié, Christophe Bugaud, Nelly Forestier-Chiron
	PP2- Gari, Eba, Attiéké	Busie Maziya-Dixon (IITA-Nigeria)	IITA-Nigeria [Gari/Eba]	Busie Maziya-Dixon	Michael Adesokan	Emmanuel Alamu, Wasiu Awoyale, Hakeem Oyedele
			NRCRI-Nigeria [Gari/Eba]	Ugo Chijioke	Ugo Chijioke	Ugo Chijioke, Justice Okoronkwo, Oluchi Achonwa
			CNRA-Côte d'Ivoire [Attiéké]	Amani Kouakou	Catherine Ebah	Sylvie N'Nan Diby
	PP3- Fufu	Ugo Chijioke (NRCRI-Nigeria)	NRCRI-Nigeria	Ugo Chijioke	Ugo Chijioke	Ugo Chijioke, Justice Okoronkwo, Oluchi Achonwa
			CIRAD	Dominique Pallet	Dominique Dufour	Didier Mbéguié-A-Mbéguié
Cooking Banana	**PP4- Boiled Plantain**	Gérard Ngoh (CARBAP-Cameroon)	CARBAP-Cameroon	Gérard Ngoh	Gérard Ngoh	Cedric Kendine
			INRA-Avignon	Agnès Rolland-Sabaté	Agnès Rolland-Sabaté	Aliénor Dutheil de la Rochère
			CNRA-Côte d'Ivoire	Amani Kouakou	Catherine Ebah	Sylvie N'Nan Diby
	PP5- Matooke	Kephas Nowakunda (NARL-Uganda)	NARL-Uganda	Kephas Nowakunda	Kephas Nowakunda (NARL)	Yusuf Mukasa, Elizabeth Khakasa
	PP6- Fried Plantain	Delphine Amah (IITA-Nigeria)	IITA-Nigeria	Busie Maziya-Dixon	-	-

Crop	Product Profile	Product Champion	Partner Institutes responsible for WP2 activities	Institute Focal Point	WP2 Team Composition	
					Product Profile WP2 Correspondent	Names of Operational Staff for WP2 Analyses (texture, sensory, biochemistry, etc.) & Data Management
Sweet-potato	PP7- Boiled Sweetpotato	Robert Mwanga (CIP-Uganda)	CIP-Uganda & Kenya [+ NARL for Sensory Analyses]	Reuben Ssali	Mukani Moyo	Mariam Nakitto
			JHI	Mark Taylor	Mark Taylor	Mark Taylor
			CIRAD	Dominique Pallet	Christian Mestres	Christian Mestres, Léa Ollier, Christophe Bugaud, Nelly Forestier-Chiron
	PP8- Fried Sweetpotato	Jan Low (CIP-Kenya)	CIP-Ghana	Jolien Swanckaert (Uganda)	-	-
			CNRA-Côte d'Ivoire	Amani Kouakou	Catherine Ebah	Sylvie N'Nan Diby
Yam	PP9- Boiled Yam	Noel Akissoe (UAC-FSA-Benin)	UAC-FSA	Noel Akissoe	Noel Akissoe	Laurent Adinsi, Imayath Djibril Moussa, Laurenda Honfozo
			IITA-Nigeria [Gari/Eba]	Busie Maziya-Dixon	Michael Adesokan	Emmanuel Alamu, Wasiu Awoyale, Hakeem Oyedele
			NRCRI-Nigeria	Ugo Chijioke	Ugo Chijioke	Ugo Chijioke, Justice Okoronkwo, Oluchi Achonwa
			CNRA-Côte d'Ivoire	Amani Kouakou	Catherine Ebah	Sylvie N'Nan Diby
			INRA-Avignon	Agnès Rolland-Sabaté	Agnès Rolland-Sabaté	Aliénor Dutheil de la Rochère
			INRA-Guadeloupe (budgeted under WP3)	Agnès Rolland-Sabaté	Dominique Rinaldo	Dominique Rinaldo
			CIRAD-Montpellier	Dominique Pallet	Christian Mestres	Christian Mestres, Layal Dahdouh, Julien Ricci, Léa Ollier, Christophe Bugaud, Nelly Forestier-Chiron
	PP10- Pounded Yam	Bolanle Otegbayo (Bowen University-Nigeria)	Bowen University-Nigeria	Bolanle Otegbayo	Bolanle Otegbayo	Olayinka Oroniran
			NRCRI-Nigeria	Ugo Chijioke	Ugo Chijioke	Ugo Chijioke, Justice Okoronkwo, Oluchi Achonwa
			CNRA-Côte d'Ivoire	Amani Kouakou	Catherine Ebah	Sylvie N'Nan Diby
Potato	PP11- Boiled Potato	Thiago Mendes (CIP-Kenya)	CIP-Uganda [+ NARL for Sensory Analyses]	Reuben Ssali	Mukani Moyo	Mariam Nakitto
			JHI-UK	Mark Taylor	Mark Taylor	Mark Taylor

2 WP2 SUMMARY NARRATIVE

Summarize progress of your WP in Period 4 (January 2021 – December 2021) focusing on ***main Activities & Achievements*** *per output [1.5 page max.].*

Reminder: Activities performed within WP2 in Period 4 should have contributed to 4 major project outputs:

- ***Output 1.2.2:*** *RTB product profiles informed with trait dissection knowledge for 5 RTB crops and 11 RTB food/processed products;*
- ***Output 1.3.1:*** *High quality SOPs to characterize and understand key users' preferred quality traits developed;*
- ***Output 1.3.2:*** *Standardized ontology established for major quality traits for 11 RTB food/processed products with objective goal defined for each attribute;*
- ***Output 1.5.2:*** *RTB physico-chemical databases developed / enriched for users' preferred quality traits with quantitative data on 5 RTB crops and 11 RTB food/processed products.*

Do not forget to systematically cite & refer to scientific papers published by WP5 partner teams. (NB: This section will be copied & pasted as is in the body of the RTBfoods Annual Report for Period 4)

For the **sensory characterization** of the RTB products by trained panels, 10 SOPs are now available (boiled cassava (2), Gari-Eba, attiéké, Fufu, boiled Yam, pounded Yam, boiled plantain, matooke, and boiled sweet potato). By implementing Quantitative Descriptive Analysis (QDA) and instrumental texture testing for almost all product profiles, RTBfoods project has met one of the stated challenges. It is now possible to extract sensory attributes, which are common between products, and on which to focus our attention in terms of physico-chemical predictor research and SMART development: firmness/hardness, stickiness, colour, smoothness, moldability and sweetness / sourness, fibrousness, moisture/mealiness, stretchability and bitterness. Two guidances were proposed to partners for preparing sensory data and establishing relationships with instrumental measurements. Firmness (or hardness or softness), whether measured in the mouth or by hand, was the sensory attribute that could be correlated with instrumental measures of texture (penetrometry, TPA, extrusion) on the greatest number of matrices and products. Correlations were better for products that did not undergo destructuring during preparation (boiled cassava, Yam, and sweet potato) than for destructured products (Gari/Eba, matooke, pounded Yam). For the first time, mealiness could be correlated with an extrusion test and with the water absorption test on boiled cassava, which remains to be validated on other matrices. Mouldability and stretchability were correlated with TPA measurements especially on destructured products (Gari/Eba and pounded Yam). Given the specificity of sensory analysis (working with tasters), COVID 19 has had an impact on the partners' activities by reducing the number of analyses or revisiting the protocols (home testing).

Three additional SOPs for **textural characterization** of food product profiles (Gari-Eba, Fufu, Pounded Yam) were developed and finalized in period 4 by IITA, BOWEN, and NRCRI Umudike, with the help and mission of CIRAD texture focal point. Raw datasets on texture generated prior to the missions were evaluated for repeatability and discriminance among the various varieties. Following this repeatability and discriminance tests, multivariate statistical analyses (e.g. PCA) of textural attributes allowed the classification of the varieties into good, intermediate and poor quality for these product profiles. In addition, several SOPs were updated, through exchanges between partners and CIRAD texture focal point: improvements were made in particular for better control of sample temperature prior to texture measurements, for increasing the number of replicates to reduce standard deviations, and for better standardization of samples size. Finally, most partners were trained in UAC/FSA (Benin) for sensory analysis, textural measurements and correlations analysis between sensory and instrumental quality attributes.

Overall, in order to capture the textural profiles of products made from a wide range of genotypes (Yam, cassava) in databases and for selection purposes, the textural characteristics of several clones were determined by TPA, texture-extrusion and penetrometry by the various RTBfoods partners. The Guadeloupe team evaluated 52 varieties of alata Yam by penetrometry and TPA; IITA evaluated 16 varieties of Yams by extrusion; CIAT and NaCRRI-Uganda evaluated respectively 30 and 38 varieties of cassava by extrusion; NRCRI evaluated 26 gari samples from 26 varieties of cassava; NARL-Uganda evaluated 30 samples of matooke from 30 genotypes of cooking banana; and BOWEN evaluated 21 Yam varieties for pounded Yam.

Significant correlations between instrumental texture measurements and sensory texture evaluation were evidenced, particularly:

- Texture parameters from penetrometry and TPA tests can predict sensory hardness attributes of all structured PPs (boiled Yam, plantain, sweet potato and cassava). In addition, the extrusion test can also predict sensory mealiness of boiled cassava.
- The hardness and cohesiveness parameters from TPA tests can predict respectively the sensory mouldability of eba and the sensory cohesiveness of pounded Yam.

In Period 4, partners continued to develop **kitchen tests protocols** for the various product profiles, building on the work initiated during Periods 2 and 3. Functional quality traits were investigated including water absorption, texture (softness, initial gradient, maximum force, distance at max force, etc.), cooking time, swelling of Gari upon addition of hot water, pounding time. Processing ability criteria were also investigated such as ease of peeling, ease of sieving the dewatered cassava mash, retting ability (softening, foaming, turbidity, pH). Collaborations among partners saw the water absorption protocol, initially developed for boiled cassava, adapted for other product profiles (boiled Yam, boiled sweet potato), as it offers relatively short analysis time (30 minutes/sample) and is easy to scale-up, requiring only additional gas stoves, kitchen utensils and balances. In the case of cassava, water absorption (WAB) is now used routinely by breeders at CIAT to characterize boiling quality of cassava breeding populations, for example the Parental collection and the Biofortified progeny (F1C1), totaling more than 4000 genotypes in 2021. This development constitutes an important contribution of RTBfoods to improving cassava breeding and selection operations of other projects, and demonstrates the potential of the RTBfoods approach to develop breeding selection tools for a variety of post-harvest quality traits.

The following correlations were identified between functional properties and processing ability and end-product quality: Water absorption with cooking time; Texture (Distance at max force, End_force:Max_force ratio) with mealiness (boiled cassava); water absorption with mealiness (boiled cassava) and chewiness (boiled Yam); cooking time with sensory hardness (boiled Yam) and dry matter with pounding time (pounded Yam). Each partner evaluated between 10 and 40 genotypes depending on the product profile. At the end of Period 4, prediction of some of the sensory attributes and quality of RTB product profiles by instrumental analyses has thus become possible, reducing the time necessary to screen genotypes for post-harvest quality traits. In particular, in the case of boiled cassava, boiled Yam and pounded Yam, the analyses put in evidence significant differences in boiling and retting behavior, allowing to identify groups of genotypes with desirable postharvest quality traits for breeding and selection.

For fermented products such as Fufu a positive correlation between penetrometer data and processors data for foaming ability and water clarity or turbidity was evidenced. This implies that evaluation of foaming ability and turbidity can be used as an intermediate method for accessing retting ability of cassava genotypes by breeders.

Biochemical proof of concept studies to elucidate the molecular mechanisms underpinning the sensory and texture quality of RTB products focused mainly on **pectins** and cell walls (CIRAD, JHI, INRAe, CIAT). A medium-throughput manual or automated chemical colorimetric procedure (20 or 50 samples/day, respectively) for assessing total pectin content and branched pectins was developed, as well as a procedure for the extraction of cell walls from Yam, plantain and sweet potato. Concerning the role of pectins, contradictory results were obtained and studies will be further developed in period 5:

- Total pectin content, evaluated as total galacturonic acid content of fresh Yam and fresh cassava, was significantly and positively correlated with texture (hardness) of steamed Yam

and steamed cassava (seven genotypes). In addition, the softening during cooking of Yam also appeared linked to the degradation of pectins,
- However total pectin content was not a good predictor of cooking time of steamed Yam and sweet potato,
- Degree of methylation appeared negatively correlated with cooking time of steamed Yam, and with firmness of cooked plantain, but not in the raw material, suggesting a putative action of pectin methylesterase (PME) during the cooking process.
- Furthermore, in green vegetables, pectins are known to complex with calcium ions (Ca^{2+}) to form a sample-wide network that strengthen texture and reduces water absorption. Experiments of boiling cassava in water in presence of Ca^{2+} demonstrated that same effect, with water absorption decreasing with increasing Ca^{2+} concentration up to 4 g/L, thus further confirming that pectins play a key role in determining cooking quality. In addition, mouldability of pounded Yam was negatively correlated with Yam ash content which may also be linked to the interaction of pectins with cations.

The role of other components of RTB was also investigated:

- **Starch content** and **dry matter content** had no direct effect on the cooking quality (texture, water absorption) of boiled cassava, but may play a role in determining texture. On the contrary, dry matter of raw sweet potato played a key role in determining the texture of boiled sweet potato, as evidenced by significant correlations with sensory firmness ($r = 0.500$) and mealiness ($r = 0.717$),
- **Amylose** content was negatively correlated with the firmness of boiled Yam,
- As hypothesized and already observed with previous experiments, **cyanide content** was highly significantly correlated with bitterness.
- The evaluation of the role of **polyphenols** on the color of RTB products and their interaction with texture has just begun, and will continue during Period 5.

3 WP2 ACHIEVEMENTS & CONTRIBUTION TO OUTPUT 1.2.2, OUTPUT 1.3.1, OUTPUT 1.3.2 & OUTPUT 1.5.2

*Fill-in the table below to provide an **update on progress made** toward the achievement of project outputs in Period 4 & list all **deliverables submitted** by WP partners – **NB: Information provided in this table will be used to update the RTBfoods Results-Tracker***

Output	Expected/Committed Targets/Milestones in Period 4	Level of Achievement in Period 4	If any difference between 'Committed' and 'Achieved': **Brief Explanation**	**Complete List of Deliverables submitted to PMU in Period 4** (apart from the *WP Scientific Progress Report*)
1.2.2	**11 new quality traits characterized** to inform RTB product profiles **with biochemical measurable indicators**	The following quality traits were investigated during Period 4: - Dry matter - Starch content - Cell wall content - Pectin content - Pectin structure - Water absorption - Cooking time - Texture profile by penetrometer - Texture profile by TPA - Texture profile by texture-extrusion - Retting ability The quality traits listed above were measured on the following 9 RTB products: Fresh cassava, boiled cassava, gari-eba, Fufu, fresh Yam, boiled Yam, pounded Yam, boiled plantain, matooke		D2.3: Proof of concept on tentative correlation between cell wall composition and textural properties of sweetpotato roots (G. MacDougall, JHI) D2.4A: Relation entre l'évolution de la texture de l'igname et les comportements de l'amidon et des pectines au cours de la cuisson (T. Simonis, CIRAD) D2.4B: Relationships Between Yam Texture Changes and Starch & Pectins Behaviours During Cooking (T. Simonis, CIRAD) D2.5: Développement et validation d'une méthode analytique par analyseur de flux automatisé. Cas spécifique du dosage des pectines (C. Miossec, CIRAD) D2.10 : Do pectins play a role in the texture of RTB products? Case study on boiled Yam & cassava (C. Mestres, CIRAD) D2.6: Etude de l'aptitude à la cuisson de diverses variétés d'ignames par mesures texturales et biochimiques (J. Ajax, CIRAD) D2.7: An Attempt to Differentiate Cassava Genotypes by their Retting Behavior using some Biophysical Indicators (G. Wakem, CIRAD/UAC-FSA) D2.8: Impact of cell wall composition on texture of boiled Yams (A. Dutheil de Larochère, INRAE) D2.9: Impact of cell wall composition on texture of boiled plantains (A. Dutheil de Larochère, INRAE) D2.11: Processing ability and textural properties of boiled Yam as related to the species and variety (D. Rinaldo, INRAE) D3.1A: Gari-Eba Full Product Profile Development Workshop Report (E. Alamu, IITA) D3.2A: Fufu Full Product Profile Development Workshop Report (U. Chijioke, NRCRI)

Output	Expected/Committed Targets/Milestones in Period 4	Level of Achievement in Period 4	If any difference between 'Committed' and 'Achieved': **Brief Explanation**	**Complete List of Deliverables submitted to PMU in Period 4** (apart from the *WP Scientific Progress Report*)
				D3.3A: Boiled plantain Full Product Profile Development Workshop Report (G. Ngoh Newilah, CARBAP) D3.4A: Boiled Yam Full Product Profile Development Workshop Report (N. Akissoe, UAC-FSA) D3.5A: Pounded Yam Full Product Profile Development Workshop Report (B. Otegabyo, Bowen) D4.1: Sensory panel evaluation and texture-extrusion analysis of boiled cassava at CIAT (T. Tran, CIAT) D4.2: Relationships between sensory texture attributes & uni-axial texture parameters (I. Djibril-Moussa, UAC-FSA)
1.3.1	**10 SOPs uploaded on CIRAD Dataverse &/or BTI repositories** for the characterization of RTB crops and products	Achieved: 11 SOPs uploaded in CIRAD Dataverse Committed: At least 2 more SOPs under development (E4.10: Sample preparation for cell wall polysaccharides analysis of raw and boiled Yam and plantain; Ex.x: Extraction of CWM from fresh cassava roots).		E4.9: Determination of galacturonic content (C. Mestres, CIRAD) E4.11: Preparation of cell wall material from sweetpotato roots (G. Mac Dougall, JHI) E4.12: Analysis of monosaccharide composition of sweetpotato cell wall material/ polysaccharides after acid hydrolysis by high performance anion exchange chromatography (HPAEC) (G. Mac Dougall, JHI) E4.13: Fourier Transform Infra-Red Spectroscopy analysis of cell walls from sweetpotato roots (G. Mac Dougall, JHI) E5.3new: Standard Operating Protocol for Textural Characterization of Boiled Sweetpotato - V.2 (with correlation with sensory to validate it is discriminating) (M. Nakitto, CIP) E5.5: Standard Operating Protocol for Textural Characterization of Eba (B. Maziya Dixon, IITA) E5.6: Standard Operating Protocol for characterization of water absorption, cooking time and closing angle of boiled cassava (T. Tran, CIAT) E5.6bis: Standard Operating Protocol for Characterization Texture of Boiled Cassava: Texture-Extrusion (T. Tran, CIAT) E5.7: Standard Operating Protocol for Textural Characterization of Fufu (U. Chijioke, NRCRI) E5.8: Standard Operating Protocol for Textural Characterization of Pounded Yam (B. Otegbayo, Bowen) E6.6: Product preparation & sensory analyses on Attiéké (S. NNan Diby, CNRA) F2.4: RTBfoods Manual - Part 2 - Tutorial. Monitoring Panel Performance and Cleaning Data from Descriptive Sensory Panels for Statistical Analysis + Annexes 1 & 2 (C. Bugaud, CIRAD)

Output	<u>Expected/Committed</u> Targets/Milestones in Period 4	Level of <u>Achievement</u> in Period 4	If any difference between 'Committed' and 'Achieved': **Brief Explanation**	**Complete List of Deliverables submitted to PMU in Period 4** (apart from the *WP Scientific Progress Report*)
				F2.4bis: Manuel RTBfoods - Partie 2 - Tutoriel. Contrôler les Performances du Panel et Préparer les Données en Analyses Sensorielles avant les Traitements Statistiques + Annexes 1 & 2 (C. Bugaud, CIRAD) F2.5 : RTBfoods Manual - Part 3 - Tutorial, Statistical Analyses (PCA and multiple regression) to Visualise the Sensory Analysis Data and Relate it to the Instrumental Data + Annex (C. Bugaud, CIRAD) F2.5bis: Manuel RTBfoods - Partie 3 - Tutoriel : Analyses Statistiques (ACP et régression multiples) pour Visualiser les Données de l'Analyse Sensorielle et les Relier aux Données Instrumentales + Annexe (C. Bugaud, CIRAD)
1.3.2	**11 new quality traits defined with lexicon & attribute goals** *(= to reflect end-user acceptability)*	Achieved: 14 dictionaries of sensory quality traits uploaded in CIRAD Dataverse Committed: 2 more dictionaries for sensory traits on boiled cassava in Benin and Uganda (F3.7) and boiled Yam in Benin and Nigeria (F3.9)	Attribute goals within some of the dictionaries are still under data collection (to be completed during Period 5)	F3.5: Dictionary for Sensory Traits for Boiled Sweetpotato in Uganda (A. Asiimwe, Bioversity) F3.8: Dictionary for Sensory Traits for Boiled Yam in Benin (A. Asiimwe, Bioversity) F3.11: Dictionary for Sensory Traits for Eba in Nigeria (A. Asiimwe, Bioversity) F3.12: Dictionary for Sensory Traits for Fufu in Nigeria (A. Asiimwe, Bioversity) F3.13: Dictionary for Sensory Traits for Pounded Yam in Nigeria (A. Asiimwe, Bioversity) F3.14: Dictionary for Sensory Traits for Attiéké in Côte d'Ivoire (A. Asiimwe, Bioversity) F3.15: Dictionary for Sensory Traits for Boiled Potato in Uganda (A. Asiimwe, Bioversity) F3.16: Dictionary for Processing Techniques on Boiled Plantain in Cameroon (A. Asiimwe, Bioversity) F3.17: Dictionary for Processing Techniques on Boiled Potato in Uganda (A. Asiimwe, Bioversity) F3.18: Dictionary for Processing Techniques on Sweetpotato in Uganda (A. Asiimwe, Bioversity) F3.19: Dictionary for Processing Techniques on Gari in Nigeria (A. Asiimwe, Bioversity) F3.20: Dictionary for Processing Techniques on Matooke in Uganda (A. Asiimwe, Bioversity) F3.21: Dictionary for Processing Techniques on Pounded Yam in Nigeria (A. Asiimwe, Bioversity)

Output	<u>Expected/Committed</u> Targets/Milestones in Period 4	Level of <u>Achievement</u> in Period 4	If any difference between 'Committed' and 'Achieved': **Brief Explanation**	**Complete List of Deliverables submitted to PMU in Period 4** (apart from the *WP Scientific Progress Report*)
				F3.22: Dictionary for Processing Techniques on Fufu in Nigeria (A. Asiimwe, Bioversity) F3.24: Report 'Creating Food Product Quality Traits in the Crop Ontology for RTBs (A. Asiimwe, Bioversity)
1.5.2	**35% of genotyped clones from WP4 populations informed with quality data available in existing RTB databases** for the 5 RTB crops	Achieved: 13 laboratory databases with quality data uploaded to CIRAD Dataverse, for the following RTB products: - Fresh cassava (blended: 2, intact: 2) - Cassava flour (2) - Gari (1) - Fresh Yam (blended: 3, HSI: 1) - Yam flour (1) - Matooke (fresh: 1) These databases cover 50% of the clones of WP4 populations.	Committed for Period 5: (1) Complete the existing databases with further quality data collected during Period 5 harvests. Some of the existing databases contain compositional data (e.g. dry matter, starch) only, and can be expanded to include e.g. texture or water absorption data. (2) In addition to data from blended RTB, add new databases with quality data linked to NIRS of intact roots and/or to HSI images. (3) Datasets for sweet potato need to be added (I.P. concerns to address).	**Laboratory database [.xlsx]:** RTBfoods_K.X.X_Spectral database_NIRS_Cooking_Time_Cassava_fresh_puree_CIAT Colombia_2021.xlsx (T. Tran, CIAT) RTBfoods_XXX_Spectral database_NIRS_Cassava_Flour_DM_Starch_Amylose_Amylopectin_Sugar_Fiber_NRCRI_P4.xlsx (U. Chijoke, NRCRI) RTBfoods_XXX_Spectral database_NIRS_Cassava_Flour_starch_DM_NRCRI Nigeria_P4.xlsx (U. Chijoke, NRCRI) *RTBfoods_XXX_Spectral database_NIRS_Cassava_Fresh_intact_DM_NRCRI Nigeria_P4.xlsx (U. Chijoke, NRCRI)* RTBfoods_XXX_Spectra database_NIRS_Fresh_Yam_DM_NRCRI Nigeria.xlsx (U. Chijoke, NRCRI) RTBfoods_WP3_Spectral_database template_NIRS_DMSTARCH_FreshblendedCassava_IITA_Nigeria_final.xlsx (E. Alamu, IITA) RTBfoods_WP3_Spectral_database template_NIRS_DMSTARCH_IntactrootCassava_IITA_Nigeria_final.xlsx (E. Alamu, IITA) RTBfoods_WP3_Spectral_database template_NIRS_Functional Properties_Gari_IITA_Nigeria_final.xlsx (E. Alamu, IITA) WP3_database_CIRAD-INRAE Gpe_YamFlour_2016-2018_516samples.xlsx (L. Desfontaines, INRAE & D. Cornet, CIRAD) Good_Bad Yam Spectra_2021.xlsx (E. Alamu, IITA & D. Cornet, CIRAD) WP3_database_CIRAD_HSI_freshYam_19042021_FD.xlsx (K. Meghar) RTBfoods_WP3_database_Fresh_matooke_Texture_DM_IITA_NACCRI.xlsx (E. NuwaYama, NaCRRI) RTBfoods_WP3_Spectral database template_NIRS_fresh_blended_Yam_IITA_Nigeria.xlsx (E. Alamu, IITA) WP3_database_CIRAD_Yam_NIRS-Texturometry-DM_Guadeloupe_02062021.xlsx (J. Ajax & M. Lechaudel, CIRAD)

4 DECIPHERING QUALITY AND FUNCTIONALITY OF RTB FOOD PRODUCTS

4.1 Boiled Cassava

4.1.1 Sensory evaluation in Uganda, Benin and Colombia

Methodology development and definition of sensory descriptors:

Two SOPs are available for the sensory characterization of boiled cassava:

1. Ephraim NUWAMANYA, (2021). Sensory Characterization of Boiled Cassava. Biophysical Characterization of Quality Traits, WP2. Kampala, Uganda: RTBfoods Laboratory Standard Operating Procedure, 18 p.
2. Laurent ADINSI, Noël AKISSOE, (2021). Sensory Characterization of Boiled Cassava. Biophysical Characterization of Quality Traits, WP2. Cotonou, Benin: RTBfoods Laboratory Standard Operating Procedure, 13 p.

At NACRRI-Uganda, the sensory descriptors generated and used by the sensory panel are:

- Appearance (Yellow, White, Homogeneity of colour, Surface Smoothness)
- Texture in mouth (softeness, moisture, smoothness, fibrousness, mealiness, *kiwuta*, stickiness)
- Texture by touch (mealiness, moldability, stickiness)
- Taste (sweetness, bitterness, bitter after taste)
- Aroma (cassava, roasted cassava)

Among these, the key priority quality traits are **softness, mealiness, sweetness/bitterness**.

At UAC-FSA-Benin, 13 descriptors were used for sensory evaluation:

- Appearance (white colour, yellow colour, presence of stripe)
- Texture (sticky, hard to break, crumbly, presence of fibers, granular, easy to chew)
- Cassava odour
- Taste (sweet taste, bitter taste and bitter aftertaste).

Among these, the key priority quality traits, based on the preference surveys by WP1, are **white colour, crumbly, easy to chew and sweet taste**. These traits must absolutely be passed on to breeders for integration as breeding priorities.

At CIAT-Colombia, the sensory attributes were adapted to the Colombian context through focus group discussions with panellists. The sensory attributes are:

- Appearance (yellow, cream)
- Texture by touch (friability, glassiness)
- Texture in mouth (hardness, moisture, fibrousness, mealiness, stickiness)
- Taste (bitterness, bitter after-taste)

NB: This list is presented in this report in English version, but for the sensory analysis sessions at CIAT, we use the Spanish version. The sensory attribute fibrousness was not discriminant and will be abandoned for the sensory analyses of Period 5. Among the sensory descriptors listed above **Mealiness** is considered a priority quality trait (PQT) to determine the adoption of improved varieties by consumers. Based on the dataset of RTBfoods progenitors at CIAT from Period 4 (first period when sensory analysis was organized), mealiness was correlated with instrumental texture parameter "End_force:Max_force ratio" (texture-extrusion SOP), and with water absorption. Mealiness may therefore be predicted by biophysical measurements (to be confirmed after repetition of these experiments with Period 5 harvests). **Hardness** may also be considered a priority trait. However the boiling protocol used for Period 4 sensory analysis (i.e. boiling each genotype until its optimum cooking time) minimized the contrasts among genotypes, resulting in limited differences according to the hardness parameter. An updated protocol, boiling all genotypes for the same time, i.e. 20 min, will be tested in Period 5 to check whether hardness can be a discriminant attribute for sensory analysis. **Bitterness** is also a key trait, as genotypes with a bitter taste are not acceptable for consumption as boiled cassava. **Moisture** and **stickiness** may also be considered as important traits describing the behaviour of boiled cassava and able to discriminate among different genotypes.

Nb of samples & Nb of genotypes characterized by the sensory panel in 2021

At NaCRRI-Uganda, no sensory analysis was undertaken in period 4 due to challenges related to the Covid-19 situation. Key results shall be presented in the next reporting period.

At UAC-FSA-Benin, 9 samples that differed by the variety and harvesting age were evaluated by the panel. Globally, 7 genotypes (from which 2 were harvested at 2 planting age) were evaluated in 2020. These samples are landraces named Agric (9 and 12 months), Alanmandou (6 months), Atinwéwé (6 months), Dossi (9 and 12 months), Hombété (8 months), Koléhaomè, (6 months) and Ofégué (8 months).

At CIAT-Colombia, 14 genotypes were harvested at 14 months after planting (June 2021) and characterized by a sensory panel of 14 judges. The texture of these genotypes was characterized by texture-extrusion at the same time, and correlations between sensory attributes and instrumental texture parameters were investigated.

Key results & graphs:

At UAC-FSA-Benin, the samples studied were similar regarding four descriptors which are yellow colour, stickiness, granular and cassava odour ($p > 0.05$). These descriptors, non-discriminating, should be considered as non-essential for boiled cassava. The other sensory attributes varied depending on variety and age at harvest. Regardless of age (9 or 12 months), the Dossi variety was found to have the highest value of white colour, crumbly in hand and easy to chew. Inversely, the Agric variety seems to show different behaviour according to harvesting age. Indeed, at 12 months after planting, Agric was found to be crumbly and easy to chew while at 9 months after planting, it was hard to break like the varieties Alanmadou (6 months), Atinwéwé (6 months), Koléhaomè (6 months) and Ofèguè (8 months). The intensity of white colour and yellow colour also varied depending on the harvesting age of the Agric variety. Among the nine samples, only Koléhaomè (6 months) was judged to have a very pronounced bitter taste.

The sensory mapping of boiled cassava (Figure 1) indicated that Dossi (9 and 12 months), Agric (12 months), and Hombété (8 months) varieties were associated with the descriptors easy to chew, crumbly, cassava odour, white colour, presence of stripe, presence of fibers, sticky and sweet taste. These descriptors were recognized in gender food mapping study as the drivers of boiled cassava overall liking. Accordingly, they can be considered as high quality boiled cassava. The varieties Alanmandou (6 months), Atinwéwé (6 months), 0fèguè (8 months) and Agric (9 months) were associated with granular, hard to break and yellow descriptors. The Koléahonmè variety (6 months) was associated with the bitter character (bitter taste and bitter aftertaste). This variety can be considered as poor quality for boiling.

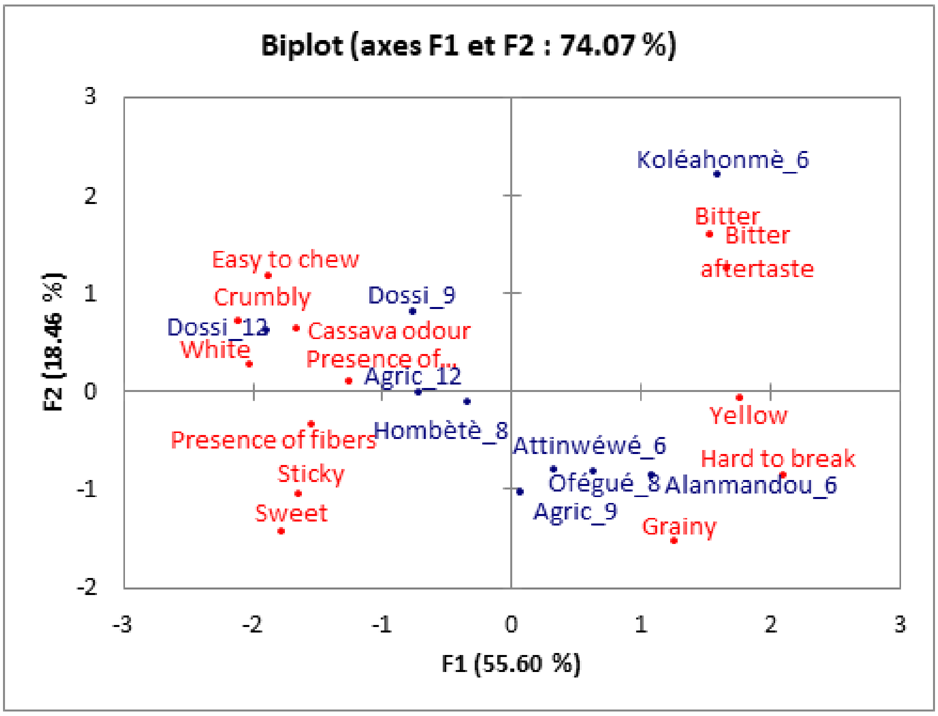


Figure 1 Sensory mapping of boiled cassava samples.

At CIAT-Colombia, Bivariate correlation analysis (Pearson) indicated that some sensory attributes are strongly correlated, in particular Mealiness and Stickiness (R = 0.86) and Mealiness and Glassiness (R = -0.94) (Figure 4). Overall the attributes selected seemed suitable to discriminate among cassava genotypes, and describe the range of their cooking behaviours, from long-cooking-time associated to hard texture, to short-cooking time associated with different types of texture (figure 2).

Full results are provided in the report "Sensory panel evaluation and texture-extrusion analysis of boiled cassava at CIAT (Tran et al., 2021)". NB: These results should be considered as preliminary because the panel was not fully trained and calibrated, resulting in high standard deviations in the evaluation of sensory attributes. This situation was due to the Covid restrictions and social unrest period that delayed the execution of the sensory analysis workplan.

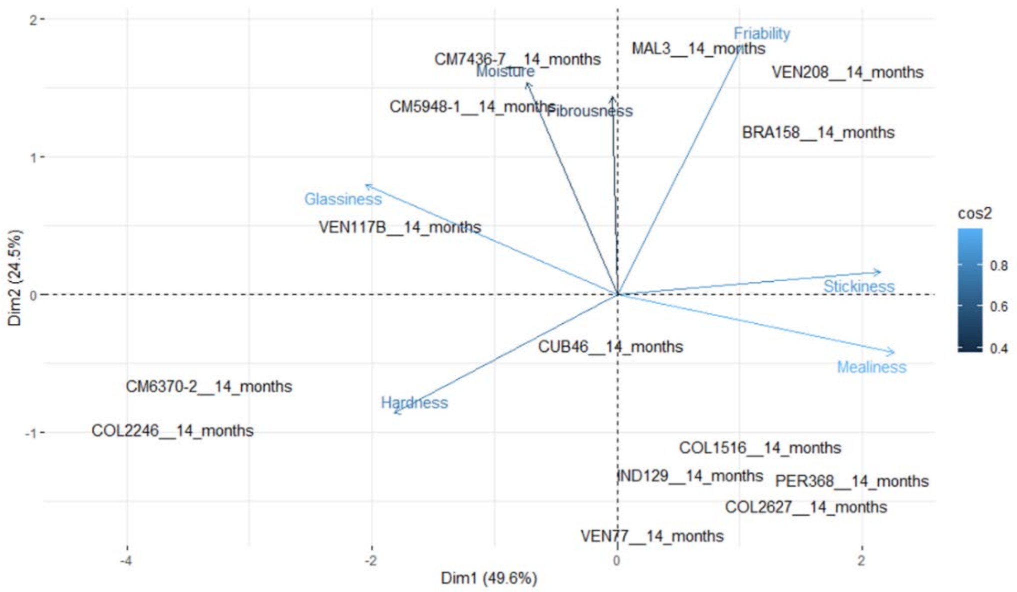


Figure 2 Principal component analysis of the sensory attributes of 14 cassava genotypes (boiled roots) of the CIAT RTBfoods progenitors collection harvested at 14 months.

4.1.2 Textural evaluation & correlation with sensory descriptors in Colombia, Uganda and Benin

A SOP for textural characterization of boiled Cassava through texture-extrusion was developed and optimized by CIAT. A first version was submitted and revised in 2019. A final version was submitted and revised during period 4 and has been adopted at UAC-FSA-Benin and at NaCRRI-Uganda, with few adjustments. In addition, penetrometry and texture profile analysis (TPA) tests were also used at UAC-FSA-Benin and NaCRRI. These tests are based on the conditions defined in the SOP developed at UAC-FSA for textural characterization of boiled Yam.

Nb of samples & Nb of genotypes characterized for texture in 2021

At NaCRRI-Uganda, 38 genotypes from two different sites were characterized by TPA period 4.

At UAC-FSA-Benin, the 9 samples aforementioned in sensory section (from 7 genotypes) were used for textural characterization as well.

At CIAT-Colombia, 30 genotypes were characterized by texture-extrusion. Each genotype was harvested at 9, 10 and 11 months after planting, resulting in 90 samples characterized in 2021. Additionally, a subset of 14 genotypes was characterized at 14 months after planting, to coincide with the sensory analysis panel.

List of key sensory descriptors for texture (generated & used by trained panellists) to be measured instrumentally:

At UAC-FSA-Benin, the key sensory descriptors for texture to be measured instrumentally are **sticky**, **hard to break, crumbly and easy to chew.** The 3 texture tests as mentioned above were used to predict these sensory descriptors for texture.

At CIAT-Colombia, **Mealiness** and **Hardness** have the best potential for measurement by texture analyser. This is because the vectorial space (analysed by principal component analysis) of the texture-extrusion parameters is organized according to two main dimensions: (i) texture parameters describing hardness of the sample, such as Maximum force, Area under curve, Linear distance, Initial gradient; (ii) texture parameters describing the breaking behaviour of the sample, such as Distance at max. force and End_force:Max_force ratio.

Level of progress in the establishment of a correlation with instrumentally measured parameters:

At NaCRRI-Uganda, due to limitations related to the Covid19 situation, no sensory analysis was undertaken on the 38 clones from RTB trials, and therefore no correlation with instrumental texture was identified. This will be carried out in period 5 for the clones that were advanced based on other traits. However, correlation analysis of texture data (TPA) showed high correlations between chewiness and resilience ($r = 0.9$), chewiness and area ($r = 0.8$), chewiness and travel 1.2 ($r = -0.9$), guminess and force 1 ($r = 0.8$), guminess and travel 2.3 ($r = 0.8$). Regarding instrumental texture analysis, the 38 genotypes evaluated all had similar properties, with no major differences, even though they were grown in two different environments (figure 3).

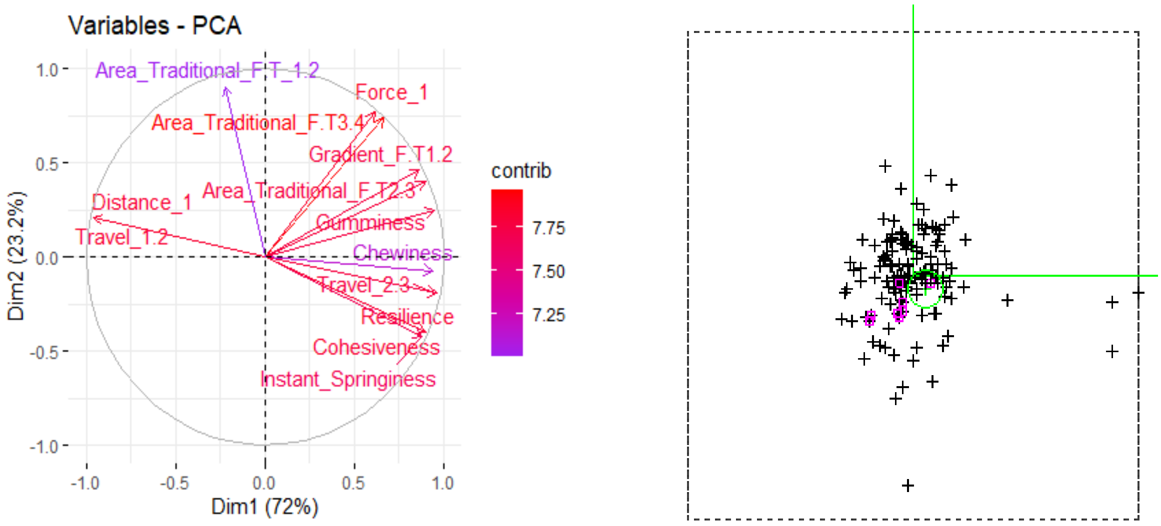


Figure 3 Principal component analysis of the instrumental texture parameters of 38 cassava genotypes (boiled roots) grown in two environments in Uganda (NaCRRI)

At UAC-FSA-Benin, a Pearson correlation test was performed to assess the relationship between the sensory texture descriptors listed above and the instrumentally measured parameters for boiled cassava (Table 1). The hard to break attribute was significantly and positively linked to maximum compression force (hardness) (r = 0.94) and energy (r = 0.945) evaluated by penetrometry test and to hardness (r = 0.99), gumminess (r = 0.993 and r = 0.826) and chewiness (r = 0.993 and r = 0.884) measured by the texture profile analysis (TPA) test. Crumbly and easy to chew were significantly and negatively correlated with hardness (r = -0.926 and r = -0.921) and energy (r = -0.936 and r = -0.929) assessed by penetrometry test, and with hardness (r = -0.979 and r = -0.990), gumminess (r = -0.956 and r = -0.974) and chewiness (r = -0.973 and r = -0.977). As far as extrusion test is concerned, the descriptor hard to break was significantly and positively linked to max slope (r = 0.94) and energy (r = 0.96) while they were significantly and negatively related to distance at max force (r = -0.88). Conversely, the descriptors crumbly and easy to chew were significantly and negatively correlated with max slope (r = -0.919 and r = -0.922) and energy (r = -0.948 and r = -0.964). Furthermore, the crumbly descriptor was significantly and negatively associated with hardness (r = -0.814) while the easy to chew descriptor was significantly and negatively correlated with distance at max force (r = 0.836). None of the instrumentally measured texture parameters was significantly correlated with the sensory descriptor ‘sticky’. These results confirm that several sensory attributes for boiled cassava can be measured instrumentally by one of the three instrumental texture methods (penetrometry, TPA, texture-extrusion) tested at UAC-FSA.

Table 1 Pearson correlation matrix between texture sensory descriptors and uni-axial texture parameters of boiled cassava

Uni-axial texture parameters		**Sensory texture descriptors**			
		Sticky	**Hard to break**	**Crumbly**	**Easy to chew**
Penetrometry test	Hardness (N)	-0.357	**0.942**	**-0.926**	**-0.921**
	Energy (N.sec)	-0.366	**0.946**	**-0.936**	**-0.929**
TPA test	Hardness (N)	-0.043	**0.999**	**-0.979**	**-0.990**
	Cohesiveness	0.216	0.590	-0.443	-0.483
	Gumminess (N)	0.024	**0.993**	**-0.956**	**-0.974**
	Springiness (N)	-0.268	0.751	**-0.866**	**-0.830**
	Chewiness (N.mm)	-0.121	**0.993**	**-0.973**	**-0.977**
Extrusion test	Max slope (N/m)	-0.064	**0.948**	**-0.919**	**-0.922**
	Hardness (N)	-0.059	0.691	**-0.814**	-0.787
	Distance at max force (mm)	-0.224	**-0.887**	0.790	**0.836**
	Energy (N)	0.072	**0.969**	**-0.948**	**-0.964**

Bold Pearson correlation coefficient are significant ($p < 0.05$).

At CIAT-Colombia, the sensory and instrumental texture dataset collected during Period 4 indicates a good correlation (R^2 = 0.67) between Mealiness and End_force:Max_force ratio, thus indicating that Mealiness can be measured instrumentally (pending repetition and confirmation of these results during Period 5):

Mealiness = -11.851+ 0.187 * End_force:Max_force ratio

On the other hand, no significant correlation was found between sensory hardness and instrumental hardness, possibly due to the use of different boiling protocols. Experiments will be repeated in Period 5 using the same boiling protocol for both sensory analysis and texture-extrusion. Correlation analysis between sensory attributes and instrumental texture-extrusion parameters is summarized in Figure 4.

Figure 4 Bivariate correlation analysis (Pearson) between sensory descriptors and texture-extrusion parameters of 14 cassava genotypes (boiled roots) of the CIAT RTBfoods progenitors collection harvested at 14 months.

Complete results are provided in the report “Sensory panel evaluation and texture-extrusion analysis of boiled cassava at CIAT (Tran et al., 2021)”.

Synthesis on progress achieved:

Institute-Country	Targeted Quality Trait / Sensory Descriptor	Instrumental Method for Measurement on Final product				
		Measurement method	SOP available? (Yes/No/ Pending)	Correlation established with sensory? (Yes/No/ Pending)	Nb of Analyses performed in 2021	Nb of WP4 clones characterized in 2021
NaCRRI-Uganda	Softness	Penetrometer based	YES	Pending	158	158
	Mealiness	Not available	NO	No	0	0
	Bitterness	Enzymatic method (Bradbury)	NO	No	393	393
	Sweetness	Not available	NO	No	0	0
	Textural properties	Texture analyser	YES	Pending	158	158
UAC-FSA-Benin	Sticky		YES	NO	9 samples * 3 cooking batches * 3 replicates	NA
	Hard to break		YES	YES		NA
	Crumbly		YES	YES		NA
	Easy to chew		YES	YES		NA
CIAT-Colombia	Mealiness	Texture-extrusion	Yes	Yes, to be confirmed with harvests in Period 5	90 + 14 = 104	
	Hardness	Texture-extrusion	Yes	No. Further experiments necessary after adjusting the boiling protocol for the sensory tests	90 + 14 = 104	

Similarities and differences between three partners have been summarized in the table below.

Steps	NaCCRI	UAC-FSA	CIAT
Samples preparation	Cassava cylinders of 5-6 cm		
Cooking preparation	Steam cooking for 55 min	Steam cooking for 45 min	Boiling between 15 and 50 min
Temperature of tasting	Tasting begins at 60-65°C		
Repeated samples	Only one cultivar replicated per session	Triplicate	No replicates
Numbers of products	25 cultivars	7 cultivars	14 cultivars
Vocabulary	21 attributes	13 attributes	11 attributes
Common attributes	White colour, yellow colour, hardness (or softness), stickiness (mouth or by hand), fibrousness, bitter, bitter after taste,		
Different attributes	Homogeneity of colour, moisture, smoothness, mealiness, moldability, sweetness, cassava aroma	Presence of stripe, granular, easy to chew, hard to break, crumbly, sweetness, cassava odour	Friability (in appearance), glassiness, mealiness, moisture
Rating scale	Structured scale 0-10	Non-structured scale 0 to 100 mm	Structured scale 0-10
Priority quality traits (PQT)	softness, mealiness, sweetness, bitterness	white colour, crumbly, easy to chew and sweet taste	Mealiness, hardness, moisture, stickiness, bitterness

The differences observed between the methodologies proposed by the 3 partners mainly concern the cooking time and the vocabulary used. This can be explained by the specificities of the panels (cultural differences, culinary differences, etc…).

Not all priority quality traits were common between partners. Softness/hardness, mealiness and bitterness were common to NaCRRI and CIAT, and sweetness to NaCRRI and UAC/FSA. Sweetness however is related in the case of boiled cassava to the absence of bitterness (i.e. cyanide compounds), and is therefore close to the bitterness attribute used by CIAT.

Several correlations have been established between sensory attributes and instrumental parameters of texture. These correlations are presented in the following table.

Sensory attributes	Textural parameters	R^2	Partner involved
Easy to chew	Penetrometry, TPA and extrusion parameters	$0.70 < R^2 < 0.98$ (depending of the textural parameters)	UAC-FSA
Hard to break	Penetrometry, TPA and extrusion parameters	$0.78 < R^2 < 0.98$ (depending of the textural parameters)	UAC-FSA
Crumbly	Penetrometry, TPA and extrusion parameters	$0.66 < R^2 < 0.98$ (depending of the textural parameters)	UAC-FSA
Mealiness	End force / max force	0.67	CIAT

All sensory attributes correlated with instrumental parameters are priority quality traits. The correlation established by UAC/FSA on hard to break can be used for predicting hardness (CIAT) and softness (NaCRRI). No correlation was found for stickiness with textural parameters.

Next steps & activities planned to measure key sensory descriptors for texture instrumentally:

In Period 5, **NaCRRI-Uganda** will conduct sensorial analyses from NARL on the selected clones and will work toward the establishment of correlations between sensory and instrumental measurements.

At UAC-FSA-Benin, no additional texture analyses are planned in period 5. However, the team plans to submit publication of results from texture analyses during the first semester of 2022.

At CIAT-Colombia, the 30 genotypes characterized in 2021 were replanted in March 2021 and will be harvested in December 2021 – March 2022 at 9, 10 and 12 months after planting (MAP). Instrumental texture-extrusion characterizations will be repeated for each of these harvests. Sensory analysis will also be conducted at 9, 10 and 12 MAP, on a subset of 15 genotypes. The sensory analysis protocol will be adjusted by reducing the cooking time to 18 minutes, to be the same as the texture-extrusion protocol and to have a better contrast between genotypes tested by the sensory panel.

The focal points for sensory and texture at CIRAD recommend:

- On texture attributes: Validate the good relationships between sensory attributes (chewiness, hardness, crumbly and mealiness) and instrumental parameters (for the 3 partners). Try to find relationships between stickiness and new textural measurements (CIAT).
- On white colour: Try to find relationships with chromameters parameters (UAC/FSA)
- On sweetness and bitterness: Try to find relationships between these attributes and chemical components (soluble sugars, polyphenols).

4.1.3 Rapid / intermediate kitchen tests to assess the processing & the cooking ability of boiled cassava

Rapid kitchen test or intermediate assessment methods investigated:

At NaCRRI-Uganda, the major behaviour of concern for the boiled cassava product is cooking ability. Comparative behaviours are being assessed among different populations to define the variations, with local varieties acting as benchmarks. Faster cooking clones are preferred coupled to soft texture in particular proportions (i.e. not so soft and not hard). The rapid assessment method being used at the moment is water absorption. NaCRRI-Uganda is using the standard procedure for determination of water absorption at 30 minutes of cooking developed by CIAT. This is quite robust and applicable both on station and under field conditions. It has been used for almost all the trials under the RTB project and the NEXTGEN cassava project. In addition, adaptability of laboratory personnel on the procedure has been smooth with reproducible results both in the field and in the laboratory. To determine root softness, the use of the penetrometer has been mainstreamed in most of the activities at NaCRRI. Using the procedures developed over time while using this instrument, values for softness are used to complement observations from water absorption in understanding the softness traits. Significant differences were thus observed between locations among the genotypes evaluated in 2021 (Figures 5 and 6): Genotypes assessed in Namulonge were softer with scores below 4.5 $N.min^{-1}$, compared to Serere where about 60% of the genotypes had softness scores ranging from 1-5 $N.min^{-1}$. The texture-extrusion protocol gave similar results (Figure 7).

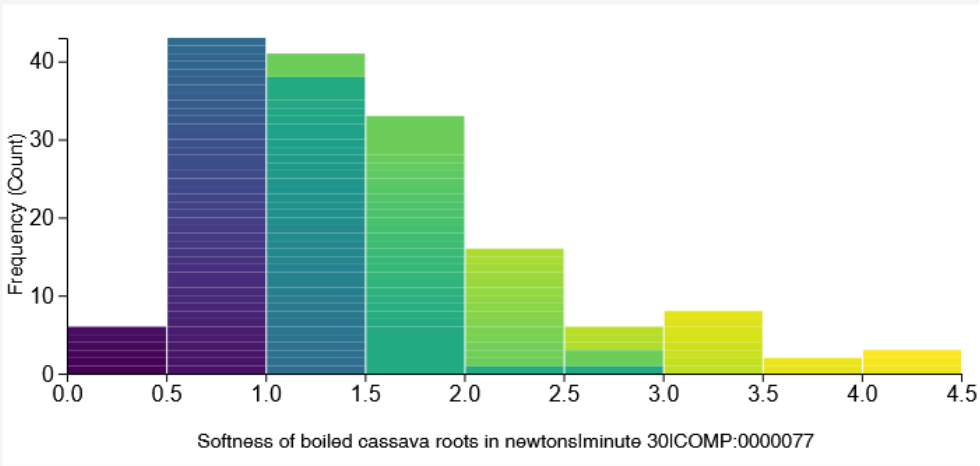


Figure 5 Penetrometry results for root softness of boiled cassava from genotypes grown in Namulonge

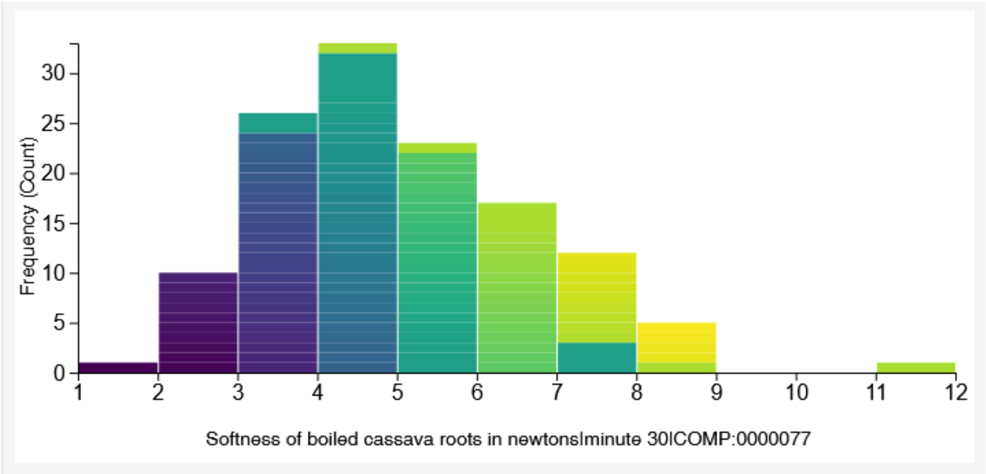


Figure 6 Penetrometry results for root softness of boiled cassava from genotypes grown in Serere.

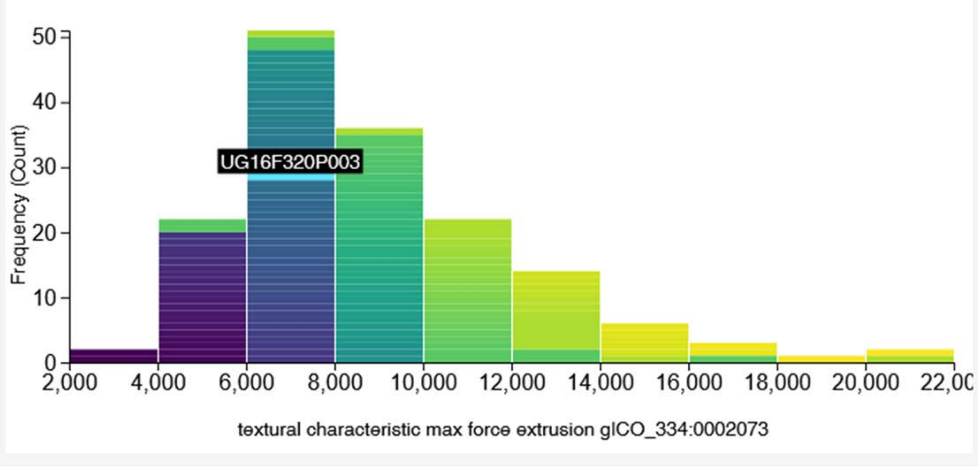


Figure 7 Texture-extrusion results for maximum force of boiled cassava from Namulongue

At CIAT-Colombia, cooking ability of boiled cassava has been investigated as well through the measurement of Water absorption (WAB) after 30 minutes, Cooking time (fork method and Mattson cooker method), Dry matter before and after boiling (30 minutes), Texture (texture-extrusion protocol).

Water absorption (WAB) was the main medium-throughput phenotyping method developed and put to use on various cassava populations of the CIAT cassava program: RTBfoods progenitors and progeny collections, diversity collection, biofortified F1C1 population. WAB showed significant negative correlations with cooking time (CT), in particular at 30 min boiling, with correlation coefficient r = -0.78 (figure 8). WAB at 30 min boiling can therefore be a faster, less subjective and more repeatable method to predict CT for cassava breeding efforts, compared to direct measurement which requires up to 60 min for some genotypes. On a practical level, measuring CT directly requires well-trained personnel and remains a somewhat subjective measurement. WAB at 30 min boiling, on the other hand, provides a simple and objective method to predict CT, requiring only weighing the roots at time 0 and after 30 min boiling, instead of a constant inspection until the roots reach the desired softening (using a fork multiple times). Up to 50 samples per day may be managed by one person using the WAB method (not including root washing & peeling, and sample preparation). Please refer to the article "*Tran et al. (2021): Correlation of cooking time with water absorption and changes in relative density during boiling of cassava roots*", and to the SOP "*Protocol for characterization of water absorption, cooking time and closing angle of boiled cassava; 14/09/2021*" for further details. The WAB method was developed at CIAT in 2019, and resulted in the writing of a SOP submitted in 2020 and updated in 2021 ("Protocol for characterization of water absorption, cooking time and closing angle of boiled cassava"; 14/09/2021; file *WP2_SOP-boiled-cassava-cookingtime-wab-angle210914.doc*). Other RTBfoods partners then adapted the SOP to their own materials (boiled cassava: NaCRRI, boiled Yam: UAC-FSA, ...). The method has been peer-reviewed and is published in the special issue of IJFST (Tran et al., 2021. Correlation of cooking time with water absorption and changes in relative density during boiling of cassava roots).

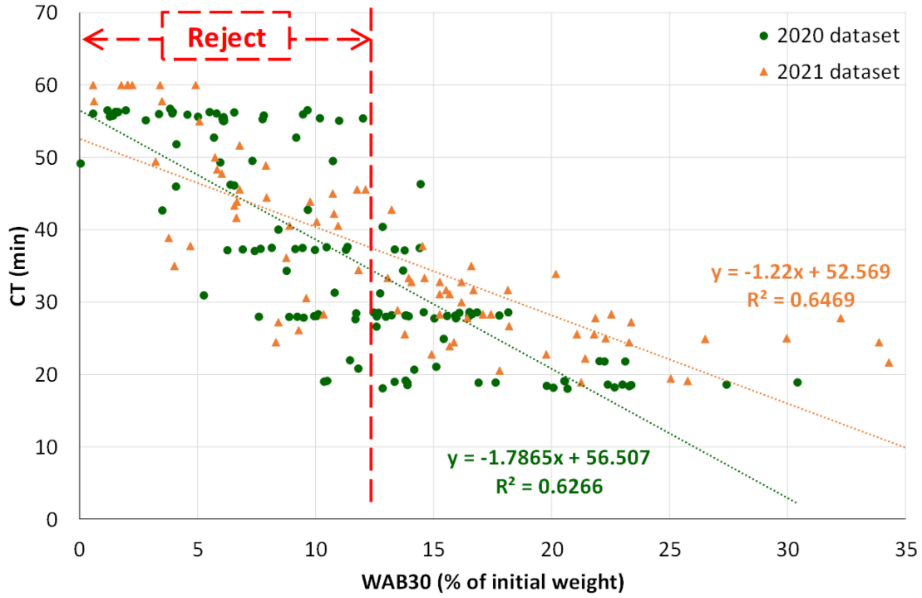


Figure 8 Correlation between cooking time (CT) determined by fork method and water absorption at 30 minutes boiling (WAB30) using the datasets from the harvests Period 3 and Period 4 (2020 and 2021, respectively).

When WAB30 is lower than 12%, the sample can be considered to take too long to cook, and be rejected as illustrated by the "Reject" label.

A **Mattson cooker** (figure BC9) was built and tested to try to replace the conventional fork method for cooking time by a more robust and reproducible method. The instrument consists of nine slots each holding a piece of cassava root (of specific, standard dimensions), and immersed in a pot of boiling water. Each piece is placed under a needle of calibrated weight. When a piece becomes soft enough, the needle goes through it and the time of the event is automatically recorded through an electronic switch detecting the movement of the needle. One experiment consists in placing nine pieces of roots of the same genotype (ideally sampled from different roots and plants for representativeness) in the Mattson cooker, and recording the cooking time of each individual piece. The cooking time of the genotypes is defined as the time when 6 out of 9 pieces are cooked, as determined by the needles going through each piece. Good correlations between Mattson and conventional fork method have been obtained, and need to be confirmed with further data collection during the 2022 harvests. The Mattson cooker method is still under development, with more data collection needed to fully demonstrate the correlation between cooking time determined by Mattson and by the conventional fork method.

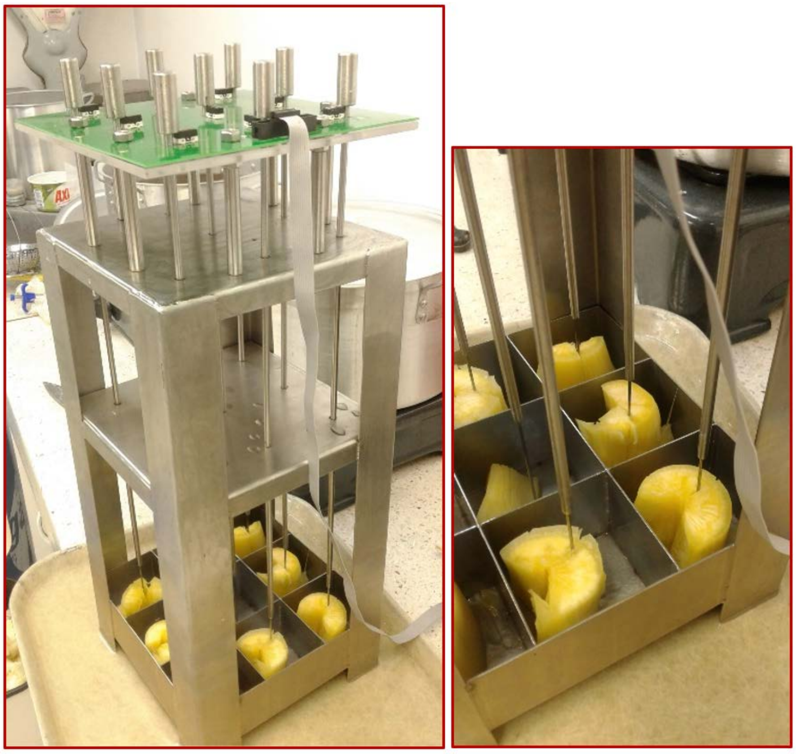


Figure 9 Mattson cooker being tested at CIAT to replace the fork method for determination of cooking time

The **texture-extrusion protocol** developed in 2020 for boiled cassava (18 min boiling) was applied to the RTBfoods progenitors collection (Period 4, 2021 harvests) and proved effective at discriminating cassava genotypes according to their texture. Several parameters were recorded and described the behaviour of boiled cassava according to two dimensions (figure 10): (1) Parameters maximum force, area under curve, linear distance and initial gradient gave information about hardness; (2) parameters distance at max force and end force:max force ratio gave information about breaking behaviour during compression (i.e. whether a sample was extruded smoothly, of whether something in the sample internal structure resisted and broke when passing through the extrusion grid). Three groups of genotypes were thus identified among the RTBfoods progenitors collection: Soft and not breaking; hard and not breaking; hard and breaking. These three groups correspond to increasingly hard to cook genotypes. Texture-extrusion measurements were thus useful in classifying genotypes in terms of ease-of-cooking. Correlations with sensory analysis of texture and with NIRS were investigated (see section on sensory analysis above, and WP3 report). A fourth group, soft and breaking, remained theoretical as no genotype was identified with such behaviour. Please refer to the SOP "*Protocol for characterization of texture of boiled cassava: Texture-extrusion; 14/09/2021*" for further details.

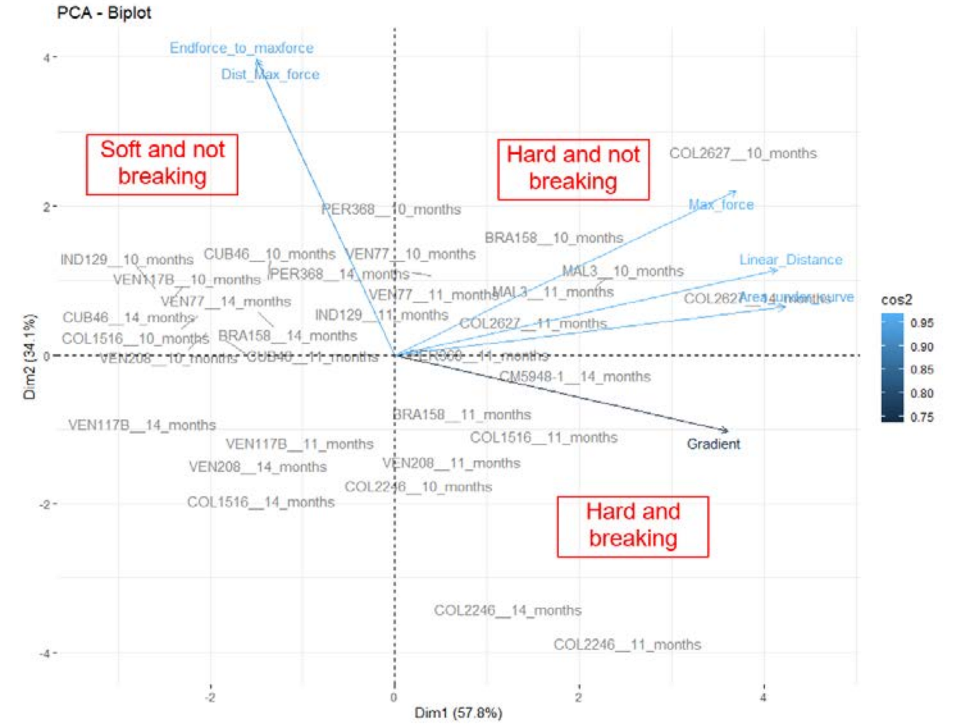


Figure 10 Principal component analysis of Period 4 texture-extrusion data, illustrating the grouping of texture parameters into two main dimensions.

(1) Hard/Soft: maximum force, area under curve, linear distance and initial gradient; (2) Breaking/Not breaking: distance at max force and end force:max force ratio.

Synthesis on progress achieved:

Following the development of kitchen tests protocols for boiled cassava during Periods 2 and 3 (water absorption, texture analysis, cooking time), one of the major achievements during Period 4 was the transition from experimental development to applying these kitchen tests routinely to characterize boiling quality of large cassava breeding populations, at both NaCRRI and CIAT. The water absorption protocol in particular (WAB) has proven relatively rapid (30 minutes/sample) and easy to scale-up, requiring only additional gas stoves, pots & strainers, and balances. As a result, one trained assistant researcher can characterize up to 50 samples per day (with 2 replications, i.e. 100 measurements in total). In addition to RTBfoods populations, two other breeding populations at CIAT were characterized with the WAB protocol including the Parental collection and the HarvestPlus biofortified progeny (F1C1), totalling more than 4000 genotypes, thus demonstrating the direct contribution of RTBfoods to improving cassava breeding and selection operations of other projects.

Next Steps in the development of rapid / intermediate kitchen assays for boiled plantain

At NaCRRI-Uganda, the main next steps are to:

1. Develop SOPs and undertake analyses on starch and amylose content as the main determinants of industrial relevance of cassava
2. Undertake sensorial based characterisation of advanced clones and clones under testing in Serere and Namulonge mainly due to logistical considerations
3. Mainstream RTB derived WP2 SOPs in analysis of broader cassava trials from other projects and initiatives
4. Produce more reference data sets to support calibration development within WP3

At CIAT-Colombia, the water absorption (WAB) method developed is now used routinely to characterize various populations of the CIAT cassava program. For the last project period the team will continue to collect WAB data of the RTBfoods progenitors and progeny collections, in order to increase our database and further investigate correlations between cooking quality, sensory analysis data, and NIRS. The main objective is to obtain enough data to reach reliable HTPP prediction by NIRS of functional quality trait such as cooking quality, in addition to already existing prediction algorithms of composition traits such as dry matter and carotenoids.

The priority activities during Period 5 consist in finalizing the standard operating protocols (SOPs) that are still pending (i.e. some of the texture analysis protocols), and in harmonizing across partners the format of the datasets collected so far, in order to deliver consistent data for upload to the RTBfoods database and/or Cassavabase. Additionally, RTBfoods partners will continue to collect new data from the harvests planned in Period 5, and integrate them to the datasets from previous years.

4.1.4 Dissection and understanding of key quality traits for boiled cassava

Quality trait(s) of focus/investigated:

At NaCRRI-Uganda, research activities focused on Bitterness/Sweetness, Cooking time, Mealiness, Industrial value. Biochemical attributes suspected of being involved and studied are: cyanogenic potential-free and bound cyanide for bitterness/sweetness; starch content/starch quality for cooking time, mealiness and industrial value; amylose content and amylose amylopectin ratio for cooking time, mealiness and industrial value; pectin content for cooking time and mealiness.

At UAC-FSA-Benin, the quality traits investigated were linked to taste (sweet and bitter) and texture (crumbly and easy to chew). Biochemical attributes suspected of being involved are: sugars and cyanide for the taste; dry matter, starch and amylose contents for texture.

At CIAT-Colombia, research activities focused on the cooking quality of boiled cassava, assessed through cooking time, water absorption and texture parameters, which is likely determined by the fibers and pectin composition of cassava roots.

At CIRAD-France, research activities focused on texture -through penetrometry test- that can be linked to easy to chew, hard to break, crumbly quality traits. Pectins were suspected of being involved.

Level of progress in the development of biochemical proof of concept / method to measure the biochemical attributes of raw material

At NaCRRI-Uganda, significant progress has been made on development of biochemical proof of concept for cyanide in cassava. The lab has been testing the relationship between cyanide determination methods of picric acid/picric paper and the enzymatic Bradbury procedure. In the picrate procedure, modifications involving both scoring and determination of absorbance of picrate paper placed in distilled water has been conducted. The lab awaits the proof of concept for pectin analysis from CIRAD for mainstreaming in laboratory analyses at NaCRRI. Through the work of the masters student (Fatumah Babirye) on the NEXTGEN project, proof of concept for determination of cassava root starch content from cassava flour and fresh roots has been undertaken. Progress on this work involves refinement of the procedures to provide for analysis of both raw samples and processed flour samples for starch content.

At UAC-FSA-Benin, 9 samples that differed by the variety and harvesting age were used for biochemical evaluation of raw (fresh) and boiled cassava through measurement of sugar, cyanide and dry matter at UAC/FSA lab. Dry matter content was determined on both raw and boiled cassava samples. Flours of both raw and boiled samples were produced by oven-drying and milling, and were then used to measure sugar and cyanide contents. Cyanide content may be underestimated because oven-drying can degrade cyanogens contained in cassava roots; however using oven-drying was necessary in Period 4 for lack of more suitable equipment such as a freeze-dryer.

The expected relationships between quality trait of boiled cassava and biochemical components of raw cassava were established (Table 2) although they need to be consolidated taking into account the number of samples used.

Table 2 Pearson correlation matrix of boiled cassava quality traits and raw cassava biochemical properties (with additional correlations between biochemical components of raw and boiled)

		Biochemical components of raw cassava		
		Sugar (%)	**Cyanide (mg/kg)**	**Dry matter (%)**
Quality traits of boiled cassava	Crumbly	0.030	0.405	-0.111
	Easy to chew	0.420	0.437	-0.051
	Sweet	0.534	-0.806	0.191
	Bitter	-0.493	0.926	-0.120
Biochemical components of boiled cassava	Sugar (%)	0.651		
	Cyanide (mg/kg)		0.955	
	Dry matter (%)			0.557

NB. The number of samples is low, variant from 9 to 6

At CIAT-Colombia, starch was ruled out as determining factor of cooking quality of boiled cassava, as no correlation was found between dry matter (i.e. starch content) and various parameters of cooking quality (figure 11).

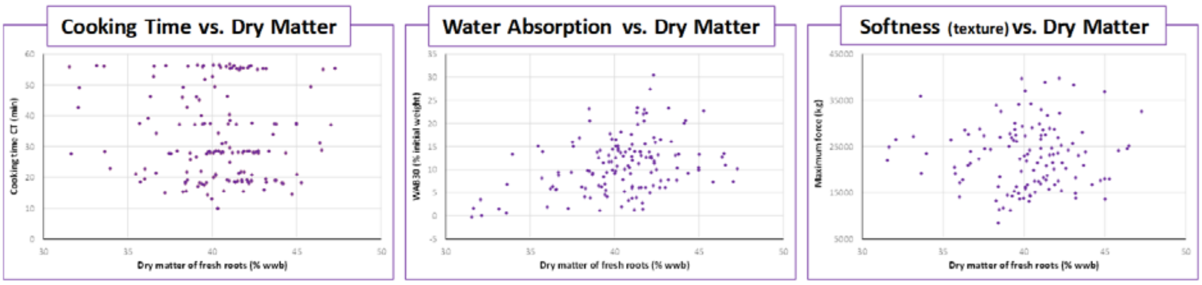


Figure 11 Absence of correlation between dry matter and cooking time, water absorption and softness (by texturometer) in boiled cassava.

Preliminary results point to cell walls components, in particular pectins, as a key factor controlling water absorption and cooking quality.

CIAT conducted experiments in 2021 to quantify the quantity of cell walls an pectins in 4 genotypes of the progenitors collection. A negative correlation was found between pectin content and water absorption. The experiments will be repeated with more genotypes in order to confirm this result. CIAT also tested the effect of various concentrations of calcium Ca^{2+} in the water used to boil a selection of four cassava genotypes, as Ca^{2+} can interact with pectins to strengthen texture. A central composite experimental design was used to investigate the effect of a range of Ca^{2+} concentrations and of boiling times on water absorption and texture (texture-extrusion protocol) of pieces of boiled cassava. The experimental design was implemented on four contrasted cassava genotypes in terms of cooking quality, from short-cooking to long-cooking. Initial results indicate a linear negative correlation between water absorption and Ca^{2+} concentration, thus pointing to a role of pectins in determining water absorption during boiling. In order to confirm these results, experiments will be repeated with more cassava genotypes in Period 5, using higher concentrations of Ca^{2+} in order to identify the limit at which minimum water absorption is reached, for each genotype.

At CIRAD-France, 7 varieties (two roots of each) were waxed and delivered fresh from CIAT. The varieties were selected to cover the range from short-cooking to long-cooking. Raw samples were sliced and sampled as explained in the SOP for texture measurement. One part was freeze-dried and analysed for pectins using the SOP. For the other part, the texture (penetrometry test) was measured on 8-15 cubes/root as detailed in the SOP. The penetrometry firmness ranged from 4 to 10 N, and two classes of samples could be evidenced. Total pectins ranged from 550 to 1100 mg/100g and a significant correlation was evidenced between total pectin content and penetrometry firmness (Figure 12).

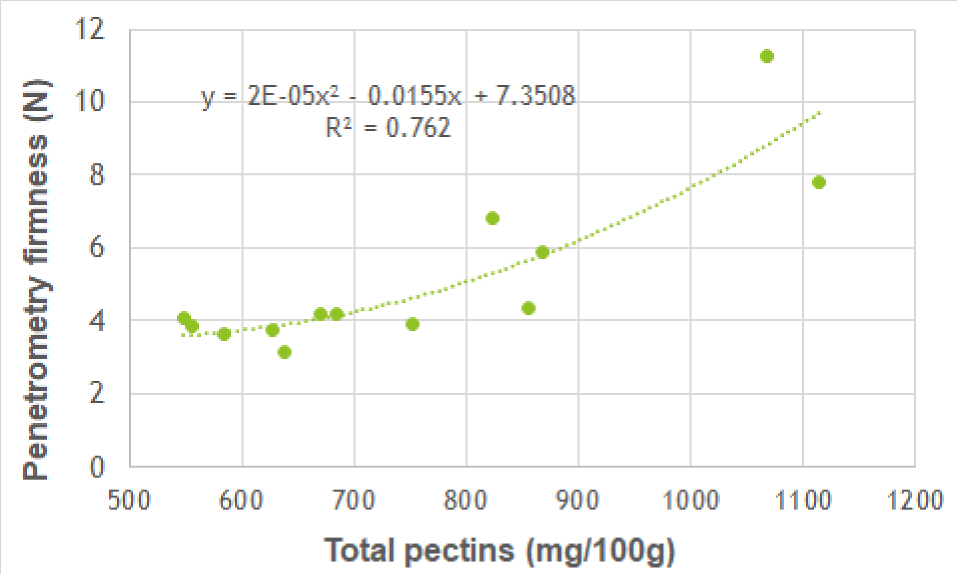


Figure 12 Relationship between total pectins and penetrometry firmness

Synthesis on progress achieved:

No direct significant correlation could be evidenced between dry matter, or starch content, and cooking behaviour. No direct significant correlation could also be evidenced between cell wall content and cooking behaviour, but the effect of pectin level and of Ca^{2+} on cooking behaviour has been evidenced on a small set of samples (7 genotypes); this tends to prove the effect of pectin on the cooking behaviour of boiled cassava. As hypothesized and already demonstrated on previous experiments, a highly significant correlation between cyanide content and bitterness has been evidenced.

Institute-Country	**Targeted Quality Trait / Sensory Descriptor**	**Suspected Responsible Biochemical Attributes of Raw Material**				
		Attribute	**Analysis Method**	**Proof of Concept established? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**SOP available? (Yes/No/ Pending)**
NaCRRI-Uganda	Softness	Starch content	Phenol sulphuric Gravimetric	pending	158	No
	Mealiness	Starch content	Phenol sulphuric Gravimetric	Pending	0	No
	Bitterness	HCN	Picrate Bradbury	No	393	No
	Sweetness	HCN	Picrate Bradbury	No	393	No
	Textural properties	Starch/ Pectin	Phenol sulphuric Gravimetric	Pending	158	No
UAC-FSA-Benin	Sweet taste	Taste	Sugar content	Yes	9 samples * 3 cooking batches * 2 replicates	Yes
	Bitter taste	Taste	Cyanide content	Yes	9 samples * 3 cooking	Pending

Institute-Country	Targeted Quality Trait / Sensory Descriptor	Suspected Responsible Biochemical Attributes of Raw Material				
		Attribute	Analysis Method	Proof of Concept established? (Yes/No/ Pending)	Nb of Analyses performed in 2021	SOP available? (Yes/No/ Pending)
					batches * 2 replicates	
	Crumbly	Texture	Starch content	No	9 samples * 3 cooking batches * 2 replicates	Pending
	Easy to chew	Texture	Dry matter content	Yes	9 samples * 3 cooking batches * 2 replicates	Yes
CIAT-Colombia	Cooking behavior	Dry matter, Starch	SOP for dry matter Tentative enzymatic SOP for starch	No No	93	Yes Pending
	Cooking quality: Water absorption, cooking time	Pectin content and structure	Pectin analysis	Pending	7 genotypes analyzed	Pending
	Cooking quality: Water absorption	Pectin content and structure	Water absorption and texture in presence of Ca^{2+} (increasing concentrations)	Pending	4 genotypes analyzed	Pending
CIRAD-France	Penetrometry test	Total pectin	Colorimetry	Under development	14	Yes

Next steps & activities planned on the establishment of proofs of concept:

A better collaboration between partners will be favoured in period 5 to promote exchanges on procedures for pectins, starch, and cyanide determinations. SOPs are available and will be shared, others should be finalized (starch) or written after exchanges between partners (cyanides).

An increased number of samples will be analysed in period 5 to confirm/improve the correlations between biophysical results and sensory or texture evaluations. Samples will be shared between partners to check analytical results.

At NaCRRI-Uganda, the priority activities for Period 5 are the refinement of laboratory procedures for determination of biochemical traits and their relevance to produce reference information for WP3 activities. The publication of proofs of concepts for cyanogenic potential, starch content and pectin content is also considered.

At UAC-FSA-Benin, due to inadequacy of acid hydrolysis method for starch evaluation, data from this analysis are not integrated. As the team still has samples from the assay conducted in Period 4, they plan to measure starch and amylose contents in raw and boiled cassava samples by enzymatic method and DSC, respectively. In addition, sugars profile will be assessed by HPLC.

At CIAT-Colombia, in period 5, further experiments of boiling cassava in presence of Ca^{2+} will be conducted in order to determine the concentration of Ca^{2+} above which water absorption reaches its minimum ($[Ca^{2+}]_{lim}$), for selected cassava genotypes. The team hypothesizes that $[Ca^{2+}]_{lim}$ of short-cooking genotypes will be lower than that of long-cooking genotypes, because the pectin network of short-cooking genotypes is assumed to be less dense and will be saturated with Ca^{2+} at a lower concentration. Verifying this hypothesis would provide additional evidence of the role of pectins in determining cooking quality of boiled cassava. Also in period 5, samples of freeze-dried fresh cassava pieces and freeze-dried boiled cassava (30 min) will be produced using the RTBfoods progenitors collection, and will be sent to CIRAD for detailed pectin analysis.

At **CIRAD-France**, upcoming activities would be to analyse cassava samples from UAC/FSA and/or CIAT already characterized for texture; to test the effect of drying condition (freeze-drying versus drying at 40-50°C) on pectin analysis and finally to communicate and/or train partners on the SOP for pectin analysis.

refer to the Next Steps paragraphs in previous sections on boiled cassava for more details.

4.1.5 Scientific collaborations between partners

Narrative on collaborations between WP teams working - Describe how the different partner teams have been collaborating (methodology development, share of equipment & facilities, share of SOP, etc.) – The list hereunder is a proposition and can be extended/modified [5-10 lines each].

UAC-FSA – Benin proposed SOPs for preparation, sensory analysis and texture analysis of boiled cassava that have been shared with partners. We received a SOP related to sample preparation and instrumental measurement of texture parameters through extrusion test from CIAT. We also received protocols of SOP for measurement of sugar content from Bowen University. Capacity building activities were carried out by CIRAD (Montpellier, France) (through virtual meeting mainly via skype discussions) in order to strengthen the skills of our research team in terms of sensory and texture data analyses. Bi-monthly webinars initiated by the PMU were great opportunities to interact with others partners involved in the RTBfoods projects

NaCRRI adopted SOPs and comparative procedures from CIAT were undertaken. More work on comparative work and ring tests for attributes related to boiled cassava product will be undertaken.

At CIRAD, **s**amples were provided by CIAT for texture analysis and pectin determinations

4.1.6 Next steps on Boiled Cassava

In Period 5 for WP2, NaCRRI, UAC/FSA and CIAT will focus on expanding the databases of boiled cassava quality traits initiated in Periods 3 and 4. NaCRRI and CIAT will conduct sensory analysis and biophysical analyses of the cassava collections currently in the fields, to be harvested in the course of 2022. The main objective is to deliver by the end of Period 5 databases with enough data (capturing as much as possible the variability of boiled cassava genotypes) to (1) identify correlations between sensory analysis parameters and instrumental parameters, and (2) develop reliable high-throughput prediction by NIRS of functional quality trait such as cooking quality, in addition to composition traits such as dry matter and starch.

The datasets of boiled cassava quality traits thus produced will be formatted according to the standard WP3 template across partners, in order to upload consistent datasets to RTBfoods database, and then to Cassavabase.

For proof of concepts, activities will focus on characterizing pectins in cassava roots, and understanding their role in determining the texture and cooking quality of boiled cassava. Finally, Period 5 will also enable to consolidate laboratory procedures, finalize standard operating protocols (SOPs) that are still pending, and publish the results obtained in the course of the project.

4.2 Gari/Eba

4.2.1 Sensory evaluation in Nigeria

NRCRI and IITA have jointly developed an SOP for the Sensory Evaluation of Eba:

Busie MAZIYA-DIXON, Hakeem OYEDELE, Emmanuel ALAMU, Wasiu AWOYALE, Michael ADESOKAN, Ugo CHIJIOKE, (2021). Sensory Characterization of Eba. Biophysical Characterization of Quality Traits, WP2. Ibadan, Nigeria: RTBfoods Laboratory Standard Operating Procedure, 13 p.

At IITA – Nigeria

The sensory descriptors generated and used by the panellists are listed below:

(i) smoothness, (ii) stickiness, (iii) mouldable, (iv)hardness, and (v) stretchable. Other characteristics are (vi) colour, (vii) taste, and (viii) texture in the mouth, i.e., Fibrousness.

Priority quality traits based on WP1 outputs are as follows:

(i) **Colour**, which relates to the appearance of the Eba. The preferred colour for Eba is a white or butter (yellow) bright colour. Any form of brownish or greyish dull colour or dark colour of the Eba is perceived as unattractive.

(ii) **Swelling volume** of Eba is an additional attribute of high ranking from the WP1 reports for Eba. It refers to the volume increase when preparing Gari into Eba. Consumers like it when this volume increase is maximal.

(iii) Textural attributes of Eba. Consumers identified **hardness/softness** of Eba, **stickiness to the hand**, **mouldability**, **stretchability** of Eba as the essential quality desired in Eba.

At NRCRI – Nigeria

(i) **Colour** (White colour, Grey colour, Cream colour)
(ii) **Taste** (sweet taste. Sour taste)
(iii) **Texture** (smoothness, cohesiveness or moldability, hardness, stickiness, stretchability)
(iv) Fibrouness

Therefore, colour, swelling power and textual attributes are the key traits to be passed on to breeders for integration into breeding priorities.

At IITA, Nigeria – Sensory Evaluation

Twenty-six (26) cassava genotypes of contrasting qualities were evaluated by fourteen (14) trained panelists in Period 4. The 26 cassava genotypes were purposively selected to include contrasting genotypes based on consumer perception of Eba quality. This also included TME 419, used as a control/check. The SOPs developed and validated in Period 3 for sensory texture profile analyses was used to analyze the Eba produced from the 26 cassava genotypes. Sensory descriptors were texture by hand (hardness/softness), adhesiveness (stickiness to the finger), mouldability, and stretchability.

4.2.2 Textural evaluation & correlation with sensory descriptors in Nigeria

At IITA, in Period 3, the first draft of SOP was developed for Textural profile analysis of Eba, and was validated in Period 4 by the CIRAD texture team: ***Busie MAZIYA-DIXON, Michael ADESOKAN, Emmanuel ALAMU, Awoyale WASIU, Ugo CHIJIOKE*** *(2021). Characterization of Texture Profile Analysis of Eba. Biophysical Characterization of* Quality *Traits, WP2. Ibadan, Nigeria: RTBfoods Laboratory Standard Operating Procedure, 13 p.*

A second release of the SOP on textural profile analysis of Gari-Eba has been delivered by IITA & NRCRI in early Period 5, using a bigger panel size to revalidate the SOP: ***Busie MAZIYA-DIXON, Michael ADESOKAN, Emmanuel ALAMU, Awoyale WASIU, Ugo CHIJIOKE, Oluwatoyin AYETIGBO*** *(2022). Characterization of Texture Profile Analysis of Eba (Release 2). Biophysical Characterization of Quality Traits, WP2. Ibadan, Nigeria: RTBfoods Laboratory Standard Operating Procedure, 15p.*

Instrumental Texture Analysis

At IITA Nigeria, twenty-six (26) cassava genotypes of contrasting qualities were evaluated for their textural attributes. The cassava genotypes were of contrasting qualities based on consumer evaluation studies. The key texture descriptors were (i) Hardness, (ii) adhesiveness(stickiness) (iii) Cohesiveness, gumminess, springiness and chewiness. Instrumental analysis was conducted with a TA. TX texture analyzer using a cylindrical compression probe. Instrumental hardness for the 26 clones ranged from 116 to 426.98g with an average of 327.64g, while adhesiveness ranged from -

890.33 to -32.30 and average values of -391.03. Hierarchical clustering analysis of the clones based on the instrumental and sensory textural profile analyses shows a clear classification of the clones (Figure 13). IITA-TMS-IBA30572 and TMS14F1035P0004 were grouped with TME 419 (Check) while TMS13F1307P0016, IITA-TMS-IBA980581, TMS14F1016P000 were clustered in another group. TMS13F1343P002 was grouped with TMS15F1482P0098 and TMS13F1053P0015 respectively. Report from the ranking of the clones from the consumer perception identified IITA-TMS-IBA30572 as the most preferred clones. This clone was grouped with TME 419 from the hierarchical clustering (Figure 13). TMS13F1343P0022 was less preferred, while TMS13F1307P0016 and TMEB3 were considered poor.

The principal component analysis describes the textural attributes that characterize the genotypes into different groups. Springiness and Cohesivesness are the attributes that classified UYT30108, UYT30109, TMS 13F113P0001, while chewiness resilience and gumminess describes the classification of TMS14F1285P0017, IBA30572 and local check TME 419. The cassava genotypes TMS13F134P0004, UYT30104, IBA98051, UYT 30104 AND TMS13F1343P0004 are grouped together and are characterized by Adhesivenss and Hardness respectively (Figure 14)

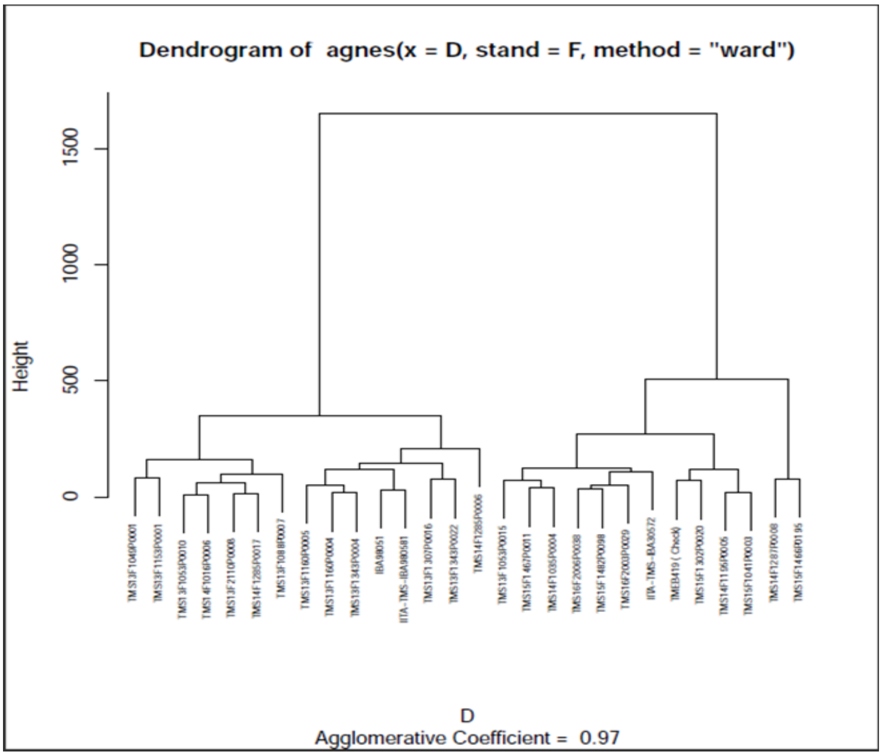


Figure 13 Cluster diagram of cassava genotypes with contrasting quality used for STPA and ITPA

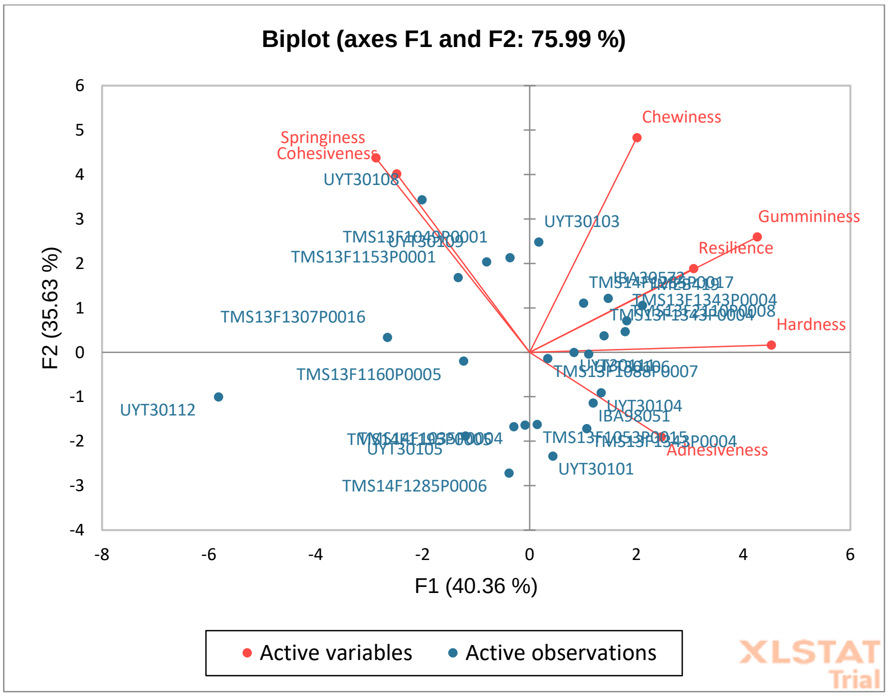


Figure 14 Principal Component Analysis of textural attributes of gari/eba

SOP Validation exercise

At IITA, a validation exercise was conducted in Period 4 by the CIRAD focal point on texture measurements using four contrasting genotypes in terms of consumer perception of eba quality. The test included two replicates per genotype and five measurements per replicate. The textural attributes showed good repeatability with no significant differences between the means of the replicates. ANOVA revealed significant differences between varieties based on all the textural parameters (except Adhesiveness).

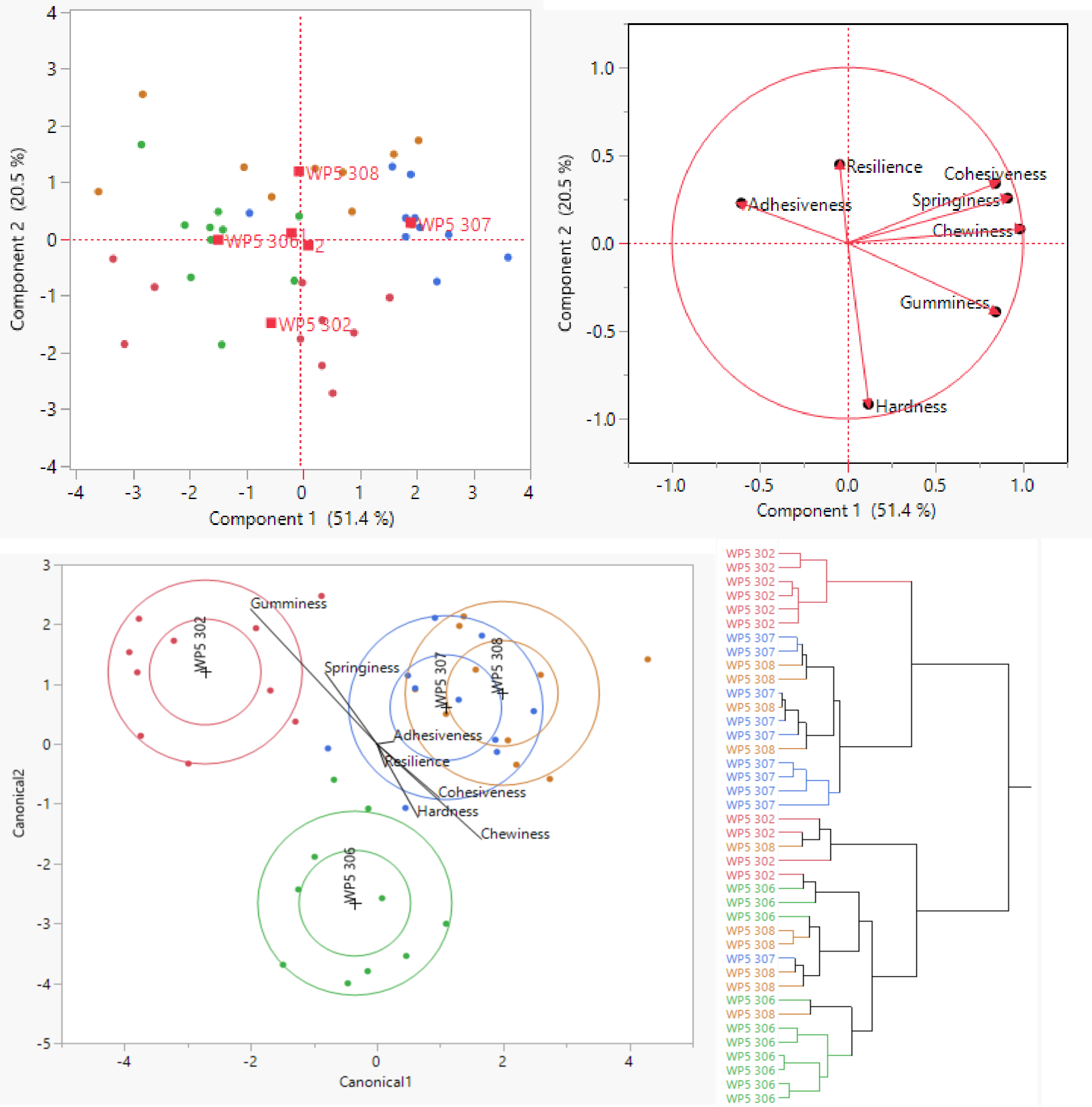


Figure 15 PCA, discriminant and hierarchical analyses for textural attributes of Gari-Eba made from Gari of 4 contrasting cassava genotypes

The four genotypes appeared fairly well separated by PCA (Figure 15), thereby confirming differences among the genotypes according to their textural attributes. The textural quality attributes that contributed most to variation among the genotypes were chewiness, springiness, cohesiveness, and hardness. For discriminance (Figure 15), the varieties were grouped separately in the canonical space, however there was an overlap between WP5 307 and WP5 308 since both genotypes are considered of intermediate quality for making eba. Discriminant analysis shows that cohesiveness, chewiness, hardness, springiness and gumminess carry more weights in discriminating between the varieties. Resilience and adhesiveness had poorer discriminating power.

Pearson Correlation of instrumental texture and sensory profile analysis

Hardness describes how hard or soft the Eba is. It is a vital textural attribute evaluated by both the trained panellists (STPA) and texture analyser (ITPA). By instrumental measurement, hardness is evaluated as the peak force required to compress the Eba dough in the first cycle of the double compression test of STPA. A positive correlation was found between sensory hardness and instrumental hardness, albeit not statistically significant (r = 0.65, Figure 16). This hints at the possibility of predicting sensory hardness with the instrumental method, provided more data is collected in order to improve the correlation. Also, ITPA hardness was significantly correlated with sensory adhesiveness (r = -0.77) and mouldability (r = 0.77), which is the ease at which the Eba dough is moulded by the hand (Figure 16).

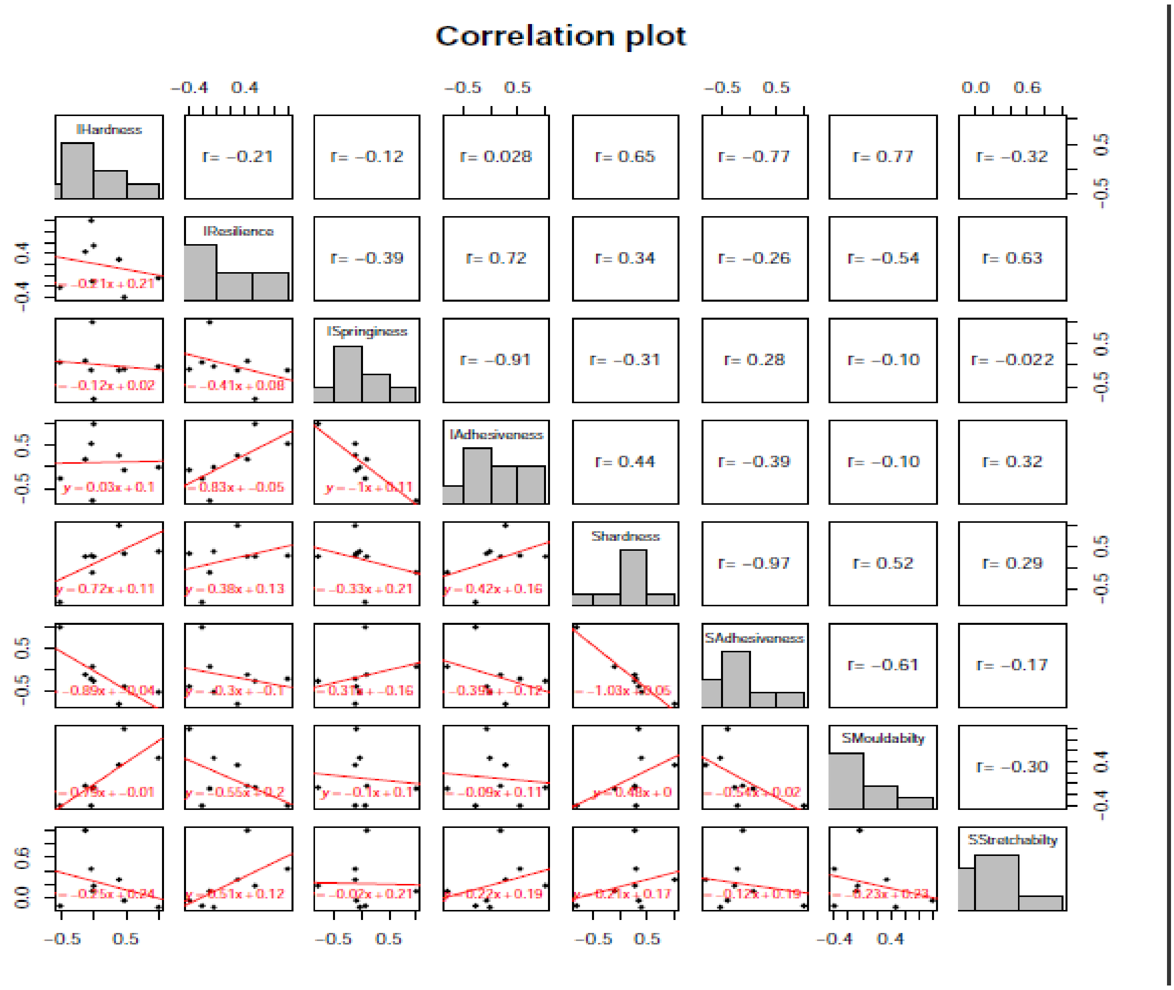


Figure 16 Pearson correlation analysis of Sensory and Instrumental texture properties for Gari-Eba

IITA summary table:

Targeted Quality Trait / Sensory Descriptor	Instrumental Method for Measurement on Final product				
	Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
Hardness	Texture Profile Analysis	Yes	Yes	26 samples	26 clones
Stickiness	Texture Profile Analysis	Yes	Yes	26 samples	26 clones
Resilience	Texture Profile Analysis	Yes	Yes	26 samples	26 clones
Texture attributes (chewiness, gumminess, springiness, hardness and cohesiveness as most discriminatory attributes)	Texture analyser (TPA test)	Yes	Pending	4 contrasting genotypes, 2 replicates per genotype, 5 measurements per replicate	4

NRCRI summary table:

Targeted Quality Trait / Sensory Descriptor	Instrumental Method for Measurement on Final product				
	Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
Colour	Chromameter	Yes	No	None	None
Texture	Instrumental texture analyzer	Pending	No	None	None

Next steps & activities planned to predict key sensory descriptors by instrumental texture:

The focal points (on sensory and texture) recommend in Period 5 for both partners:

- To clearly identify a reduced list of 4 or 5 sensory priority quality traits (PQT).
- To finalize SOPs for texture based on the suggestions provided by CIRAD texture team.
- To collect further texture and rheological datasets. Some measurements can be done at CIRAD when the relevant equipment are not available at the partners'.
- To prioritize the identification of correlations between PQT and instrumental texture parameters.
- To investigate relationships between colour attributes and chromameter parameters.
- To investigate relationships between sweetness / sourness and chemical components (soluble sugars, organic acids, acidity).

4.2.3 Rapid / intermediate kitchen tests to assess the processing & the cooking ability of RTBs

At IITA – Nigeria

The behavioural concern is the colour changes that occur during the processing of cassava roots into Gari. A proof of concept on the effects of polyphenols and polyphenol oxidase (PPO) activity on these colour changes is ongoing. Another critical behavioural concern is the swelling of Gari when it turns into Eba. A rapid kitchen test for Gari/Eba is the measurement of color of Gari using a chromameter. The Eba is prepared as described in the developed SOP for textural quality measurements of Eba. The colour parameters (Hunter Lab) are measured using Chromameter, and the Calibration model was developed for measurements of color (L*, a and b) in fresh blended roots using NIRS.

The colour parameters (Hunter Lab) are measured using Chromameter. Colour has possible interaction with soluble sugars, polyphenols, and polyphenol oxidase (PPO) in the raw roots. However, an ongoing PoC will establish the link between the polyphenols and PPO, and raw root or mash color with the final product (Eba). Ten (10) cassava genotypes *were* purposively selected from the outcome of the WP5 consumer's evaluation of the Eba produced from each of the genotypes on the field. The cassava roots were processed to Gari using the standardized laboratory-scale method. Colour changes at each processing stage were monitored using a chromameter. The link between the colour parameters and the biophysical parameters (PPO, Polyphenols, sugars) will be established. The major challenge with the progress of the PoC is the lack of capacity for the profiling of polyphenol oxidases in the samples. We are discussing with CIRAD to backstop in carrying out the Polyphenols profiling of the samples.

Gari tends to absorb water when soaked in cold or hot water and swells. Swelling of Eba means the volume increase of the Gari when turning it into Eba. A relation with swelling power in hot water was a good indicator. a proof of concept to confirm the correlation between the swelling power in hot water and the volume increase of the Eba during dough formation is being proposed.

At NRCRI – Nigeria

Ease of peeling of cassava roots and ease of sieving dewatered cassava mash for Gari processing

In period 4 NRCRI conducted a preliminary study to show the relationship between dry matter content of cassava root and product yield during unit operations of Gari processing (weight of peel, weight of chaff, the weight of dewatered mash and weight of Gari after toasting). The result of the preliminary study shows that only the weight of chaff is positively related to dry matter content of cassava root ($p<0.05$). The study will, however be repeated with precision in period 5.

10 cassava genotypes at the UYT breeding stage were harvested from the Nextgen cassava breeding programme experimental field at Otobi Benue State, Nigeria. 10kg of fresh cassava/clone was collected from two reps in the field and peeled, and weights of the peeled roots were taken. The peeled roots were washed, grated, bagged and dewatered in a hydraulic press. The weight of the dewatered mash was taken 72h after, dewatered mash was pulverised with hand and sieved with a plastic sieve with even aperture. Chaff or seviette was collected and weighed; sieved mash was toasted manually until properly gelatinised. The Gari samples were allowed to cool and the toasted Gari granules' weight was recorded.

The dry matter content of the samples was determined using the method described below. Clean weighing moisture can was placed in an oven and dried at $80^{0}C$ for about 30min, cooled in a desiccator and weighed (W_0). Approximately 10g of each sample was weighed into the moisture can and reweighed (b). The can with the sample was dried in an oven at $105^{0}C$ for 16 hours, cooled in a desiccator. The procedure was repeated until a constant weight (c) was achieved. Dry matter content was calculated as moisture content subtracted from the 10g of sample

In Period 4, key properties and processing behaviours likely to impact acceptability of new cassava varieties for Gari-Eba were identified as follows (based on experiments with two sets of 10 cassava genotypes each): Concentration of polyphenols and polyphenol-oxidase, dry matter of fresh roots, ease of peeling, ease of sieving the dewatered cassava mash, and swelling of Gari upon addition of hot water for preparation of Eba. Preliminary tests were conducted to confirm the link between these behaviours and the processing quality of Gari-Eba.

In Period 5, full experimental protocols to characterize quantitatively these processing behaviours will be developed, and applied to confirm the effects of the targeted processing behaviours on quality and acceptability of Gari-Eba products, using at least 10 contrasted cassava genotypes.

In period 5 factors influencing the ease of peeling cassava root (such as time of peeling, root geometry), also factors related to the ease of sieving dewatered cassava mash and swelling of Eba will be extensively investigated. SOPs for measuring ease of peeling of cassava root and sieving cassava mash will be developed for breeding programs.

In period 5 NRCRI, Umudike in collaboration with IITA will study the relationship between the total phenol and sugar content of fresh cassava roots and colour of Gari and Eba using the chromameter

4.2.4 Dissection and understanding of key quality traits for Gari/Eba

At IITA – Nigeria

The quality traits of importance for Gari/Eba are Color and textural attributes and swelling volume of Eba. The biochemical characteristics suspected to relate to these priority quality traits are dry matter, starch content, bulk density, swelling power of Gari.

The cassava roots have been processed to Gari using standard protocols while the colour changes were monitored during processing. The fresh roots were freeze-dried and will be sent for polyphenol profiling with CIRAD. Biochemical analysis such as starch content, soluble sugar, amylose, bulk density, pasting property, swelling power, and other functional parameters was conducted on the Gari(milled). At the same time, dry matter and colour were conducted on the fresh raw cassava roots. The relationship between the biochemical parameters and the polyphenols of the fresh roots will be established after the polyphenol proofing analysis is completed.

A PoC to investigate the correlation between the amylose content and textural quality of Eba will be completed in Period 5.

Ten selected cassava genotypes provided by the IITA RTBfoods project (WP4 and WP5) were used to establish the relationship between amylose and textural quality of Eba. The cassava roots have been processed to Gari using standard protocols. The fresh roots and the gari samples were freeze-dried and ready for amylose content analysis. Textural measurements of Eba from all the Gari samples are to be taken. The activity will be completed in Period 5.

SOPs:

i) Develop SOP for product yield measurements will be developed, validated, and delivered to the Breeders at the end of the project life
ii) Development of SOPs for biophysical traits related to swelling of Gari; SOP for Amylose determination, SOP for Water Absorption Capacity, and SOP for Swelling power

Colour: PoC will also be conducted to establish the relationship between colour and sensory measurement for appearance

Texture: Revalidation of SOPs for Instrumental textural profile (ITPA) and sensory texture profile analysis (STPA) will be conducted on 30 selected cassava varieties, and a relationship between ITPA and STPA will be established. A PoC to investigate the effect of amylose content of gari on the textural qualities of Eba will be completed in Period 5.

Swelling volume: A PoC to investigate the correlation between the volume of Eba and swelling power of Gari will be completed in Period 5. A total of 30 cassava varieties of contrasting qualities will process into Eba. The volume of Eba will be compared to the swelling power of the intermediate product for each variety.

At NRCRI - Nigeria

The quality traits of importance for Gari/Eba is Product yield. The biochemical characteristics suspected to relate to these priority quality traits are dry matter

cassava genotypes at UYT stage replicated twice were harvested from Nextgen cassava trial in Otobi, Nigeria. Three roots of different sizes (big, medium and small) were sub-sampled, washed, peeled, chipped. 5g of chipped root was sub-sampled and oven dried at 105°C for 16h until a constant weight was achieved to obtain dry matter content of fresh root. The 10kg root of average size per clone was weighed, tubers were washed, air dried and peeled using a knife, the weight of the peel and peeled tubers were measured on a scale. The roots were washed with clean water, grated dewatered and fermented for 72h, sieved and toasted. Gari yield per clone was calculated to obtain product yield. Dry matter content was determined using approved protocol developed under RTBfoods project.

Perform the biophysical analyses planned in period 5 on 10 (preferably more) and 44 genotypes by IITA and NRCRI, respectively

In period 5 NRCRI. Umudike will collaborate with IITA in the following areas:

Develop SOP for product yield measurements will be developed, validated, and delivered to the Breeders at the end of the project life.

Development of SOPs for biophysical traits related to swelling of Gari; SOP for Amylose determination, SOP for Water Absorption Capacity, and SOP for swelling power.

- **Colour**
    - PoC will also be conducted to establish the relationship between colour and sensory measurement for appearance
- **Texture**
    - Revalidation of SOPs for Instrumental textural profile (ITPA) and sensory texture profile analysis (STPA) will be conducted on 30 selected cassava varieties, and a relationship between ITPA and STPA will be established.
    - These two SOPs will be delivered as a method to be used by breeders:
        - SOP for sensory profile analysis of Eba
        - SOP for instrumental textural profile analysis
- **Swelling**
    - A PoC to investigate the correlation between the volume of Eba and swelling power of Gari will be completed in Period 5. 30 cassava varieties of contrasting qualities will process into Eba, and the volume of Eba will be compared to the swelling power ofthe intermediate product for each variety.
    - The SOP for swelling power measurements will be delivered as a method used by breeders.

IITA summary table:

Targeted Quality Trait / Sensory Descriptor	**Suspected Responsible Biochemical Attributes of Raw Material**				
	Attribute	**Analysis Method**	**Proof of Concept established? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**SOP available? (Yes/No/ Pending)**
Color	Appearance	Chromametric measurement	Ongoing/pending	26 genotypes	Yes
Texture	Hardness, adhesiveness, cohesiveness, resilience	Texturometer	Ongoing	26 genotypes	Yes
Swelling volume	Water Absorbed	Pycnometer	Ongoing	26 genotypes	Pending

NRCRI summary table:

Targeted Quality Trait / Sensory Descriptor	**Suspected Responsible Biochemical Attributes of Raw Material**				
	Attribute	**Analysis Method**	**Proof of Concept established? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**SOP available? (Yes/No/ Pending)**
Swelling power of Gari	Water absorption capacity	Water absorption capacity	No	44 genotypes	Yes

4.2.5 Scientific collaborations between partners

There was an effective interaction between IITA, NRCRI, and CIRAD especially in the area of development and sharing of protocols (SOP for texture analysis of Eba and boiled Yam). Also, there was sharing of information during the RTBfoods In-country Partner's meeting facilitated by IITA.

In addition, CIRAD visited IITA, and CIRAD tested the SOP for instrumental texture and proposed improvements.

4.2.6 Next steps on Gari/Eba

At IITA - Nigeria

In period 5, the SOP validated for instrumental texture measurement will be used to investigate the relationship between the key sensory texture descriptors and the instrumental texture parameters. Any of the sensory descriptors which gives good correlation with the instrumental texture measurement will be measured instrumentally using the correlation equation between sensory and instrumental texture.

In addition, Gari samples from five genotypes were collected by CIRAD focal person for further texture and rheological measurements in Montpellier during period 5, in order to establish discrimination in textural attributes between genotypes, and possibly sensory correlations with rheological properties.

The activities to be completed before the end of period 5 are as follows:

- To complete the proof of concept on the effects of polyphenols and polyphenol oxidase (PPO) in the roots on the enzymatic and non-enzymatic browning of Gari-Eba.
- Conduct PoC to establish the relationship between colour and sensory measurement for appearance (colour) of Eba.
- Conduct Instrumental textural profile (ITPA) and sensory texture profile analysis (STPA) using the developed SOPs and establish the relationship between ITPA and STPA.
- Conduct PoC to establish the relationship between the volume of eba (during preparation) and swelling power (biophysical) of gari.
- Establish the threshold for all the prioritized quality traits for raw, intermediate and final products by WP1.

At NRCRI – Nigeria

- Four cassava genotypes with contrasting Eba quality will be evaluated with the SOP for instrumental texture analysis developed by IITA and NRCRI.
- Sensory evaluation with trained panellist, biophysical, NIRS and consumer studies will also be carried out on these clones.
- Correlation analysis will be conducted to establish relationships between instrumental texture attributes and data generated during sensory analysis.
- In collaboration with IITA, NRCRI Umudike will conduct PoC on relationship between color and sensory measurement for appearance using 18 Nextgen cassava genotypes at late stage of breeding.
- In collaboration with IITA, NRCRI Umudike will conduct PoC on enzymatic and non-enzymatic browning of Gari/Eba using 18 Nextgen cassava genotypes at late stage of breeding.

As IITA and NRCRI shared their SOP, they have common sensory attributes for Eba: smoothness, stickiness, mouldability, hardness, and stretchability for texture traits, white, grey, and cream for color, fibrousness and sweet and sour taste.

CIRAD texture team has contributed to identify technical issues related to the TPA test. The data recovery and automated analysis was improved and the texture test was optimized. Special attention should be given to the analysis of texture data obtained during period 5 to make sure that these data are relevant (in particular to avoid mistakes by the automated macro program). In addition other texture tests such as Lubricated Squeezing Flow, and/or rheometer will be tested to better describe, if possible, the texture of pasted product such as gari/eba.

Consequently, correlations between sensory attributes and textural parameters identified in Period 4 need to be confirmed through a analysis of a higher number of samples in period 5.
The focal point recommends:

- Improve the robustness of the existing SOP for texture: temperature control, define minimum number of replications, etc.
- To perform other texture measurements (Lubricated Squeezing Flow) and/or rheological measurements on a set of IITA samples at CIRAD.
- To investigate relationships between colour attributes and with chromameters parameters.
- Analyse the biochemical properties (starch, polyphenols, soluble sugars, etc.) of eba and/or fresh cassava roots, in order to establish PoC of biochemical bases determining the quality of Eba, that could be used as MTPP or HTPP (by NIRS by WP3) by WP4.

4.3 Fufu

4.3.1 Sensory evaluation in Nigeria

One SOP is available for sensory evaluation of Fufu, Ugo CHIJIOKE, Nwamaka OGUNKA, Oluchi ACHOMWA, Justice OKORONKWO, (2021)**.** Sensory Characterization of Fufu. Biophysical Characterization of Quality Traits, WP2. Umudike, Nigeria: RTBfoods Laboratory Standard Operating Procedure, 18 p.

At NRCRI-Nigeria, the sensory descriptors generated and used by panellists are:

- Colour (white, off white, grey, light grey, light cream, light yellow, yellow, deep yellow)
- Texture (smoothness, stickiness, mouldable, stretchability, hard)
- Aroma (Fufu odour)

Colour and texture of dough have indeed been considered as key priority quality traits during preference surveys carried out in Nigeria.

Nb of samples & Nb of genotypes characterized by the sensory panel in 2021

At NRCRI-Nigeria, no sensory characterization of cooked Fufu was conducted in 2021 because the first draft SOP for instrumental texture analysis is just being validated in Period 5. Sensory and textural analyses for 11 genotypes are currently being conducted in Period 5.

4.3.2 Textural evaluation & correlation with sensory descriptors in Nigeria

The first draft SOP for the textural characterization of Fufu is available and has been reviewed by the focal person on texture from CIRAD.

Nb of samples &Nb of genotypes characterized for texture in 2021:

In the preliminary experiments **at NRCRI-Nigeria,** 4 cassava genotypes (wonono, chenke, 0518, 1368) with contrasting textural properties were used to develop the SOP for instrumental texture analysis of cooked Fufu. A validation exercise carried out by CIRAD focal point on texture considered 3 cassava genotypes (wonono, 0505, 1368).

List of key sensory descriptors for texture (generated & used by trained panellists) to be measured instrumentally:

At NRCRI-Nigeria, the key sensory descriptors for texture to be measured instrumentally are:stickiness, mouldability, stretchability, hardness.

Level of progress in the establishment of a correlation with instrumentally measured parameters:

At NRCRI-Nigeria, no sensory–texture correlation has been established yet in Period 4 (2021). Correlation analysis between the instrumental parameters and sensory descriptors is to be conducted in early Period 5.

The preliminary instrumental texture analysis of Fufu was obtained using four cassava genotypes with five measurements per replicate, and two replicates per genotype. Results (Figure 17) showed good repeatability between replicates within each genotype, good discrimination between the genotypes regarding the textural attributes, and significant differences between the genotypes by ANOVA. The textural attributes resilience, gumminess, springiness, chewiness, hardness and cohesiveness were the most relevant to discriminate between genotypes, based on PCA and discriminant analysis. In contrast, adhesiveness was not discriminating between genotypes.

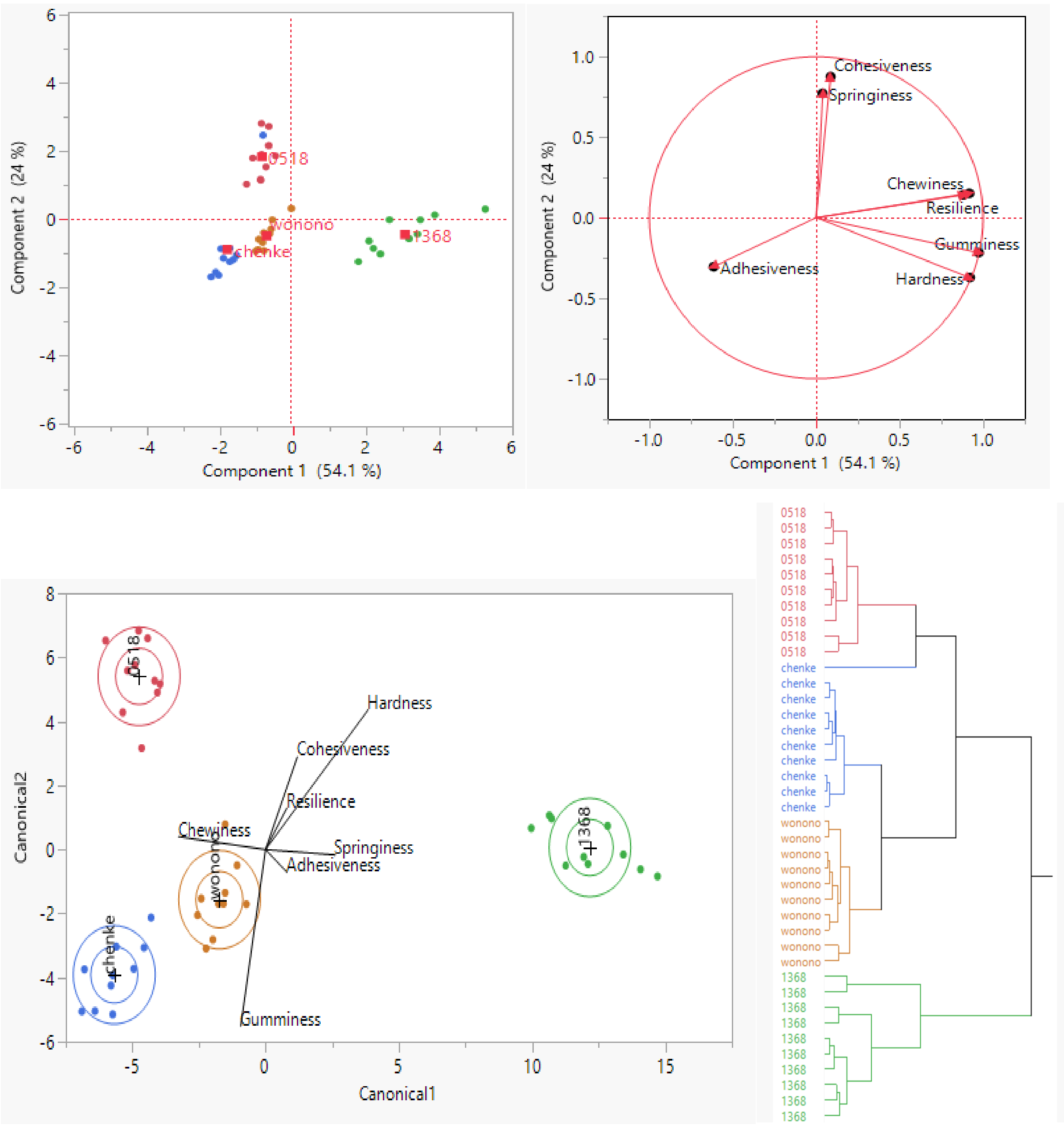


Figure 17 PCA, discriminant and hierarchical analyses for textural attributes of Fufu made from 4 contrasting cassava genotypes.

A validation of the instrumental textural SOP for Fufu was conducted in Period 4 by the focal person for texture in CIRAD (Oluwatoyin Ayetigbo) using 3 cassava genotypes of contrasting textural attributes. Six measurements per replicate, 2 replicates per genotypes were considered. Again, results (Figure 18) showed good repeatability between replicates within each genotype, good discrimination between the genotypes regarding the textural attributes, and significant differences between the genotypes by ANOVA. Generally, adhesiveness and springiness are poor discriminating textural quality attributes between the genotypes. The more discriminatory textural attributes are resilience, chewiness, gumminess, hardness and cohesiveness.

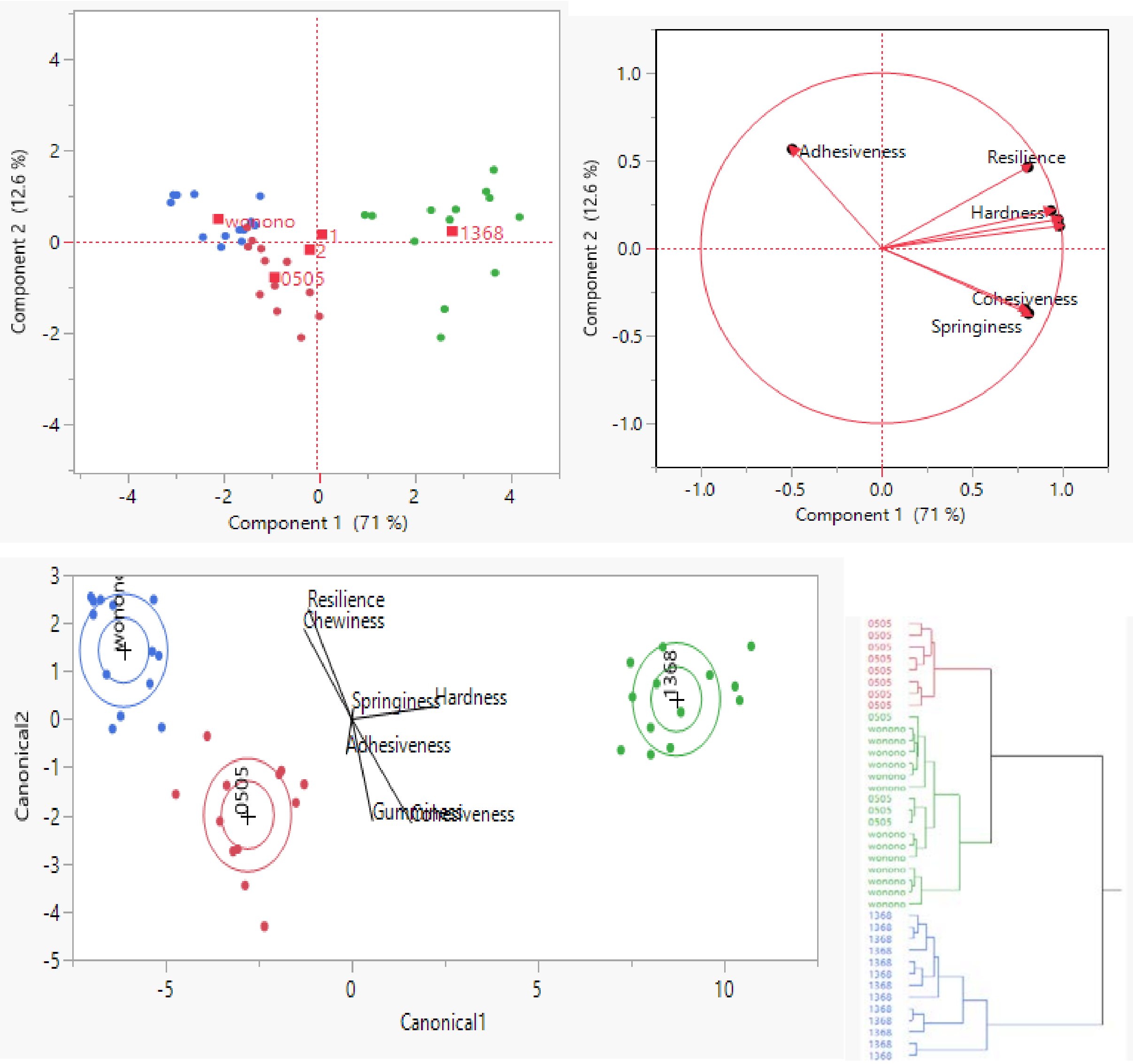


Figure 18 Validation results of PCA, discriminant and hierarchical analyses for textural attributes of Fufu made from 3 contrasting cassava genotypes.

Overall, the common more discriminatory textural attributes between both preliminary measurements and validation measurements were hardness, cohesiveness and resilience.

Synthesis on progress achieved:

NRCRI-Nigeria has developed a textural analysis method. There is a current version of first draft that has been reviewed by focal person on texture from CIRAD. Before validating this method, it will be necessary to conduct sensory analysis to establish correlations with texture parameters.

	Targeted Quality Trait / Sensory Descriptor	**Instrumental Method for Measurement on Final product**				
Institute-Country		**Measurement method**	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones charact erized in 2021**
NRCRI-Nigeria	Colour (white, off white, grey, light grey, light cream, light yellow, yellow, deep yellow)	Visual, and chromameter	Yes	No	None	None
	smoothness hardness, stickiness, mouldability, stretchability, hard	Visual, texture	Yes	pending	None	None
	hardness, cohesiveness, resilience, gumminess, springiness	TPA test	first draft available	Pending	4 genotypes, 2 replicates per genotype, 5 measurements per replicate	4
	hardness, cohesiveness, resilience, chewiness, adhesiveness	TPA test	first draft available	Pending	3 genotypes, 2 replicates per genotype, 6 measurements per replicate	3

Next steps & activities planned to measure key sensory descriptors for texture instrumentally:

Instrumental texture analysis and sensory analysis will be conducted in period 5 using 4 cassava genotypes having contrasting Fufu quality sensory properties. These clones to be evaluated will be preferred and less preferred genotypes identified during WP1 activity 4 and Nextgen Cassava genotype at NCRP breeding stage. Sensory studies will be conducted using approved vocabularies generated which contains the prioritized quality traits identified by WP1. Instrumental texture analysis will be done concurrently with the sensory analysis and correlation analysis will be conducted to establish relationship between the Fufu sensory descriptors and the instrumental parameters.

The focal points on sensory and texture recommend:

- To clearly identify a reduced list of priority quality traits (4-5 PQT).
- To establish correlations between sensory and instrumental textural attributes
- To explore other textural measurement (Lubricated Squeezing Flow), and/or rheometer that could be more relevant to evaluate the textural properties (viscoelastic attributes) of this type of product (pasty).
- To try to find relationships between colour attributes and chromameter parameters

4.3.3 Rapid / intermediate kitchen tests to assess the processing & the cooking ability of Fufu

Rapid kitchen test or intermediate assessment methods investigated:

NRCRI-Nigeria is developing different rapid kitchen tests for the evaluation of retting ability of cassava roots.

- Manual evaluation of rate of softening by processors
- Evaluation of softening by physical measurement with Penetrometer
- Visual evaluation of Foaming/ appearance of bubble on retting water
- Clarity/turbidity of water
- Water pH and titratable acidity

Preparation of cassava roots for retting assessment: 18 different cassava genotypes from regional UYT and 4 varieties identified during WP1 survey were used for the retting ability studies. 4kg weight of uniform sized (medium sized root) freshly harvested cassava roots were weighed from each genotype. The roots were peeled and 3kg of each peeled genotype was weighed into fermenting vessel of uniform size. Equal volume of water was poured into each fermenting vessel containing the roots and was allowed to stand for 72 hours.

Penetration test using hand-held Penetrometer: for each cassava variety, five different roots per genotype were evaluated for Hardness (2 penetrations reading on opposite sides of each root, hence 10 penetrations readings per genotype).

Manual evaluation by champion processors: the four champion processors were given a structured questionnaire to evaluate the progress of retting from day 0 to day 3 of fermentation by hand feel. Hardness of the five roots per clone was evaluated by each processor on a scale of 1 to 5; from 1 = extremely soft to 5 = very hard. By visual observation, rate of bubble formation and water clarity/turbidity were also evaluated and rated on a scale of 1 to 5; from 1 = extremely foamy/extremely turbid, to 5 = no foam/extremely clear.

Evaluation of water clarity using spectrophotometric method: the spectrophotometer was switched on and set to measure transmittance. About 1ml of the fermenting liquor was poured into a cuvette and the transmittance was read. This procedure was carried out every day during the three days of fermentation. The transmittance of water was used as reference.

Synthesis on progress achieved:

At NRCRI-Nigeria, the proof of concept has been established by NRCRI that retting ability of cassava roots can be measured using the hand-held penetrometer. At NRCRI in period 4, the retting ability of 21 Nextgen cassava clones were characterized during Fufu processing using hand-held penetrometer. Significant differences in retting behavior among the 21 clones evaluated at NRCRI were identified with the penetrometer method, allowing a classification into three groups: fast, intermediate and slow retting. The results obtained showed that hand-held penetrometer is effective for clustering cassava genotypes into different categories based on their retting abilities (fast, intermediate and slow retting clones). The study also showed correlations between penetrometer data and processors evaluation of foaming ability and water clarity or turbidity (Fig 21). This implies that evaluation of foaming ability and turbidity can be used as an intermediate throughput method for accessing retting ability of cassava genotypes by breeders.

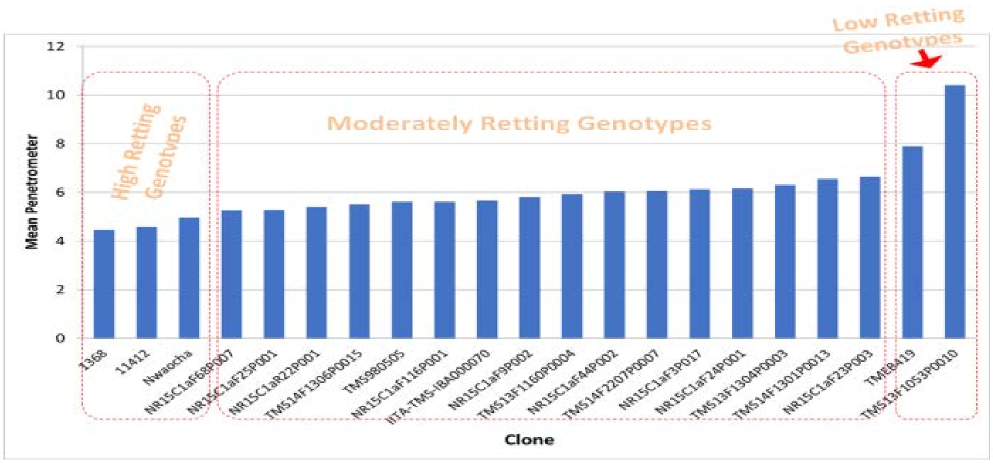


Figure 19 Cluster diagram showing Retting/ softening ability of 17 Nextgen cassava genotypes and 4 cassava varieties with contrasting quality determined with Handheld Penetrometer

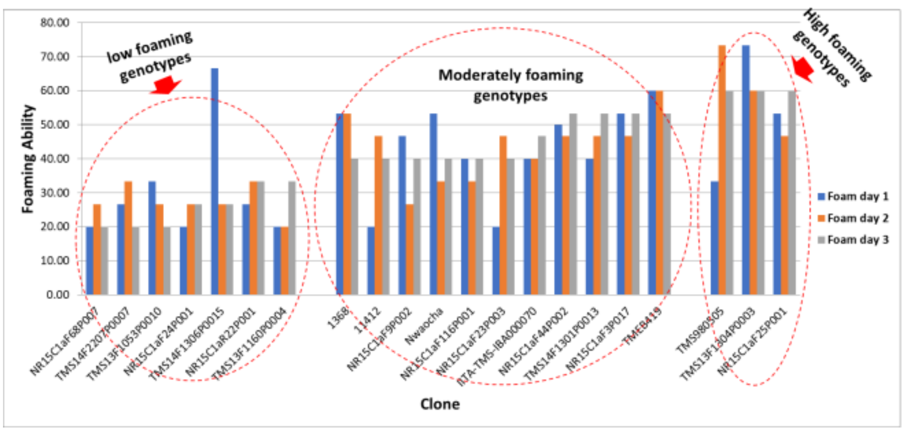


Figure 20 Cluster diagram showing Retting/ softening ability of 17 Nextgen cassava genotypes and 4 cassava varieties with contrasting quality measured by visual observation of appearance of foam/ appearance of bubbles on retting water.

Retting property	meanpen	pen_day_1	pen_day_2	pen_day_3	meanfoam	Foam_day_1	Foam_day_2	Foam_day_3	dispersibility
meanpen	1								
pen_day_1	0.58**	1							
pen_day_2	0.71***	-0.13	1						
pen_day_3	-0.26	-0.72***	0.14	1					
meanfoam	-0.23	-0.29	-0.05	0.24	1				
Foam_day_1	0.12	0.12	0.08	0.09	0.78***	1			
Foam_day_2	-0.35	-0.35	-0.53 **	-0.04	0.83***	0.36	1		
Foam_day_3	-0.42	-0.42	-0.35	-0.2	0.90***	0.52*	0.79***	1	
dispersibility	0.45*	0.55**	0.16	-0.61 **	-0.1	0.22	-0.4	-0.15	1

Figure 21 Pearson correlation coefficients between instrumental (penetrometer) and sensory (visual) attributes of retting ability of cassava roots during Fufu processing

Next Steps in the development of rapid / intermediate kitchen assays for Fufu

In Period 5, **NRCRI-Nigeria** and **CIRAD-France** will collaborate to develop lots of different rapid or intermediate kitchen assays for key quality traits for Fufu. Regarding the assessment of cassava root retting ability, the key priority action points in period 5 will be to complete retting ability evaluation, develop and validate a SOP for retting ability (NRCRI and CIRAD to collaborate). NRCRI shall try to develop a calibration method for pH as an indicator for retting and deliver to breeders.

Regarding the assessment of easiness of peeling of cassava roots, the priority will be to complete the proof of concept on the relationship between root-quality attributes (Root weight, shape (length, circumference and smoothness of the skin) and ease of peeling. (NRCRI-Nigeria and CIRAD-France to collaborate). NRCRI-Nigeria will also develop and validate a SOP for ease of peeling cassava genotypes and deliver to breeders (NRCRI and CIRAD to collaborate).

Regarding the colour of the flesh, NRCRI-Nigeria will develop and validate SOP to monitor colour of root flesh in relation with colour of the dough (NRCRI-Nigeria and CIRAD-France). The teams will collaborate to establish relationships between colour of flesh and polyphenol content (NRCRI and CIRAD). Threshold for colour of root flesh should be established. A validated SOP for measuring colour using chromameter will be delivered to breeders.

Regarding the easiness of sieving, NRCRI-Nigeria will develop and validate a SOP to be delivered to the breeders (NRCRI –Nigeria and CIRAD-France to collaborate).

Regarding the easiness of dough formation, NRCRI-Nigeria and CIRAD-France will work on the characterization of cohesiveness during cooking and will try to develop a SOP for easiness of dough formation.

Regarding the smoothness of the dough, a proof of concept on relationships between smoothness and some biophysical properties of raw material (dispersibility, solubility,dry matter, water absorption capacity, fiber content) will be worked out until validation. NRCRI-Nigeria, CIRAD-France and IITA-Nigeria will also collaborate on a proof of concept establishing relationships between the crude fibre content and the actual fibre obtained after sieving (fibre removal during processing). Validated SOPs on fibre content and water absorption shall be delivered to breeders

Regarding the textural traits of Fufu dough (mouldability, stretchability, hardness, adhesiveness), NRCRI-Nigeria will complete a proof of concept on relationships between cell wall properties and textural properties.

4.3.4 Dissection and understanding of key quality traits for Fufu

Quality trait(s) of focus/investigated:

At NRCRI-Nigeria, research activities in period 4 focused on the retting ability of cassava roots. The biochemical attributes suspected of being involved and analysed are: dry matter content of fresh cassava roots; crude fiber, pectin, starch, amylose and sugar contents of oven dried flour from same cassava roots; pH of fermenting liquor, mash and fresh roots; total titratable acid of fermenting liquor and mash.

pH and titratable acidity of the fermenting water were measured with duplicates for each cassava clone from day 0 to day 3 of fermentation

At CIRAD-France, research activities focused on the identification of biochemical indicators of cassava retting ability. Roots from contrasted cassava genotypes including 2 biofortified ones with yellow pulp (01/0040-27, 01/1797) and 2 varieties genotypes with white pulp (92/0326, LMR) were used. These genotypes were identified based on their softening during retting process measured by penetrometer using a TA-XT2 textural analyzer [1], 01/0040-27 and 92/0326 were softer at 24h after the beginning of the retting process compared to 01/1797 and LMR (figure 22).

[1] Mbéguié-A-Mbéguié, D., Dahdouh, L., Ricci, J. Dufour, D., Fliedel, G. and Tran, T. 2019. Softening measurement of Cassava: Development of a method to evaluate the retting process. Montpellier (France). CGIAR Research Program on Roots, Tubers and Bananas (RTB). RTB Working Paper. No. 2019-1. Available online at: www.rtb.cgiar.org

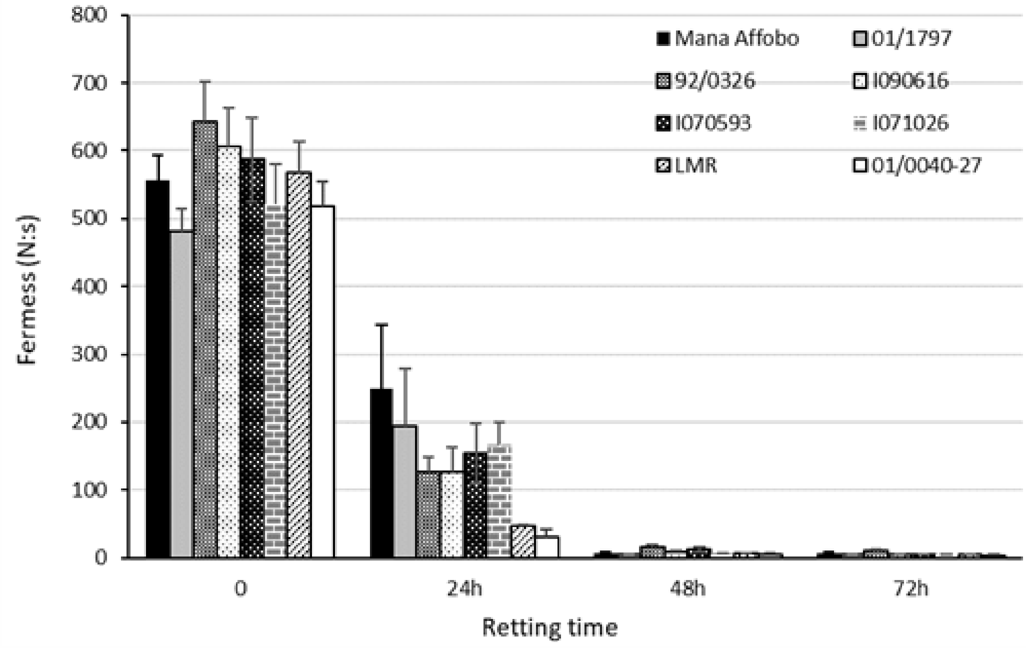


Figure 22 Root softening measurement during retting process of different cassava varieties harvest in Mbalmayo

Cassava roots were peeled, rinsed with water and ret at room temperature in tap water. Root softening was measured by penetrometer using a TA-XT2 textural analyzer. The area under the texture evolution curve was calculated to express root softening in Newton per second (N.s-1).

For each genotype, approximately 30 kg of roots of each genotype were harvested, peeled, washed, divided into 3 batches of approximately 10 kg each, and soaked in tap water for 3 days. Every day, two ret roots were randomly sampled from each batch, frozen, freeze-dried and ground. The resulting flours were used for the biochemical analysis. The following biochemical parameters were targeted: soluble sugar and organic acid measured using the HPLC SOP; amylose content measured using the DSC SOP**; starch content through the measurement of** free glucose and of starch content by HPLC after enzymatic hydrolysis; **starch functional properties** through RVA analysis; **total free and bound phenol content**. RVA analysis was used to assess changes of the starch functional properties of ret cassava flour. 2g of cassava flour was weighed into in a metal RVA canister containing 25 mL of distilled water containing $AgNO_3$, enzyme inhibitor. Viscosity was recorded using the following temperature profile: holding at 50 °C for 1min, heating from 50 °C to 90 °C at 6 °C.min^{-1}, holding at 90 °C for 5 min and then cooling down to 50 °C at 6 °C.min^{-1} with continuous stirring first at 960 rpm for 10 s and then at 160 rpm throughout the rest of the experiment.Four parameters were measured on the visco-amylogram: pasting temperature (PT), peak viscosity (PV), lowest hot paste viscosity or holding strength (HS) and final viscosity (FV). Two additional parameters were then calculated: breakdown (BD) estimated as PV-HS and setback (SB) estimated as FV-HS[2]. The analyses were performed in triplicate and mean values were calculated.

For total free and bound phenol content, a method of extraction of total free and bound phenolic compounds has been tested on cassava. 1g of cassava flour was weighed and homogenized in a methanol/water buffer (80% v/v). The mixture was centrifuged and the supernatant used for determination of free total phenolic acids Folin-Ciocalteu colorimetric method according to the standard protocol ISO 14502-1:2005.For bound phenolic compound, the pellet obtained after centrifugation step was dissolved in 3M HCL buffer, then incubated at 90°C for 20 min. The mixture

[2] Monthe, O. C., Grosmaire, L., Nguimbou, R. M., Dahdouh, L., Ricci, J., Tran, T., & Ndjouenkeu, R. (2019). Rheological and textural properties of gluten-free doughs and breads based on fermented cassava, sweet potato and sorghum mixed flours. Lwt, 101(March 2018), 575–582. https://doi.org/10.1016/j.lwt.2018.11.051

was cooled at room temperature. Extracted and measured phenolic compound was done as described above. A first draft of the corresponding SOP has been proposed but remains to be finalized.

Level of progress in the development of biochemical proof of concept / method to measure the biochemical attributes of raw material

At NRCRI-Nigeria in period 4, the 21 Nextgen cassava clones characterized during Fufu processing using hand-held penetrometer were analysed for dry matter, starch, sugar, pH, water absorption capacity of the fresh root. A correlation was found between dry matter of fresh cassava roots and hardness during retting. No other correlations with composition parameters (sugar, organic acids, starch, amylose) could be evidenced (Fig 23).

Retting property	bulk_density	%dm__f_root	Starch	amylopectin	amylose	crude_fibre	Sugar	cyanide_f_rt
meanpen	-0.11	0.55**	0.3	-0.16	0.16	-0.3	0.3	-0.37
pen_day_1	-0.18	0.73***	0.31	0.02	-0.02	-0.26	0.31	-0.31
pen_day_2	-0.11	0.11	0.16	-0.27	0.27	-0.05	0.16	-0.19
pen_day_3	0.53*	-0.66***	-0.36	0.17	-0.17	-0.15	-0.36	0.2
meanfoam	0.07	-0.36	-0.26	-0.02	0.02	0.13	-0.26	-0.13
Foam_day_1	-0.01	-0.07	0.16	-0.07	0.07	0.14	-0.06	-0.38
Foam_day_2	0.17	-0.46 *	-0.03	0.1	-0.1	-0.09	-0.33	0.16
Foam_day_3	0.02	-0.41	0.24	-0.06	0.06	0.28	-0.3	-0.06
dispersibility	-0.35	0.33	0.38	-0.4	0.4	-0.04	0.43	-0.16

Figure 23 Pearson correlation between retting ability (evaluated by penetrometer and visual evaluation of foam/bubbles) and biochemical properties of cassava roots

In addition, 14 cassava genotypes at UYT stage replicated twice were harvested from Nextgen cassava trial in Otobi, Nigeria. Three roots of different sizes (big, medium and small) were sub-sampled, washed, peeled, chipped. 5g of chipped root was sub-sampled and oven dried at 105c for 16h until a constant weight was achieved to obtain dry matter content of fresh root. 100g of fresh cassava roots were oven dried 45c to constant weight. The dried chipped roots were milled and used for analysis of amylose, water absorption, crude fiber, starch and sugar using SOP validated in RTBfood in NRCRI. Fresh roots were also sub-sampled and freeze dried for pectin analysis and other cell wall component analysis to be conducted in CIRAD.

At CIRAD-France, measurements on the four cassava roots harvested at Mbalmayo indicated that total free phenolic content decreased during retting, and that the roots softened (figure 24A). However, at harvest time, the four genotypes displayed comparable quantity of total bound phenolic compounds suggesting that there is no relationship between the behavior of cassava during retting process and total bound phenol content at harvest. Considering the total free phenol content extracted from root cassava since the harvest time to the end of retting period, the data showed a significant difference between varieties, LMR variety which softens faster and 01/1797 the opposite one presented the lower and higher free phenolic content respectively (figure 24B). No marked changes were observed on bound phenolic content (figure 24C).

No correlation was observed between firmness changes during retting process and sugar, organic acid, starch and amylose content. However, retting process affected starch functional properties parameter including RVA peak viscosity, breakdown and setback (table 3). Because of the pandemic situation that impacted the implementation of the experiments, analysis of the cell wall components as well as the polyphenol profiling were not carried out in period 4 as planned initially. These experiments are planned in period 5.

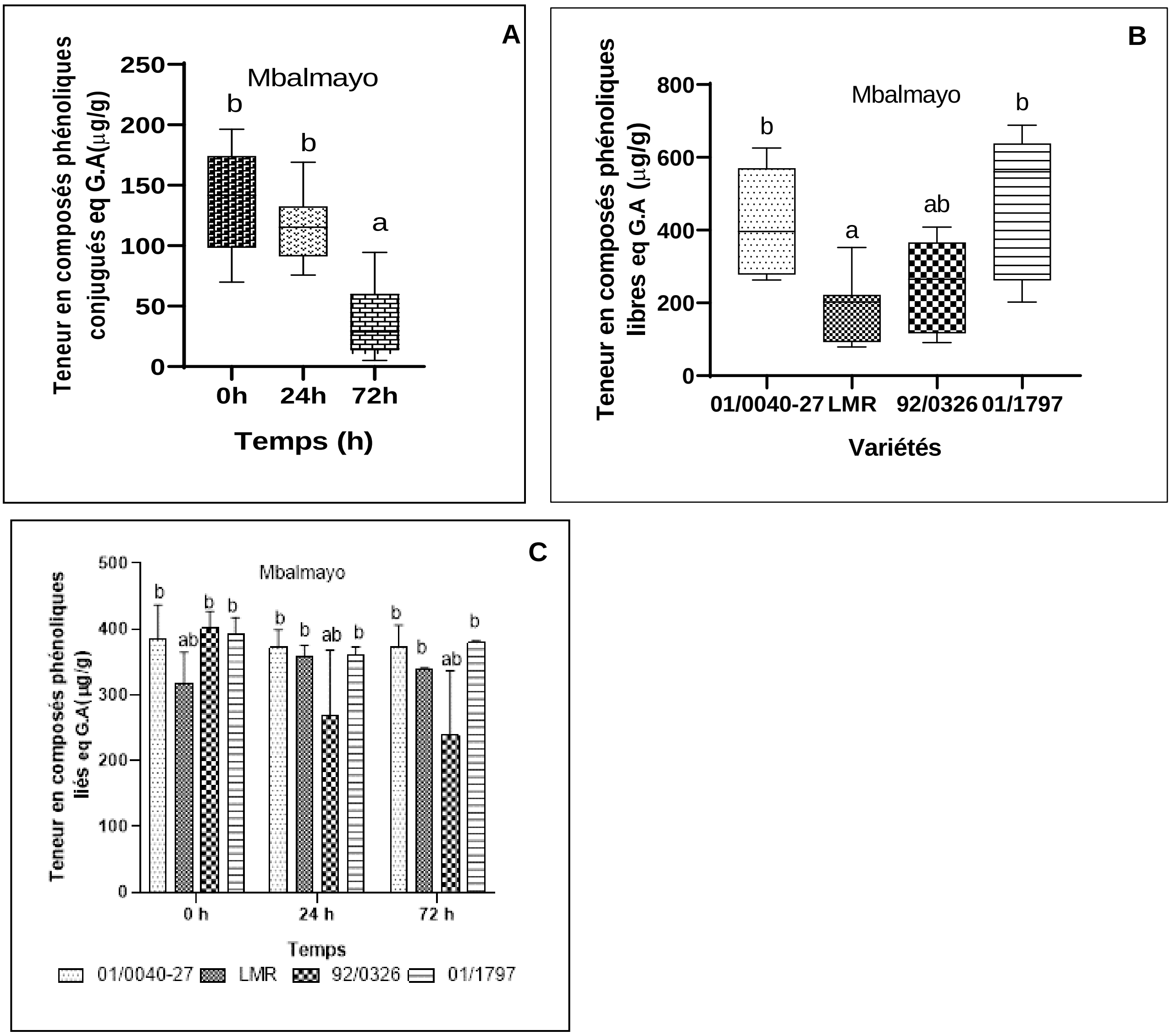


Figure 24 A-B-C Changes of phenolic content of cassava root during retting process.

The content of free phenolic compound was analyzed globally as function of retting time and whatever the variety (A). The total free phenol content extracted from root cassava since the harvest time to the end of retting period is presented in graph B while the change of bound phenolic compound during retting period was presented in graph C.

Variables	Varieties	Retting time		
		0h	24h	72h
PV (cP)	01/0040-27	1737.5±131.26^{ab}	1640.25±365.77^{ab}	1657.75±99.35^{ab}
	LMR	1791.75±231.25^{ab}	1784.75±473.14^{ab}	1822.5±15.77^{ab}
	92/0326	2049.25±48.36^{b}	1785.00±259.23^{ab}	1774.75±138.69^{ab}
	01/1797	1587.00±36.65^{ab}	1329.5±399.03^{a}	1782.75±110.76^{ab}
BD (cP)	01/0040-27	498.25±196.62^{abc}	588.25±310.39^{abc}	304.25±47.06^{ab}
	LMR	409.0±373.55^{abc}	559.75±330.02^{abc}	205.75±40.18^{a}
	92/0326	841.00±13.24^{c}	712.5±250.05^{bc}	283.25±120.82^{ab}
	01/1797	540.00±113.52^{abc}	607.75±25.13^{abc}	210.00±43.5^{a}
SB (cP)	01/0040-27	481.75±54.65^{abc}	463.5±155.8^{ab}	369.5±52.44^{ab}
	LMR	547.75±26.01^{bc}	427.25±168.43^{ab}	455.5±29.03^{ab}
	92/0326	723.25±30.55^{c}	519.5±151.26^{abc}	487.0±67.84^{abc}

Variables	Varieties	Retting time		
		0h	24h	72h
	01/1797	411.25±31.29^{ab}	269.25±199.03^{a}	255.75±126.19^{a}
PT (°C)	01/0040-27	67.00±0.00^{d}	66.87±0.02^{c}	68.30±0.05^{i}
	LMR	66.57±0.02^{a}	66.72±0.02^{b}	66.72±0.02^{b}
	92/0326	66.95±0.04^{cd}	66.92±0.02^{cd}	67.75±0.04^{e}
	01/1797	67.91±0.06^{f}	68.15±0.04^{h}	68.06±0.02^{g}
Peak T (°C)	01/0040-27	89.93±0.02^{e}	88.45±0.04^{b}	90.00±0.00^{f}
	LMR	89.91±0.02^{e}	89.8±0.00^{d}	90.00±0.04f
	92/0326	89.76±0.02^{cd}	89.72±0.02^{c}	90.01±0.04^{f}
	01/1797	89.9±0.00^{e}	76.81±0.02^{a}	90.01±0.02^{f}
Peak time (s)	01/0040-27	518.00	460.00	543.00
	LMR	501.00	495.00	566.00
	92/0326	486.00	473.00	559.00
	01/1797	484.00	344.00	567.00

Table 3 RVA analysis of flour of cassava roots taken at different retting time

PV: Peak viscosity, BD: Breakdown viscosity, SB: Setback viscosity, PT: Pasting temperature, Peak T: Peak temperature. Each value corresponds to the avairage ± standard deviation of the variable concerned. For each square (variable) the means with the same letters are not statistically different at the 5% level (Turkey test)

Synthesis on progress achieved:

Between **NRCRI-Nigeria and CIRAD-France,** several potential indicators of retting ability, a key determinant of the quality of Fufu, were investigated in Period 4, including: Softening during retting (by penetrometer and by manual evaluation by processors); foaming, turbidity and pH of retting water; sugars and organic acids formed during retting; starch content; amylose content; RVA; phenol content. Experiments were conducted on 21 clones at NRCRI and 4 clones at CIRAD.

At NRCRI-Nigeria and CIRAD-France, several analytical results have been obtained, however no correlation between polyphenol profile content, sugars or starch contents and retting abilities could be evidenced.

Institute-Country	**Targeted Quality Trait / Sensory Descriptor**	**Suspected Responsible Biochemical Attributes of Raw Material**				
		Attribute	**Analysis Method**	**Proof of Concept established? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**SOP available? (Yes/No/ Pending)**
NRCRI-Nigeria	Retting ability of cassava root	Hardness	Penetrometer	Pending	65 cassava genotypes	Pending
		Dry matter	Oven drying	Pending	65	Yes
		Crude fiber	Acid/Alkaline boiling	Pending	65	No
		pH	pH meter	pending	65	No
		TTA	Titration method	pending	65	No
		Pectin			21	Pending
		Starch	Acid hydrolysis	pending	65	Yes
		sugar	Acid hydrolysis	pending	65	Yes
CIRAD-France	Retting ability properties	Starch….	Enzymatic method	Yes	48 (4 cassava genotypes)	No

Institute-Country	Targeted Quality Trait / Sensory Descriptor	Suspected Responsible Biochemical Attributes of Raw Material				
		Attribute	Analysis Method	Proof of Concept established? (Yes/No/ Pending)	Nb of Analyses performed in 2021	SOP available? (Yes/No/ Pending)
		Amylose	DSC method	Yes	48 (4 cassava genotypes)	Yes
		RVA	Monthe et al 2019	pending	48 (4 cassava genotypes)	No
		Soluble sugar	Acid hydrolysis	pending	48 (4 cassava genotypes)	Yes
		Organic acids	Acid hydrolysis	pending	48 (4 cassava genotypes)	No
		Total free Phenol content	Methanol extraction	Pending	48 (4 cassava genotypes)	Pending
		Total bound Phenol content	Acid hydrolysis and Methanol extraction	Pending	48 (4 cassava genotypes)	Pending
		Polyphenol profile	HPLC analysis		48 (4 cassava genotypes)	Pending
		Pectin content			48 (4 cassava genotypes)	Pending

Next steps & activities planned on the establishment of proofs of concept:

The objective for period 5 is to test other analytical procedures (pectin, cell wall analysis) to investigate potential links with retting ability. This will need an improved collaboration, discussion, exchanges of samples between partners.

At CIRAD-France in period 5 biochemical analysis of ret cassava genotypes will be completed. Experiments will be carried out at CIRAD Montpellier. The PhD student G. Wakem should also perform laboratory activities at CIRAD Montpellier, for her last doctoral stay at UMR Qualisud (co-funding from the French Embassy and CIRAD). This will be done in partnership with ENSAI (Ngaoundéré University) and Yaoundé University 1. Analyses will focus on other indicators that could putatively influence the retting ability. These include: cell wall component analysis and polyphenol profiling.

4.3.5 Scientific collaborations between partners

NRCRI and CIRAD have been in touch in activities related to retting ability of Fufu and texture analysis of Fufu.

In addition, CIRAD visited NRCRI, tested the SOP for texture and proposed improvements.

4.3.6 Next steps on Fufu

Results obtained in Period 4 on predicting retting ability and Fufu quality will be repeated and consolidated with further experiments and data collection, using the cassava harvests planned in Period 5 by NRCRI and IITA. Additional processing behaviours and quality parameters likely to impact acceptability of new cassava varieties for Fufu will also be investigated, including Ease of peeling, Ease of sieving retted, pressed cassava, Ease of Fufu dough formation, Smoothness of

Fufu dough, Color of the Fufu dough compared to the color of the fresh cassava roots, Fiber content, Water absorption during forming Fufu dough. For the most relevant quality traits predicting Fufu quality and processing ability, SOPs will be established.

In Period 5, sensory analysis will be focused on a reduced list (4-5) of priority sensory quality traits (PQT). Sensory analysis and instrumental texture analysis of at least 11 cassava varieties will be done simultaneously at NRCRI. Correlation analysis between sensory and texture attributes will be conducted, and correlation equations established.

Other textural measurement protocols will be explored, such as Lubricated Squeezing Flow, and/or rheometer that could be more relevant to evaluate the textural properties (viscoelastic attributes) of Fufu and discriminance between varieties.

By end of Period 5 databases will be delivered with biophysical data capturing as much as possible the variability of cassava genotypes for Fufu, enabling (1) to identify correlations between sensory analysis parameters and instrumental parameters, and (2) to develop high-throughput prediction by NIRS of at least composition traits such as dry matter, and possibly also functional quality traits of Fufu. The datasets of Fufu quality traits will be formatted according to the standard WP3 template with the help of the new RTBfoods data manager Amos Asimwe, in order to upload consistent datasets to RTBfoods database, and then to Cassavabase.

For proof of concepts, activities will focus on developing rapid or intermediate throughput methods to characterize retting ability, such as texture analysis or penetrometry, pH (etc.) to deliver to breeders. The role of fibers, cell walls and pectins in determining retting ability and Fufu dough characteristics, such as smoothness, mouldability, stretchability, hardness, adhesiveness, will also be investigated. Period 5 will also enable to consolidate laboratory procedures, finalize standard operating protocols (SOPs), and publish the results obtained in the course of the project.

Please refer to the Next Steps paragraphs in previous sections on Fufu for further details.

Country	Institute	Product Profile	Activity Description	Nb of Genotpyes Characterized	Nb of Genotpyes Characterized	Nb of Genotpyes Characterized	Deliverable	Accountable Person for Delivery
Nigeria	NRCRI AND CIRAD	Fufu	1. Complete proof of concept on the relationship biophysical properties of cassava roots and retting ability of cassava genotypes	18	18	5	Genotypes with good sensory traits selected	Ugo Chijioke and Didier Mbeguie
Nigeria	NRCRI	Fufu	2. Validate result of relationship between sensory properties and instrumental texture attributes of Fufu	18	18	5	Establish relationship between sensory and instrumental textural properties of cooked Fufu.	Ugo Chijioke
Nigeria	NRCRI	Fufu	3. Determine the relationship between retting ability of Nextgen cassava genotypes at early breeding stage and texture attributes of cooked Fufu	43clones x 2 location (40 improved genotypes and 3 checks)	43 clones x 2 location (40 improved genotypes and 3 checks)		Classify the different genotypes into 3 categories namely; Fast retting, intermediate retting and slow retting.	Ugo Chijioke
Nigeria	NRCRI	Fufu	4. Proof of concept between colour of cooked Fufu ,tannin con tent of fresh cassava roots and colour of fresh cassava roots .				To obtain threshold of tannin for good, intermediate and poor cassava genotypes with respect to colour Fufu mash and cooked Fufu	Ugo Chijioke
	NRCRI		5. Consumer studies on cooked Fufu in Nigeria	200 cassava genotypes from Nextgen breeding population			Identify cassava genotypes that have key priority traits required by consumers	Tessy Madu
			6. Conducting QTL and heritability studies on KPQ traits on cooked Fufu					Chiedozie Egesi, Damian Njoku and Lydia Ezenwaka

4.4 Attiéké

4.4.1 Sensory evaluation in Côte d'Ivoire

One SOP for sensory characterization of Attiéké was developed by CNRA-Côte d'Ivoire and has been approved.

The sensory descriptors generated and used by panellists are:

- Appearance: orange color, Brightness, Homogeneity of grains, Size of grains, Presence/absence of fibers, Presence of lumps
- Texture by hand: Firmness, Stickiness, Cohesiveness, Modellable
- Texture in mouth: Granulosity (granularity), Masticability, Mouth filled sensations
- Taste/ Sensation: Sweet, Sourness, Fermented, Taste of Red Palm oil, Undesired taste
- Aroma/Odor: Attieke Aroma, Red Palm Oil, Sourness, Off-odours

Indeed, most of them have been identified as key priority quality traits during preference surveys in Côte d'Ivoire: appearance (brightness, absence of fibers); texture by hand (stickiness, cohesiveness, Mouldable); aroma/odour (Attiéké aroma); taste/ Sensation (Sweetness, Sourness).

Nb of samples & Nb of genotypes characterized by the sensory panel in 2021

At CNRA-Côte d'Ivoire, six varieties (Bocou 2, Yace, Yavo, I083774, Agbablé, Boucou 6) were characterized by sensory analysis in Period 4. Key results will be reported in Period 5.

Attiéké being a granular product, no instrumental texture protocol was available in Period 4 for texture characterizations.

Next steps & activities planned to measure key sensory descriptors instrumentally:

The focal points for sensory and texture at CIRAD recommend:

- On texture attributes: As Attiéké is a granular product, it would be relevant that CNRA prospects which textural measurements are used for similar products like the semolina of couscous, in order to develop a specific SOP for attiéké texture evaluation.
- On colour: Try to find relationships between brightness and chromameter parameters
- On taste: try to find relationships between sweetness / sourness and sugar content (or total soluble solids), organic acids and acidity.

4.4.2 Rapid / intermediate kitchen tests to assess the processing & the cooking ability of Attiéké

CNRA-Côte d'Ivoire has just completed WP1-Step3 'Participatory processing diagnosis' which highlighted that the fermentation ability of cassava roots is a key aspect for processing ability. Processors use boiling or roasting of sample pieces of cassava as an indicator of fermentation ability: Good-cooking varieties, i.e. varieties that become friable in a short time during cooking, ferment better than non-friable ones. Consequently, processors will increase the quantity of ferment starter when they encounter poor-cooking varieties, in order to still achieve the desired quality of fermentation. CNRA-Côte d'Ivoire plans to investigate the fermentation ability of roots and possibly link it to sensory and consumers testing scores.

4.4.3 Dissection and understanding of key quality traits for Attiéké

Quality trait(s) of focus/investigated:

At CNRA-Côte d'Ivoire, research activities focused on understanding sweetness, sourness, colour and texture. Biochemical attributes suspected of being involved are: reducing sugars, acidity, crude fibers and starch contents. No analyses have been performed so far but some could be performed in period 5, to study functional properties of cassava flour, starch components and starch properties of cassava.

Next steps & activities planned on the establishment of proofs of concept:

It could be interesting to study the correlation between starch properties, pectin and cohesiveness of grains and swelling power; the correlation between starch composition and easiness to roll grains; and the correlation between sensory evaluation and instrumental (chromameter) evaluation of color and brightness.

	Targeted Quality Trait / Sensory Descriptor	**Instrumental Method for Measurement on Final product**				
		Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
CNRA-Côte d'Ivoire	Sweetness	Reducing sugars	yes	pending	2	
	Sourness	pH, Titrable acidity, organic acid	yes	pending	2	
	Color brightness	Colorimetry	yes	pending	2	
	Presence of Fibers	Crude Fiber, Cellulose	No	pending	2	
	Cohesiveness	Starch components et properties	pending	pending	Components (2) Properties (0)	
	Stickiness			pending		
	Moldability			pending		

4.4.4 Next steps on Attiéké

Period 5 will be devoted to correlating the sensory attributes of colour and taste with chromametric and biochemical measurements. For texture attributes, as Attiéké is a granular product, it would be relevant to prospect which textural measurements are used for similar products like the semolina of couscous in order to develop a specific SOP for attiéké texture evaluation.

4.5 Boiled Yam

4.5.1 Sensory evaluation in Benin and Nigeria

One SOP is available: Laurent ADINSI, Noël AKISSOE, (2021). Sensory Characterization of Boiled Yam. Biophysical Characterization of Quality Traits, WP2. Cotonou, Benin: RTBfoods Laboratory Standard Operating Procedure, 13 p.

It is used by 5 laboratories: UAC-FSA-Benin, NRCRI-Nigeria, IITA-Nigeria, INRAe-France and CIRAD-Guadeloupe with little adjustments

The sensory descriptors used for sensory evaluation are:

1. Colour (White, off white, light grey, yellow, light yellow, brown, purple)
2. Texture by hand (Sticky to the finger, hard to break)

3. Texture in the mouth (Easy to chew, crumbly/friable)
4. Odour (Boiled Yam aroma)
5. Taste and after taste (sweet taste, Bitter taste, Bitter after taste, tasteless)

Key priority Quality Traits at UAC-FSA-Nigeria are that must absolutely be passed on to breeders for integration as breeding priorities are **white colour, crumbly, easy to chew and sweet taste**.

Key priority Quality Traits at NRCRI-Nigeria are: Sweet taste, attractive creamy white or yellow colour, softness after boiling, good Yam aroma.

Key priority Quality Traits at IITA-Nigeria are: colour, textural attributes, cooking time. Ease of cooking is one of the essential attributes highlighted by the consumers. They prefer clones that cook quickly to save time and resources.

Nb of samples & Nb of genotypes characterized by the sensory panel in 2021:

At UAC-FSA-Benin, 15 samples from 5 contrasting varieties (Ala, Dèba, Gnidou, Kpètè and Laboko landraces) were cut into three sections (proximal, central and distal) have been evaluated by the sensory panel in 2021.

At NRCRI- Nigeria, due to Covid-19, no sensory activities have been performed in 2021. This is to be conducted within the first quarter of 2022 due to the growth cycle of Yam, having completed cross lab validation of texture analysis of boiled Yam at NRCRI by WP2 texture focal person from CIRAD.

At IITA-Nigeria, 16 contrasting genotypes of Dioscorea rotundata were evaluated for sensory profile analysis using 14 trained panellists in 2021.

Key results & graphs:

At UAC-FSA-Benin, the interaction between Yam variety and the cutting section on the sensory descriptors of boiled Yam was significant for white colour, purple colour, sticky, fibrous, granular, sweet taste and bitter taste. Contrary, no significant interaction effect was evidenced for the descriptors brown colour, presence of black dots, hard to break, crumbly, easy to chew, Yam odour and bitter aftertaste. Hence, the effect of Yam varieties and cutting sections on these descriptors were explored separately. Considering the effect of Yam varieties, the five varieties showed significant differences ($p < 0.05$) for focused descriptors. Therefore, the effect of cutting sections of focused descriptors was assessed for each Yam variety separately. The cutting section effected the presence of black dots, the easiness to chew of Laboko variety, and the hardness to break of Kpètè variety. The distal section of the Laboko variety had more black dots and was easier to chew than the central and proximal sections. However, the ease of chewing and the presence of black dots at the central and distal sections are similar for this variety. The central section of Kpètè variety was less hard to break than the proximal and distal sections, while the distal section was the hardest section.

A principal component analysis (Figure 25) revealed three major groups of boiled Yam samples with specific sensory attributes. The first group is represented by the three sections of Ala and Laboko varieties which are characterized by the descriptors white colour, sticky, crumbly, easy to chew, Yam odour and sweet taste. The second group is represented by the three sections of the variety Kpètè which are characterized by the descriptors brown colour, purple colour and granular. The third group consists of the three sections of Gnidou and Dèba varieties, characterized by the descriptors, presence of black dots, hard to break, bitter taste and bitter aftertaste. These varietal differences have been reported by findings of WP1, which reported that Laboko and Ala varieties were rated as very good quality by consumers. In contrast, the Gnidou and Déba varieties are classified as intermediate and the Kpètè variety is rated as poor quality. Therefore, descriptors associated with good quality varieties can be considered as preference descriptors and those associated with Kpètè as descriptors that can be a source of rejection by consumers.

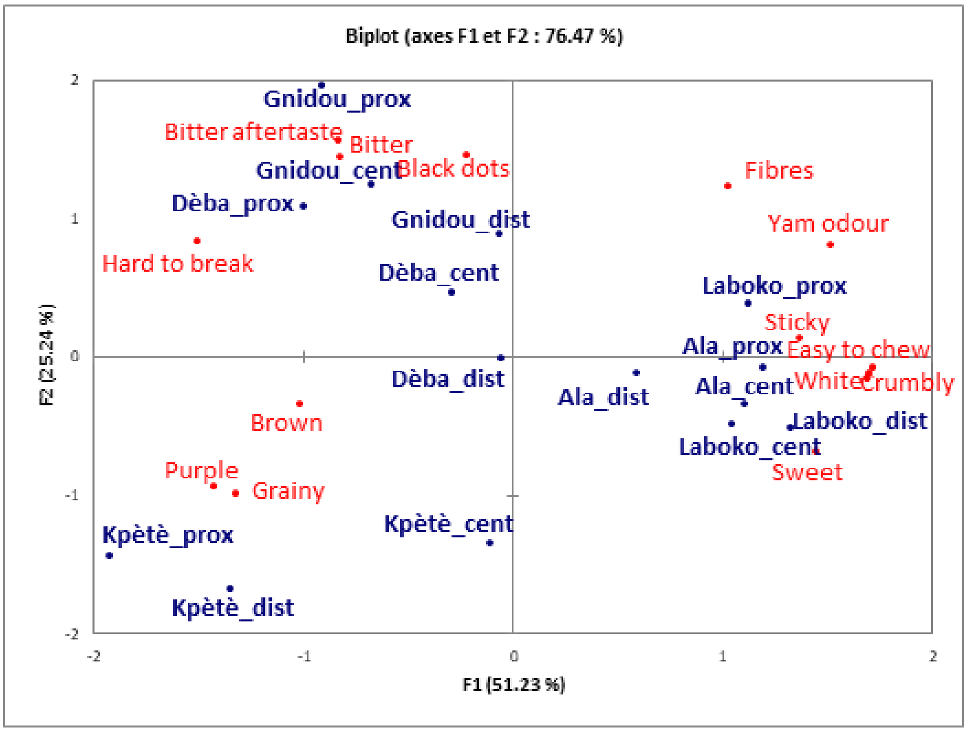


Figure 25 Sensory mapping by PCA of the boiled Yam samples from varieties (Ala, Dèba, Gnidou, Kpètè, Laboko) and cutting sections: proximal (prox), central (cent), distal (dist)

At IITA, Nigeria: 16 Yam varieties were evaluated using 14 trained panellists for sensory analysis. The identified sensory descriptors for boiled Yam were color, taste, hardness/softness, stickiness to the hand, and presence of black spot. Boiled Yam pieces from each variety were served in duplicate to the panellists at a serving temperature of 45^{0}C. The principal component analysis(PCA) (Figure 26) shows three classifications of the boiled Yam samples viz-a-viz the sensory descriptors. The Yam clones; TDr 1400359, TDr 1401419, TDr 1400159 and TDr 1100180, were characterized by taste, color and stickiness to the hand. Also, ease of chewing was the descriptor that explains the classification of TDr 1100128, TDr 1100055, TDr 1000021 and TDr 0900135 respectively. The local checks, TDr Meccakusa and TDr Ojuiyawo were grouped with TDr 1401220, which was identified to be a good variety from the consumer evaluation of the Yam clones. Hardness is another sensory descriptor that describes the third group of Yam varieties, namely TDr 1400158 and TDr 1401161.

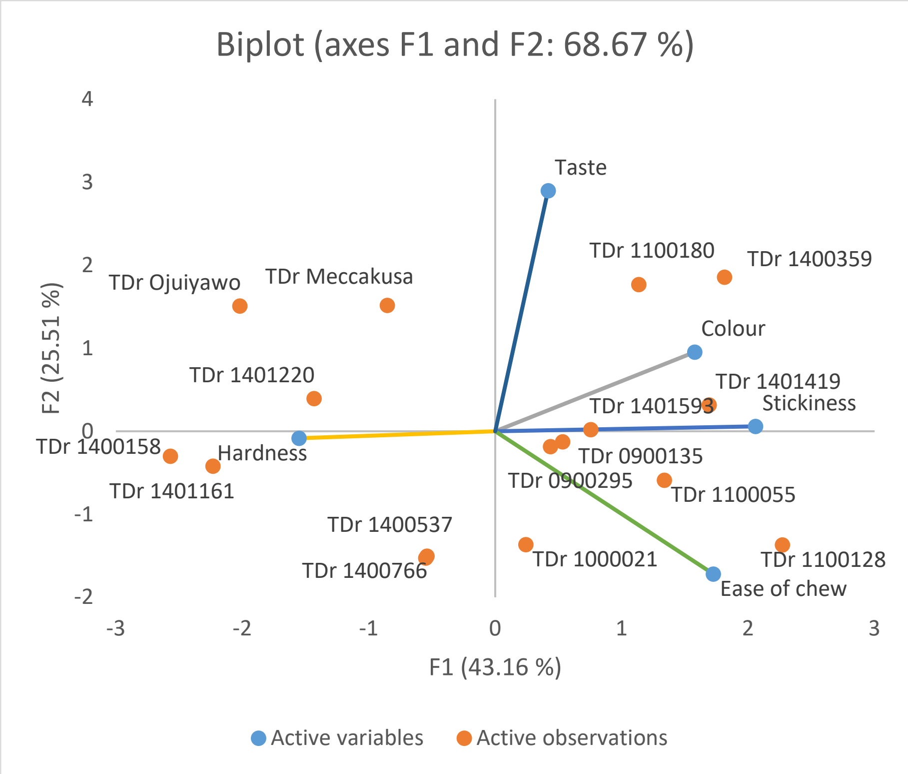


Figure 26 PCA of sensory analysis of the boiled Yam from 16 D. rotundata

4.5.2 Textural evaluation & correlation with sensory descriptors in Benin, Nigeria, France-Guadeloupe

One SOP for Sample Preparation and Texture Analysis of Boiled Yam through penetrometry, extrusion, and TPA, is available; it was developed by UAC-FSA and validated by CIRAD texture focal point:

All institute partners are using this SOP within the RTBfoods framework (UAC-FSA-Benin, NRCRI-Nigeria, IITA-Nigeria, INRAe-France/Guadeloupe and CIRAD-France/Guadeloupe) with some bit of adjustments regarding sample preparation and analyses (TPA, penetrometry, extrusion).

Nb of samples & Nb of genotypes characterized for texture in 2021:

At UAC-FSA-Benin, 15 samples differed by the variety (Ala, Dèba, Gnidou, Kpètè and Laboko) and the cutting sections (proximal, central and distal) were used for textural characterization of boiled Yam.

At NRCRI-Nigeria, 3 Yam varieties with contrasting boiled Yam (Good, intermediate and poor) attributes were characterized in 2021. 6 measurements per replicate and 2 cooking replicates were considered.

Texture analysis of 12 other genotypes (5 alata, 5 rotundata) and 2 landraces harvested in December 2021 from the African Yam project will be completed within the first quarter of 2022.

At IITA-Nigeria, 16 clones of Dioscorea rotundata; TDr 1401161, TDr 1401220, TDr 1100180, TDr 1400158, TDr 1100128, TDr 1400537, TDr 0900135, TDr 1401419, TDr 1400359, TDr 0900295, TDr 1401593, TDr 1000021, TDr 1100055 and TDr 1400766 including two local checks namely; TDr Ojuiyawo and TDr Meccakusa were characterized for their textural attributes. The sample panel consisted of clones with contrasting cooking qualities, and the samples were divided into three sections (proximal, middle and axial). Each section of the Yam was cut into a regular shape using a stainless-steel plunger with a dimension 6cm x 2 cm. A compression/extrusion test was conducted on each sample using a five-blade Ottawa cell plunger mounted on the TA.XT texture analyser. The texture attributes were hardness and the energy expended during extrusion.

In addition, at IITA, four varieties of Yam (TDr1401220, TDr1401593, TDr1400158 and Meccakusa) were steamed (to simulate boiled Yam with more controlled conditions) and characterized during a mission of CIRAD expert with IITA, in order to assess the repeatability and discriminance of the texture SOP of boiled Yam. The experiment included two cooking replicates per variety, and six measurements per replicate. Only central sections of the Yam tubers were used, from which cubes of 22 mm side were cut. Measurements of texture were carried out at 45 ^{o}C at two different cooking times (20 min and 38 min) to determine if cooking time influences texture.

At CIRAD-Guadeloupe, 52 varieties from *D. Alata* were harvested and studied in Guadeloupe-CIRAD. SOP for texture of boiled Yam (penetrometry and TPA, UAC/FSA) was used after slight modifications. 3 tubers per variety were sampled. Each tuber was divided, as far as possible, into 3 sections (proximal, distal, central). Each section was used to produce 3 cubes of 23 mm edge for penetrometric measurements, and 3 additional cubes were sampled at the central section for TPA analysis. The cooking time was 15 min, followed by a cooling time of 7 min (corresponding to a cube temperature of 45°C).

List of key sensory descriptors for texture (generated & used by trained panellists) to be measured instrumentally:

At UAC-FSA-Benin, the key sensory descriptors for texture to be measured instrumentally are **sticky, hard to break, crumbly, and easy to chew.**

At NRCRI-Nigeria,the key sensory descriptors for texture to be measured instrumentally aretexture in the hand (**Sticky to the finger, hard to break)** and texture in the mouth **(Easy to chew, crumbly/friable)**

At IITA-Nigeria, the vital sensory *descriptors* for texture measured instrumentally were (i) **texture by hand (hardness)** and (ii) **ease to chew** (energy required to compress the boiled Yam through the extrusion blade of the Ottawa cell.

Level of progress in the establishment of a correlation with instrumentally measured parameters:

At UAC-FSA-Benin, a Pearson correlation test was performed to assess the relationship between the sensory texture descriptors and the instrumentally measured parameters for the 15 boiled Yam samples (Table 4). Regarding penetrometry test, significantly positive correlations were found only between the descriptor hard to break and hardness (r = 0.677) and energy (r = 0.760). No TPA parameters were significantly correlated to sensory descriptors.

Table 4 Pearson correlation matrix of texture sensory descriptors and uni-axial texture parameters of boiled Yam

Uni-axial texture parameters		**Sensory texture descriptors**			
		Sticky	**Hard to break**	**Crumbly**	**Easy to chew**
Penetrometry test	Hardness (N)	-0.357	**0.677**	-0.376	-0.372
	Energy (N)	-0.381	**0.760**	-0.445	-0.478
TPA test	Hardness (N)	-0.360	0.113	-0.071	-0.275
	Cohesiveness	-0.100	0.172	-0.037	-0.009
	Gumminess (N)	-0.441	0.183	-0.156	-0.252
	Springiness (N)	-0.141	-0.143	0.261	0.354
	Chewiness (N.mm)	-0.369	0.052	0.015	-0.034

Bold Pearson correlation coefficient are significant ($p<0.05$).

At NRCRI-Nigeria, currently, no correlation between instrumental and sensory textural attributes has been established because no sensory analyses have been performed in Period 4. Although preliminary data was generated for texture analysis of 3 Yam genotypes collected from the African Yam breeding project. Results (Figure 19) showed good repeatability between replicates within each genotype, but no discrimination regarding the textural attributes, and no significant differences between the genotypes by ANOVA, except for adhesiveness. This could mean instrumental TPA may not be the best textural measurement technique to discriminate between genotypes of contrasting textural attributes. This hypothesis should be confirmed with complementary trials to make sure that the low discriminating ability of TPA is not due to an overcooking of Yam samples.

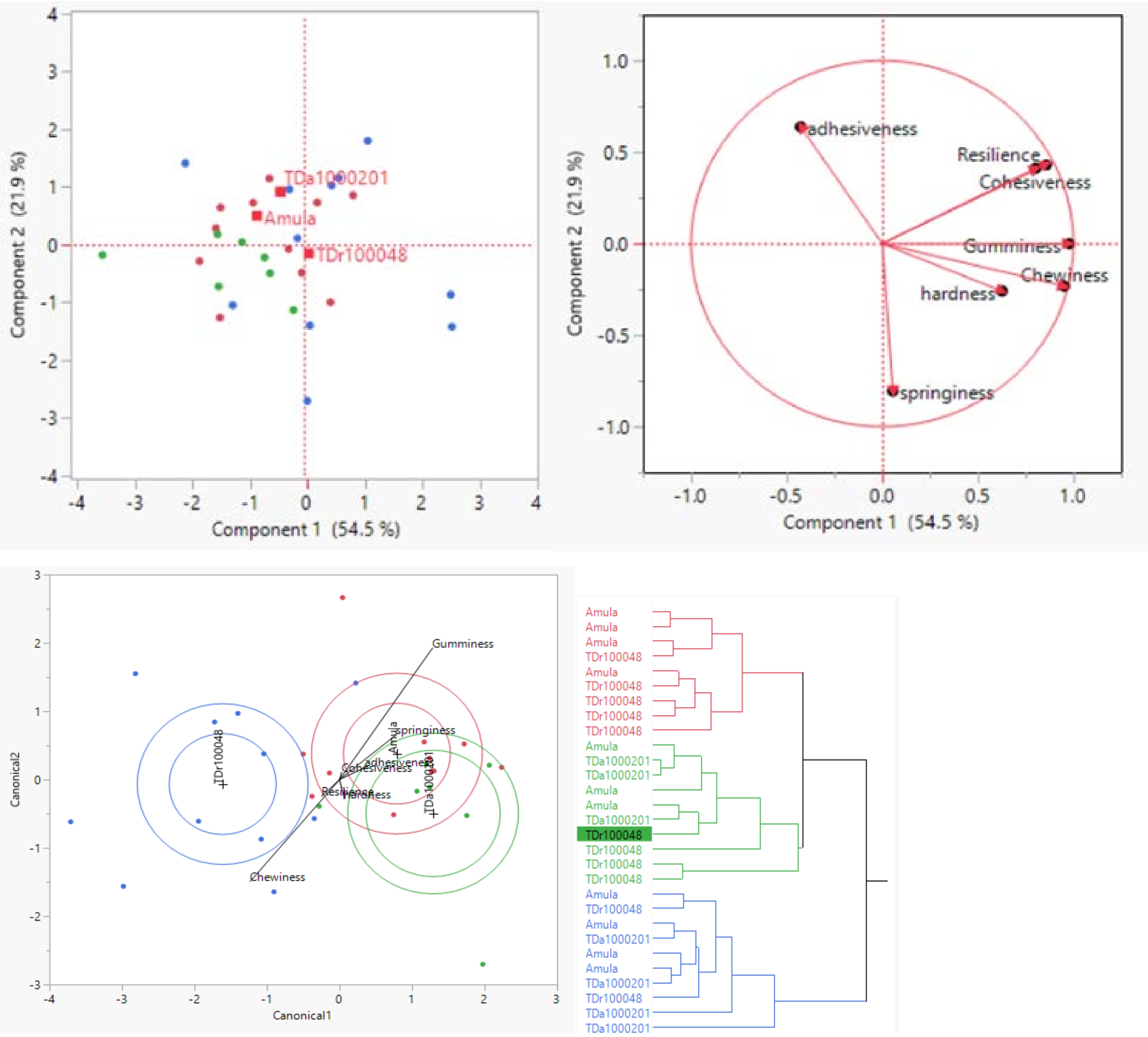


Figure 27 PCA, discriminant and hierarchical analyses for textural attributes of boiled Yam made from 3 contrasting Yam genotypes.

At IITA-Nigeria, the compression/extrusion test on the 16 Yam genotypes resulted in hardness and work during extrusion ranging from 7437 to 23900g and 117002 to 335177 g.sec, respectively. Significant differences (p<0.0001) were found by ANOVA among the textural attributes (Table 5 and 6) for different Yam genotypes, thus demonstrating the discriminance of the compression/extrusion protocol. Repeatability among replicate measurements within each genotype was good as well, with no significant (P>0.05) effect of replicate measurements. The Pearson correlation analysis between sensory descriptors and instrumental texture parameters of the 16 Yam genotypes showed a correlation between sensory hardness and instrumental hardness, albeit not significant (r = 0.47, Table 8).

Table 5 Analysis of Variance for hardness(g)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
genotype	15	1048818287	69921219	8.1485	<.0001*
Error	48	411880696	8580847.8		
C. Total	63	1460698983			

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
replicate	1	9721822.67	9721822.7	0.4154	0.5216
Error	62	1450977160	23402857		
C. Total	63	1460698983			

Table 6 Analysis of Variance for work done by extrusion

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
genotype	15	1.4994e+11	9.9961e+9	3.5634	0.0004*
Error	48	1.3465e+11	2.8052e+9		
C. Total	63	2.8459e+11			

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
replicate	1	4585675889	4.5857e+9	1.0154	0.3175
Error	62	2.8e+11	4.5162e+9		
C. Total	63	2.8459e+11			

Table 7 Mean values of hardness and work done by extrusion

Genotypes	Hardness (g)	Work (g.sec)
TDr 1400158	24419.8^{a}	260152.4^{a}
TDr 1100055	21983.4^{ab}	264277.7^{a}
TDr 1401161	20474.2^{abc}	203712.9^{ab}
TDr Meccakusa	19628.4^{abc}	191996.8^{abc}
TDr 1100180	18880.3^{abc}	173229.3^{abc}
TDr 1401419	18420.7^{abcd}	203460.5^{ab}
TDr 0900295	18225.7^{abcd}	191658.1^{abc}
TDr 1000021	18111.2^{abcd}	156192.3^{abc}
TDr 8902665	17263.7^{abcd}	204557.0^{ab}
TDr 1401593	15794.0^{bcd}	165014.5^{abc}
TDr 1100128	15136.4^{bcd}	145790.2^{abc}
TDr 1400766	14613.2^{bcde}	143953.1^{abc}
TDr 0900135	14289.6^{cde}	164925.6^{abc}
TDr 1401220	13529.9^{cde}	140510.6^{abc}
TDr 1400537	11289.4^{de}	116482.2^{bc}
TDr 1400359	7368.3^{e}	64518.8^{c}

Values in this table are average of replicates measurements. Values with the same letters are not significantly different

Table 8 Pearson correlation of STPA and ITPA of boiled Yam

	I-hardness	S-hardness	S-chewiness
I-hardness	1		
S-hardness	0.47	1	
S-chewiness	-0.37	-0.37	1

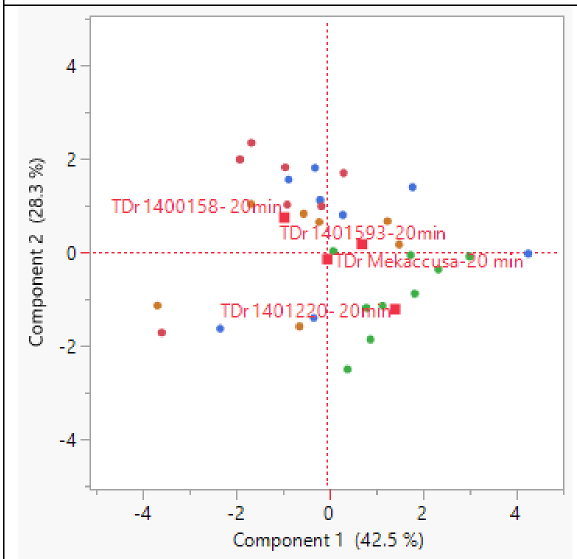
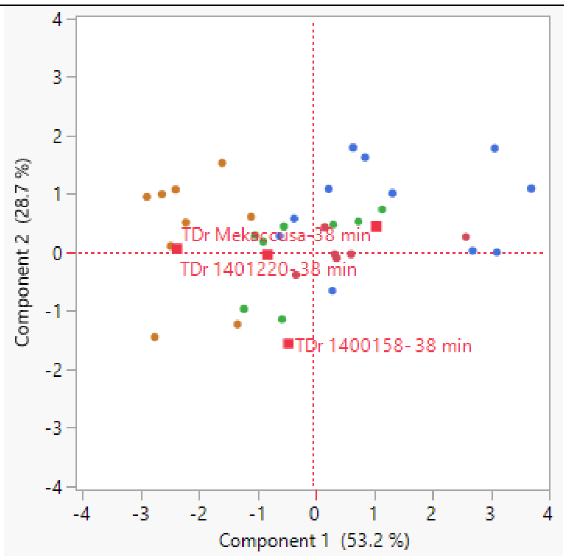
I-Hardness (Instrumental hardness); S-Hardness: (Sensory hardness); S-Chewiness (Sensory chewiness)

At IITA with the partnership of CIRAD, using the TPA compression method, the textural attributes of the varieties at 20 min and 38 min cooking time showed good repeatability with no significant differences between the cooking replicates. The variety had a slight effect on textural attributes of boiled Yam at both cooking times, especially at 38 min, but these effects were not largely significant between the variety pairs, and varied with each textural attribute. Cooking time did not significantly affect the values of all the textural attributes of the varieties.

A PCA (Figure 28) did not distinctly separate the varieties within the PC1 and PC2 space, thereby showing that the varieties had similar textural attributes, particularly at 20 min cooking time. A discriminant analysis (Figure 28) did not separate the varieties either within the canonical space, confirming that the TPA method was not discriminant at both cooking times, with this set of varieties. Also, the varieties were not classifiable into separate clusters within the hierarchical pattern (Figure 28).

Further analysis of TPA method in comparison with other textural methods such as penetrometry and texture-extrusion across the various partner locations should be investigated in order to select the most discriminant method for boiled Yam. A different set of more contrasted Yam genotypes may also be evaluated, in order to confirm the present observations regarding discriminance of the TPA method.

No correlation analysis between TPA instrumental texture attributes and sensory attributes has been performed yet.

20 min cooking time	**38 min cooking time**
	

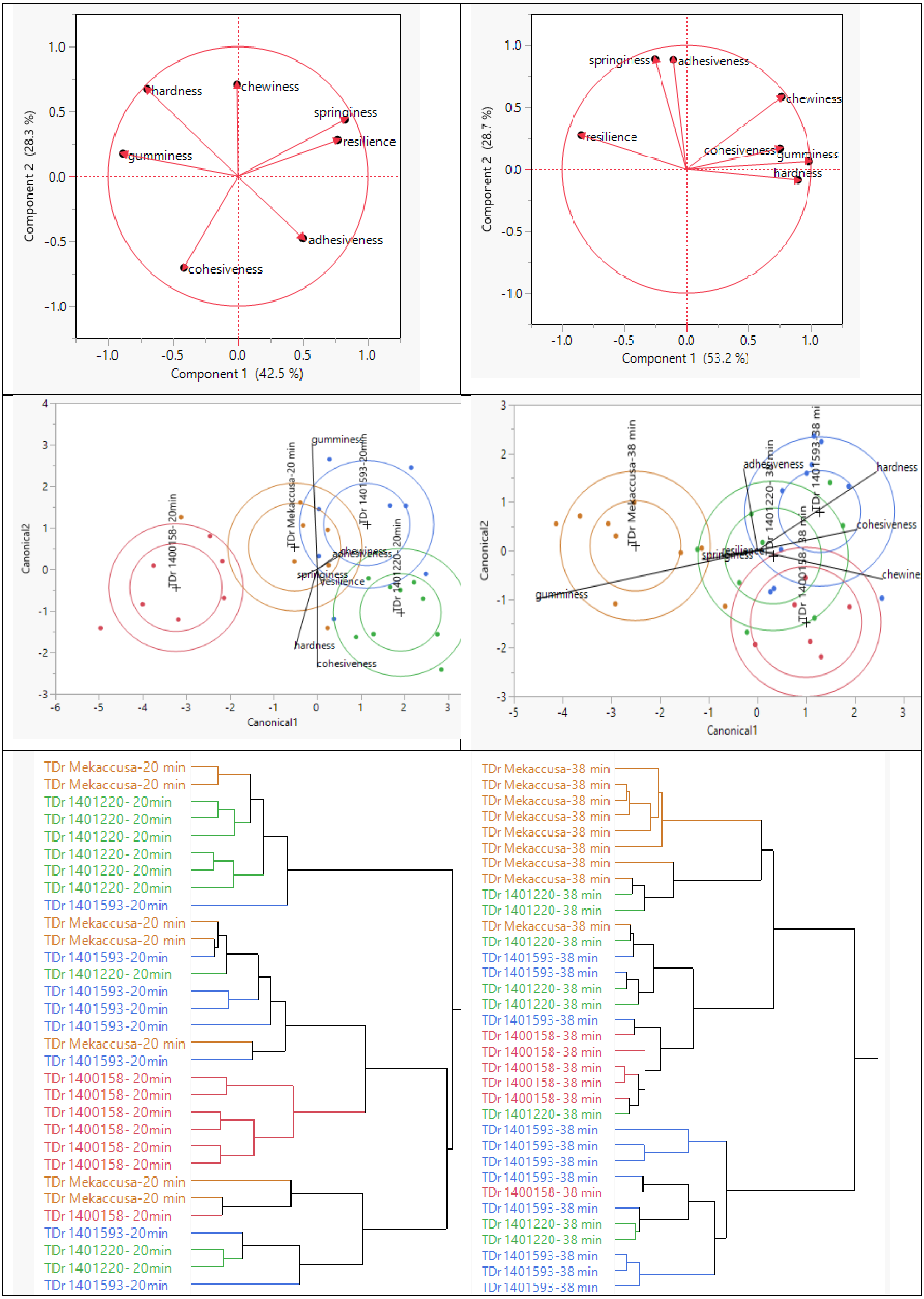


Figure 28 PCA, discriminant and hierarchical analyses for textural attributes of boiled Yam made from 4 contrasting Yam genotypes at IITA.

At INRAe-France/Guadeloupe, the cooking time measured for the 10 varieties of Yam by boiling varied from 11 to 23 min. The species D. trifida and D. esculenta showed distinct characteristics as compared to D. alata. They were easier to peel, disintegrated less and gained more weight during boiling. The hardness of the raw pulp was highest in D. trifida. After steaming for 15 min, *D. alata* had the hardest pulp.

At CIRAD-France/Guadeloupe, a gradient of maximum force (measured by penetrometry and by TPA) was observed from the proximal to the distal sections in 52 varieties, with the highest values at the proximal section. The effect of tuber was small, but confirmed the importance of performing measurements on a minimum of 3 tubers per variety in order to obtain representative results. Three groups of varieties with significantly different instrumental texture characteristics were identified by penetrometry (Figure 29) and TPA.

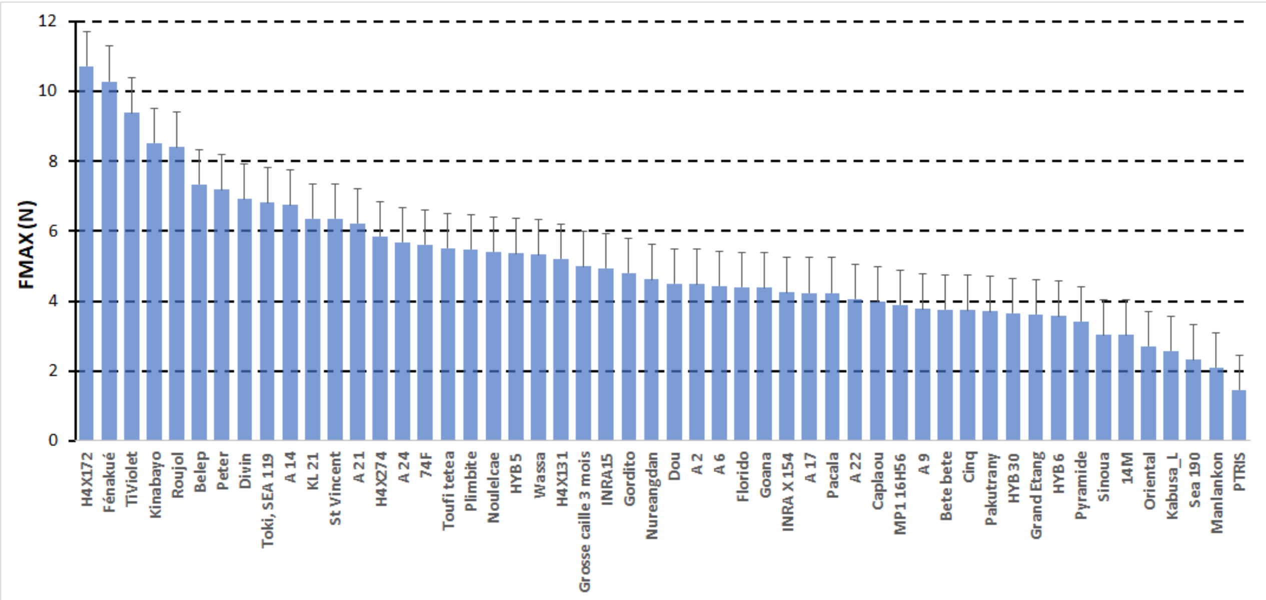
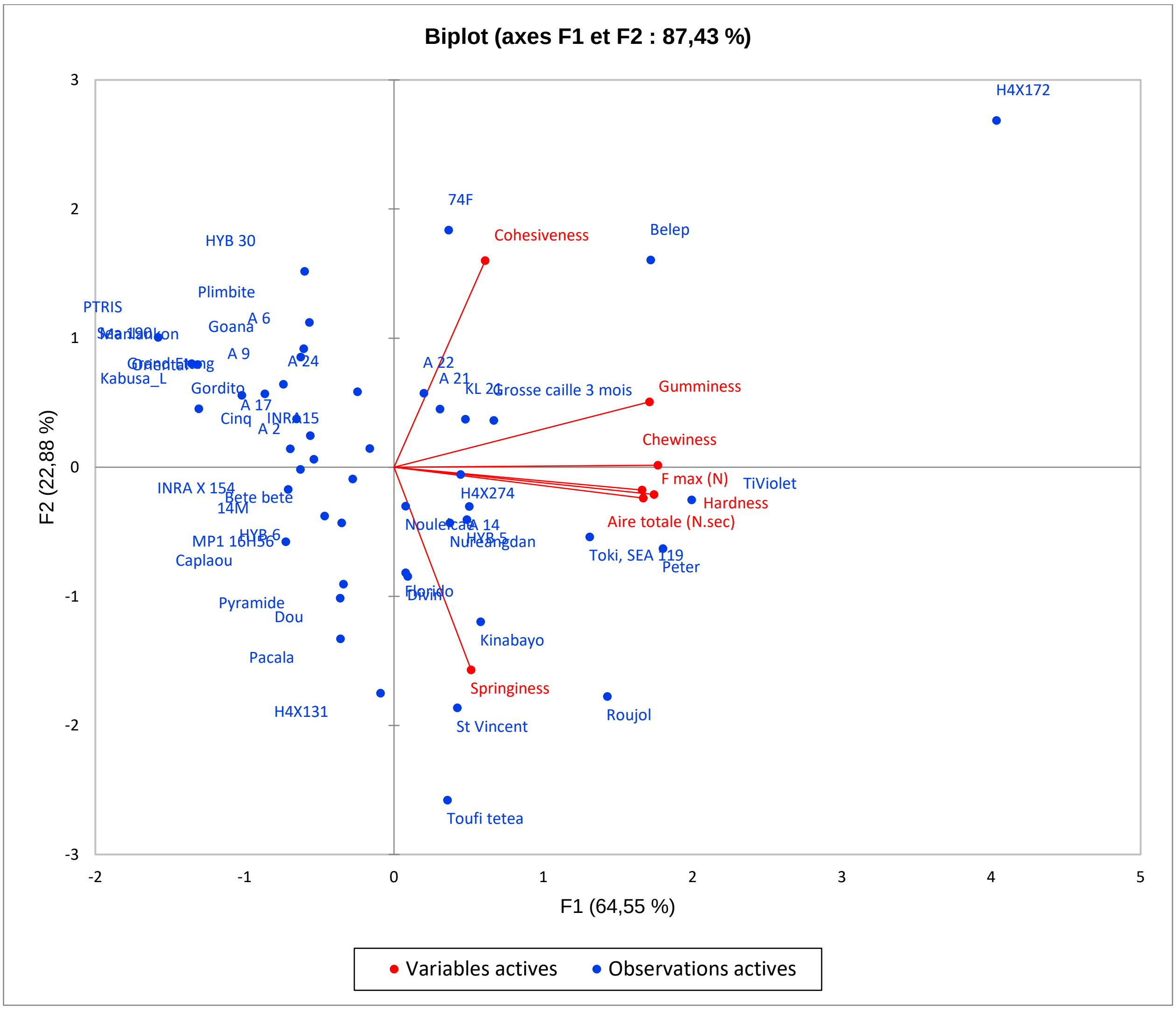


Figure 29 changes in Hardness measured by penetrometry as a function of genotype.

In order to compare penetrometry and TPA compression methods, 48 similar varieties among the 52 varieties were selected. ANOVA connecting letter grouping showed that penetrometry and TPA grouped the 48 varieties into unique letter groups, depending on the attribute (table below). Hence, both methods can practicably be used to discriminate varieties into groups having similar textural quality.

	Penetrometry	**TPA**
Max F (N)	20	
Total area	18	
Hardness		30
Cohesiveness		15
Gumminess		20
Springiness		17
Chewiness		23



The PCA of penetrometry and TPA attributes superimposed with the varieties reveal that penetrometry attributes Max Force (F Max N) and Total area (Aire totale N.sec) are highly related to TPA Hardness, Chewiness and Gumminess, and are the more important attributes in discriminating between the varieties (along PC1, carrying 64.5% of the total variance). On the PCA space, different varieties were clustered with various textural attributes. Varieties H4X172, Belep, A21, and 74F were more associated with attributes gumminess and cohesiveness, while Florido, St Vincent, and Peter were associated with Hardness and Chewiness. The Hardness attributes were correlated with PC1, and Springiness and Cohesiveness with PC2, thus showing that the penetrometry and TPA tests capture at least two dimensions of the texture of boiled Yam, the combination of which may define up to four types of textural behaviors: “hard and springy”, “hard and cohesive”, “soft and springy”, and “soft and cohesive”.

Synthesis on progress achieved:

For boiled Yam, SOPs were developed by UAC/FSA and transferred to other partners (NRCRI, IITA). The list of sensory descriptors is the one proposed by UAC/FSA. The priority quality traits differed between partners related to cultural specificities.

Both UAC-FSA and IITA found few correlations between sensory attributes and textural parameters from respectively 5 and 16 genotypes. The best correlations were found between the descriptor hard to break and penetrometry hardness (r = 0.68) and energy (r = 0.76). TPA parameters or extrusion force did not appear the most relevant instrumental procedures to predict texture sensory attributes.

Institute/ Country	Targeted Quality Trait / Sensory Descriptor	Instrumental Method for Measurement on Final product				
		Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
UAC-FSA/ Benin	Sticky	Penetrometry	Yes	No	15 samples * 3 cooking batches * 3 replicates	Not applicable
		TPA		No		
	Hard to break	Penetrometry		Yes		
		TPA		No		
	Crumbly	Penetrometry		No		
		TPA		No		
	Easy to chew	Penetrometry		No		
		TPA		No		
NRCRI	Easy to chew, crumbly/friable, Sticky to the finger, hard to break	TPA test	Yes	Pending	3 genotypes, 6 measurements per replicate and 2 replicates were considered	3
IITA-Nigeria	Hardness/ Softness	Compression/ Extrusion	Yes	Yes	16 clones	16 clones
	Ease of Chew	Compression/ Extrusion	Yes	Yes	16 clones	16 clones
INRAe-France/ Guadeloupe	Texture	Penetrometry	Yes	No	66 tubers	None
CIRAD-France/Guadeloupe		Penetrometry TPA	Yes	No	52 cultivars * 3 tubers * 3 parts	52

Next steps & activities planned to measure key sensory descriptors for texture instrumentally:

At UAC-FSA-Benin, 4 additional Yam varieties will be characterized regarding the biophysical and texture parameters in order to consolidate the previous results. We plan to submit WP2 results for publication during the first semester of 2022.

At NRCRI-Nigeria, sensory and texture of boiled Yam will be conducted using 15 genotypes (7 alata, 7 rotundata from African Yam project and a preferred land race) in period 5. Data generated will be used to establish a correlation between the sensory properties of boiled Yam (especially the prioritized traits) and related texture parameters. Possibly, the rheology of boiled Yam may be conducted in CIRAD as an additional textural technique to discriminate between the genotypes.

At IITA-Nigeria, in period 5, the SOP for boiled Yam will be validated in close interaction with the UAC-FSA and the CIRAD texture analysis focal person for the RTBfoods project. In the 2022 harvesting season, the freshly harvested genetic materials from WP4 will be evaluated for Instrumental and sensory texture profile analysis, emphasizing the key priority quality traits highlighted by WP1.

At INRAe-France/Guadeloupe, no textural and sensory analyses planned in P5 at INRAe Guadeloupe

At CIRAD-France/Guadeloupe, a replication of the experiment of period 4 on the 52 varieties will be performed; however, only one instrumental textural analysis (penetrometry test) will be performed (with an increasing number of replicate).

4.5.3 Rapid / intermediate kitchen tests to assess the processing & the cooking ability of RTBs

Rapid kitchen test or intermediate assessment methods investigated:

At IITA-Nigeria, the rapid kitchen test for boiled Yam developed in period 4 measured **cooking time and water absorption during cooking**. This involves cutting the Yam into regular pieces and subject to controlled cooking. Each Yam tuber was divided into proximal, middle, and axial sections. Then a small piece of about 6cm by 2 cm was cut from each section using a stainless-steel cutter to determine the cooking time and water absorption. The water absorption was measured by the increase in the weight of the Yam pieces after being cooked, while the cooking time is the time the Yam becomes softened using a fork test.

The time required to cook Yam varies from one genotype to another, and it has become an essential criterion for acceptability for boiled Yam consumers. Sixteen clones (16) of Dioscorea rotundata were evaluated for *cooking time and Water absorption* while cooking; the Yam genotypes' average cooking time and water absorption are 10.6±2.5 minutes and 2.7±1.0 %, respectively (Figure 30).

The Pearson correlation analysis between CT, WAB, and instrumental texture analysis showed (Table 9) a slight but not significant correlation between sensory chewiness and water absorption (r = 0.43). No significant correlation was found between sensory hardness and cooking time and water absorption of boiled Yam.

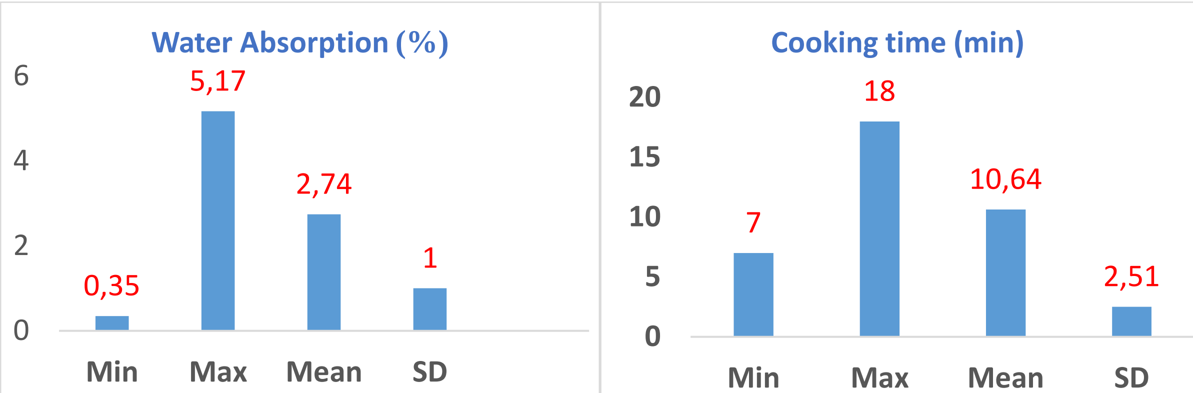


Figure 30 Cooking and Water Absorption of boiled Yam

Table 9 Pearson correlation analysis between cooking time, water absorption and sensory attributes of boiled Yam

	Cooking time	S-hardness	S-chewiness	Water absorption
Cooking time	1			
S-hardness	-0.167	1		
S-chewiness	-0.172	0.186	1	
Water absorption	0.276	0.144	0.432	1

In Period 5, freshly harvested Yam genotypes (2021/2022 harvesting season) will be used to further investigate the relationship between cooking time, water absorption, and the sensory attributes of boiled Yam.

At NRCRI-Nigeria, cooking time and softness of different Yam genotypes were evaluated using a hand-held penetrometer and a fork. 11 Yam genotypes (D. alata and D. rotundata) harvested from African Yam project multilocation trial cited in two locations namely Uyo and Umuahia in Nigeria were stored for 3 months before analysis. 3 tubers per genotype were selected and their length measured. Each of the tubers was cut into proximal, central and distal and thereafter peeled and washed. From the proximal and distal parts, 1/10 and 3/10 was cut off at the bottom and head section and discarded while for the central part, 2/10 was cut off from both ends of the central section and discarded. Each part (6/10) is divided following the length into two subsections for steam-cooking and penetrometer test. To measure the optimum cooking time, four pieces of the Yam were steam-cooked for a minimum of 20 mins, and thereafter tested for every 5 mins using a fork until it was properly cooked and the penetrometer reading was taken. The SOP used in this study by NRCRI, Umudike was developed by UAC-FSA-Benin.

The preliminary study (Tables 10 & 11) indicates that the handheld penetrometer can discriminate between Yam genotypes, both D. alata and D. rotundata. No clear relationship between biochemical properties (dry matter, amylose/amylopectin) and cooking behaviour can be drawn from these first results. Further data will be collected in Period 5 to confirm these results.

Table 10 Effect of genotype on processing parameter and some physicochemical properties of stored D. alata clones cooked at an optimized cooking time

CLONE	Cooking time (min)	Penetrometer reading (kg/n)	Pounding time (min)	DM (%)	Amylose (%)	Amylopectin (%)
TDA 1100224	26.77^{c}	1.80^{de}	6.41^{a}	36.16^{a}	25.34^{a}	74.66^{b}
TDA 1415201	20^{a}	2.72^{bcde}	4.50^{a}	31.84^{c}	19.10^{b}	80.90^{a}
TDA 1401253	23.50^{b}	3.43^{bc}	8.67^{ab}	31.96^{bc}	18.13^{b}	81.87^{a}
TDA 1100247	20^{a}	2.13^{de}	8.50^{ab}	36.31^{a}	23.44^{a}	76.66^{b}
TDA 1400307	20^{a}	3.51^{bc}	7.00^{a}	33.68^{abc}	18.58^{b}	81.42^{a}
TDA 1100374	20^{a}	2.50^{cde}	5.67^{a}	36.49^{a}	20.27^{b}	79.73^{a}
TDA 1100250	20^{a}	2.99^{bc}	6.67^{a}	30.92^{c}	20.00^{b}	80.00^{a}
TDA 0000194	20^{a}	4.96^{a}	7.00^{a}	31.65^{c}	19.58^{b}	80.42^{a}
TDA 1412030	20^{a}	3.72^{b}	19.83^{c}	35.42^{ab}	19.25^{b}	80.75^{a}
TDA 291	18.53^{c}	1.52^{e}	12.57^{b}	30.84^{c}	19.74^{b}	80.26^{a}

Table 11 Effect of genotype on processing parameter and some physicochemical properties of stored D. rotundata clones cooked at an optimized cooking time

CLONES	Cooking Time (min)	Penetrometer reading	Pounding time (min)	Dry matter	Amylose (%)	Amylopectin (%)
TDR 0900295	20^{a}	4.598^{gh}	4.83^{d}	47.29^{a}	19.64^{cde}	80.36^{abc}
TDR AMOLA	20^{a}	3.300^{cde}	7.33^{bc}	38.11^{ef}	21.92^{bcd}	78.08^{bcd}
TDR 1400158	20^{a}	3.463^{cdef}	9.33^{a}	40.57^{de}	19.18^{de}	80.82^{ab}
TDR 1000021	20^{a}	5.106^{h}	6.78^{c}	42.54^{bcd}	18.08^{e}	81.92^{a}
TDR 1000128	20^{a}	2.748^{bcd}	7.50^{bc}	42.32^{bcd}	23.94^{b}	76.06^{d}
TDR 0900135	20^{a}	4.248^{efgh}	6.83^{c}	45.03^{ab}	19.09^{de}	80.91^{ab}
TDR 1401161	20^{a}	2.567^{bc}	4.50^{d}	39.74^{de}	23.89^{b}	76.11^{d}
TDR 89/02665	20^{a}	3.700^{defg}	6.22^{c}	44.46^{abc}	19.57^{cde}	80.43^{abc}
TDR 1100180	20^{a}	4.492^{fgh}	6.50^{c}	46.65^{a}	22.08^{bcd}	77.92^{bcd}
TDR 1400359	20^{a}	2.490^{bc}	8.83^{ab}	41.46^{cd}	22.94^{bc}	77.06^{cd}
TDR 1400537	20^{a}	1.980^{ab}	6.17^{c}	35.23^{fg}	22.10^{bcd}	77.90^{bcd}
TDR 1400766	20^{a}	3.348^{cde}	8.17^{ab}	44.22^{abc}	27.89^{a}	72.11^{e}
TDR 1401220	20^{a}	1.943^{ab}	6.33^{c}	40.63^{de}	19.14^{de}	80.86^{ab}

CLONES	Cooking Time (min)	Penetrometer reading	Pounding time (min)	Dry matter	Amylose (%)	Amylopectin (%)
TDR 1401593	20[a]	1.948[ab]	7.33[bc]	34.76[g]	17.11[e]	82.89[a]
TDR 1401785	20[a]	1.268[a]	8.50[ab]	41.47[cd]	19.08[de]	80.92[ab]
TDR 1100055	20[a]	3.130[cd]	8.17[ab]	39.91[de]	19.69[cde]	80.31[abc]
GRAND MEAN	**20**	**3.146**	**7.08**	**41.52**	**20.96**	**79.04**
P VALUE	**-**	**<.001**	**0.224**	**<.001**	**<.001**	**<.001**
LOCATION	**-**	**0.441**	**0.003**	**<.001**	**0.100**	**0.100**
SECTION	**-**	**0.004**	**<.001**	**<.001**	**0.015**	**0.015**

In Period 5, other kitchen tests should be developed at NRCRI-Nigeria: a SOP for measuring ease of peeling and ease of slicing Yam. This will help characterize easy to peel ability of different Yam spp from AfricaYam multilocation trials. The data will be related to productivity, product yield and reduction of drudgery during processing of boiled Yam.

Synthesis on progress achieved:

In Period 4, indicators of boiled Yam quality likely to impact acceptability of new Yam varieties were identified as follows (based on experiments with 11 and 16 genotypes): Cooking time, softness (instrumental characterization by fork and penetrometer) and water absorption. Two of these, water absorption and cooking time, were linked with sensory attributes of boiled Yam, with a positive but not significant correlation between water absorption and chewiness. Prediction of sensory attributes of boiled Yam by instrumental analyses may therefore be possible, thus reducing the time necessary to screen Yam genotypes for boiling quality.

4.5.4 Dissection and understanding of key quality traits for boiled Yam

Quality trait(s) of focus/investigated:

At CIRAD-France, research activities focused on the relationship between the physio-chemical properties of raw Yam (dry matter, pectins, amylose and starch contents) and the cooking behaviour (texture at different cooking time).

At INRAe-France,research activities focused on the relationship between the fine composition of cell wall polysaccharides (in raw and cooked Yam) and cooking ability (texture and cooking time). Relationship between soil properties and cooking properties of tubers are also investigated. Biochemical attributes analysed are: dry matter content, starch,amylose, cell wall, pectin and lignin content, methylation degree, galacturonic acid and neutral sugars composition, soluble pectin molar mass distribution, procyanidin composition, pectinolytic enzymatic activities (pectinmethyl esterase (PME) and polygalacturonase (PG)), protein, ash.

At UAC-FSA-Benin, the quality traits investigated are related to colour (white and brown), texture (crumbly and easy to chew) and taste (sweet and bitter). The biochemical components suspected of being involved in boiled Yam colour (brown or white) are the activity of **polyphenols oxidase** as well as the **polyphenols content**. The **dry matter, starch and amylose contents** are suspected of being involved in boiled Yam texture (crumbly and easy to chew) while **sugar content** is suspected of being involved in the sweet taste of boiled Yam. The polyphenols content can also be associated to bitter taste.

At IITA-Nigeria, the quality traits evaluated for boiled Yam are **water absorption, cooking time, colour, texture (hardness/softness) and taste**. The suspected biochemical traits to be linked to the cooking qualities of boiled Yam were **dry matter, starch content, pectin, cell wall, and soluble sugars.**

Level of progress in the development of biochemical proof of concept / method to measure the biochemical attributes of raw material

At CIRAD-France& CIRAD-Guadeloupe,several biophysical analyses were performed on the 52 varieties:

- Dry matter following SOP on the 52 varieties,
- Pectin levels on 24 samples using a SOP that has been validated,
- Amylose content following SOP and starch content (using drafted SOP) on 6 samples.

Results clearly showed on the 468 samples (52 cultivars*3 tubers*3 sections) no direct correlation between dry matter content and texture values (Figure 31). It should however be noticed that samples with low dry matter content do never evidence high firmness.

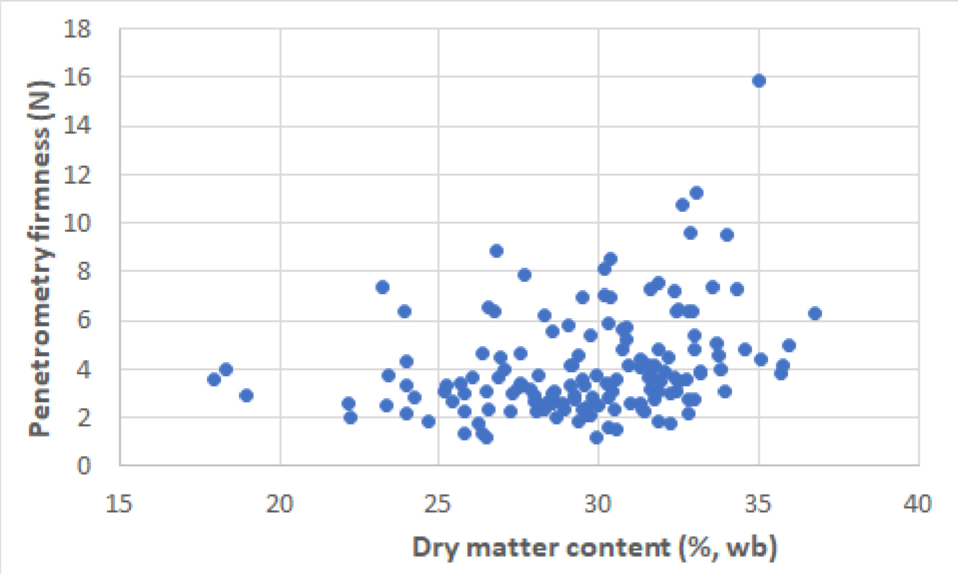


Figure 31 Relationship between dry matter content and penetrometry firmness

Among the 24 samples already analysed, total galacturonic level (main component of pectins) ranged from 232 to 3100 mg/100 g. A good correlation was evidenced between galacturonic content and texture (Figure 32); firmness increases with total galacturonic content, with however two outliers that have high very galacturonic level but moderate firmness. Modelling firmness was improved by the addition of dry matter content variable: firmness indeed increases with dry matter content for samples having similar pectin content.

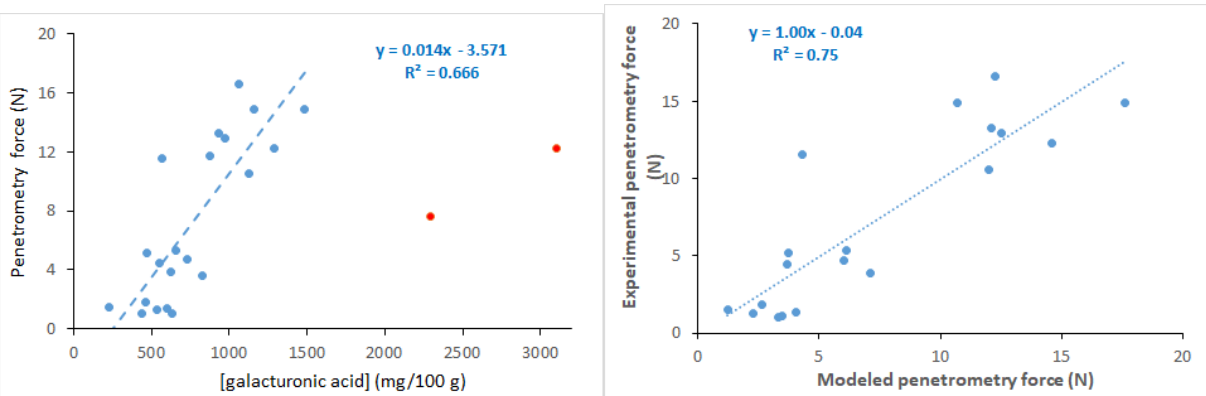


Figure 32 Relationship between galacturonic acid content and penetrometry firmness, and model of penetrometry firmness from galacturonic acid and dry matter content

A SOP for the extraction and colorimetric assessment of galacturonic acid has been designed and validated. It recommends triplicates and standard deviation between replicate typically ranges by 10%.

Starch and amylose contents have been additionally determined on 6 samples, including the two outliers. It tentatively shows that amylose content should have a role on the texture of cooked Yam (Figure 33)

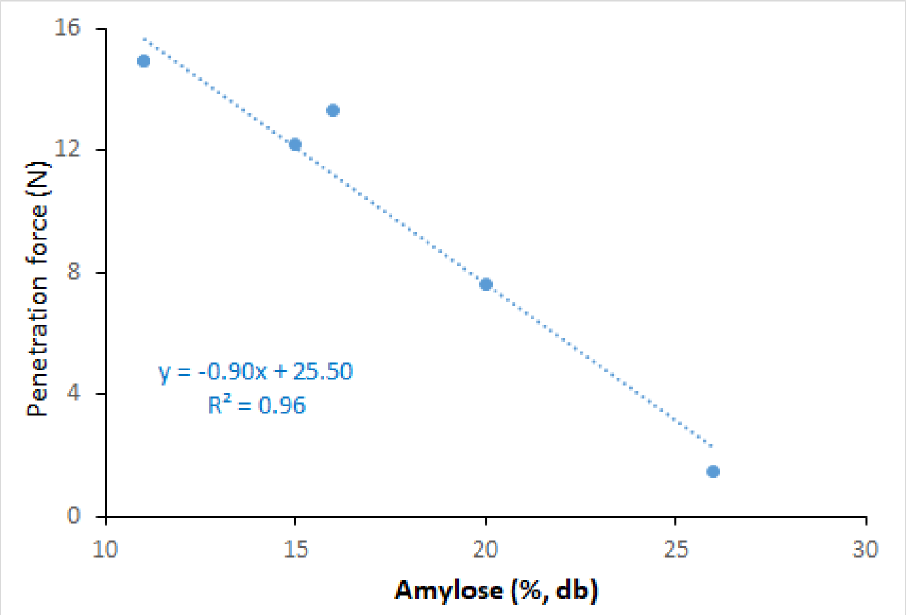


Figure 33 Relationship between amylose content and penetrometry firmness of boiled Yam

In order to apprehend the mechanism of cooking several parameters have been followed during cooking: core temperature, gelatinization level, soluble pectins, and penetrometry hardness (Figures 9 and 10). Soluble pectin level increases after 5 minutes of cooking; it can be modelled by a zero order kinetic (Figure 35a). The gelatinization appeared complete after 5 minutes of cooking while the core temperature of the cube of Yam is over 80°C (Figure 35b). The hardness decreases after 5 minutes (Figure 34) while the gelatinization is already achieved but can be tentatively linked to the degradation of pectins and thus their higher solubility.

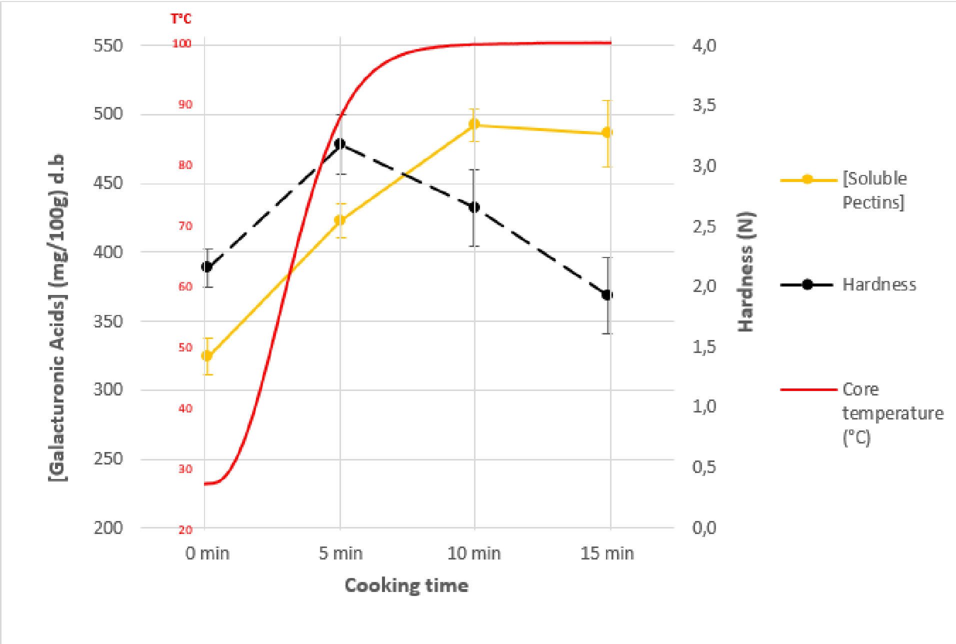


Figure 34 Impact of cooking on the biophysical parameters of Yam (Uncertainty bar = 1 standard error)

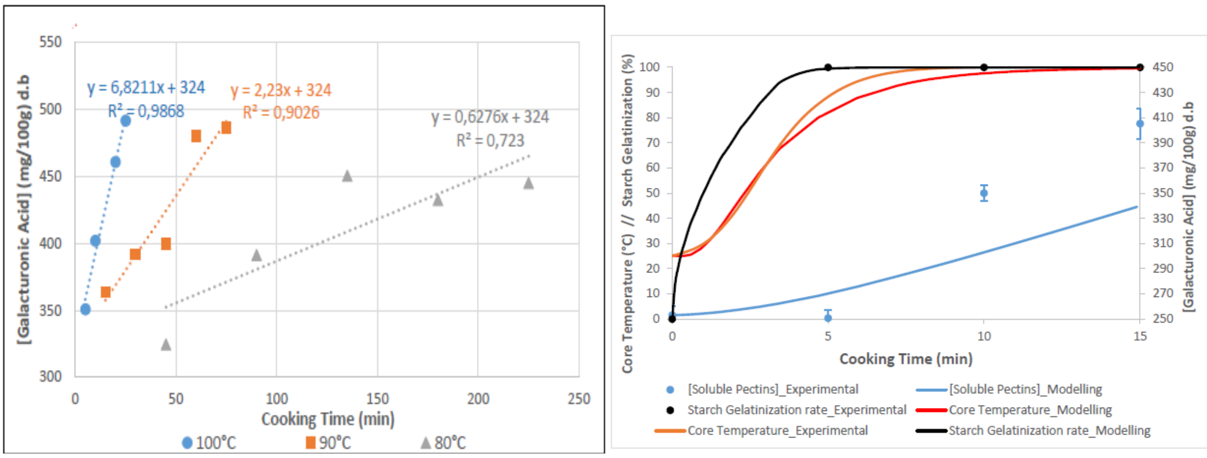


Figure 35 (a) Evolution of the soluble pectin content during cooking as a function of the cooking temperature, (b) Comparison of modelling and experimental measurements

At INRAe-France, samples of raw Yam and starch control sample from Megazyme have been sent to CIRAD Montpellier for a**mylose analysis** by DSC method in order to validate formula to estimate **starch** content by this SOP. We are waiting for these results by DSC to consolidate the relevance of using starch data and to develop prediction model of amylose and starch content by NIRS in WP3. Futhermore, 37 samples corresponding to 37 tubers of nine varieties of *D. alata*, harvested in 2020 at Duclos (INRAE site), have been analyzed for amylose content after sample solubilization at room temperature during 16h as previously selected. Raw Yam flour of A2, Grand Etang, Sinoua, Pacala, Kinabayo, Pyramide, Inra15, Florido, TouffiTetea, are amylose content ranged from 15 to 26% DM. These data are used to complete Database for amylose prediction in raw Yam flour by NIRS (see WP3 scientific report). The WP2 database of all data collected from P2 to P4 is in progress. The report on the Impact of cell wall composition on texture of boiled Yams is under writing.

INRAE Avignon and INRAE Guadeloupe defined methods, strategy and selected samples to analyse in order to study the **impact of cell wall and starch on Yam cooking ability and texture**. Yam production and cooking evaluation takes place in Guadeloupe whereas polysaccharide, lignin and procyanidin analyses are carried out in Avignon. INRAE Avignon used the methods (SOPs

CW_extraction_Yam plantain & Cell wall analysis) and strategy developed in P3 and P4 in order to study the impact of cell wall, lignin, procyanidin and starch on cooked Yam texture.Texture and cooking ability were done on Period 4 samples produced at INRAE Guadeloupe in INRAE Guadeloupe while cell wall, starch and procyanidin analysis were done on freeze-dried P3 samples, i.e. cooked and raw Yam samples prepared and characterized in terms of cooking ability and texture in P3 by INRAE Guadeloupe (see results reported in P3).5 varieties (Kabusah, Pacala, Sinoua, Assam, Spindle) and 2 species (D. alata and D. esculenta) of P3 samples were analysed for polysaccharide and procyanidin composition at INRAE Avignon in P4. These samples were harvested in P3 from the site of Godet and from Duclos'site.

Tubers from Godet have a longer cooking time and are firmer after cooking than the ones from Duclos.Yams from this collection contain between 2.5 and 6.2 mg/g galacturonic acid on dry basis. Samples from P2 (2019 harvest, fresh samples) had similar galacturonic acid content (4.6mg/g in 2019 vs 3.9 mg/g in 2020 for Pacala). This finding is consistent with the values found by C Mestres (CIRAD Montpellier) with another method on its own set of Yam samples. The rhamnogalacturonan portion of pectin is highly dominated by galactose, which is similar to potato pectin but different from cassava and sweet potato. Raw Yam pectins have a low to medium methylation degree (DM), comprised between 37 and 58%. *D. esculenta* samples contain significantly more mannose than *D. alata* samples. This is due to *D. esculenta* mucilage which is mainly composed of glucomannan. Considering the two harvesting sites, the composition of CWP was similar whereas the methylation degree of Godet's samples was lower.

No pectinmethylesterase (PME) activity was found in the collection (fresh samples kept at -80°C for enzyme analyses), however polygalacturonase (PG) activity was present in fresh samples, thus confirming the results obtained on samples harvested in 2019.

At INRAe-France, **no correlation was found between total pectin content in raw tubers neither in steamed tubers and cooking time**, on the selected samples. The total pectin content is the sum of galacturonic acid, galactose, fucose, arabinose and rhamnose respective contents. Moreover, no correlation was found between galacturonic acid content and cooking time.

However, a negative correlation between cooking time and methylation degree of raw Yam tubers has been found, as shown in Figure 36. As the Assam tuber tested has an abnormally long cooking time (81 minutes), it is an outlier and is not displayed here. The cooking behaviour of the Assam tuber is different, which shows that there are other mechanisms involved in cooking ability. It has to be noted that there were no repetitions for cooking time measurement of Spindle Yam (in orange).

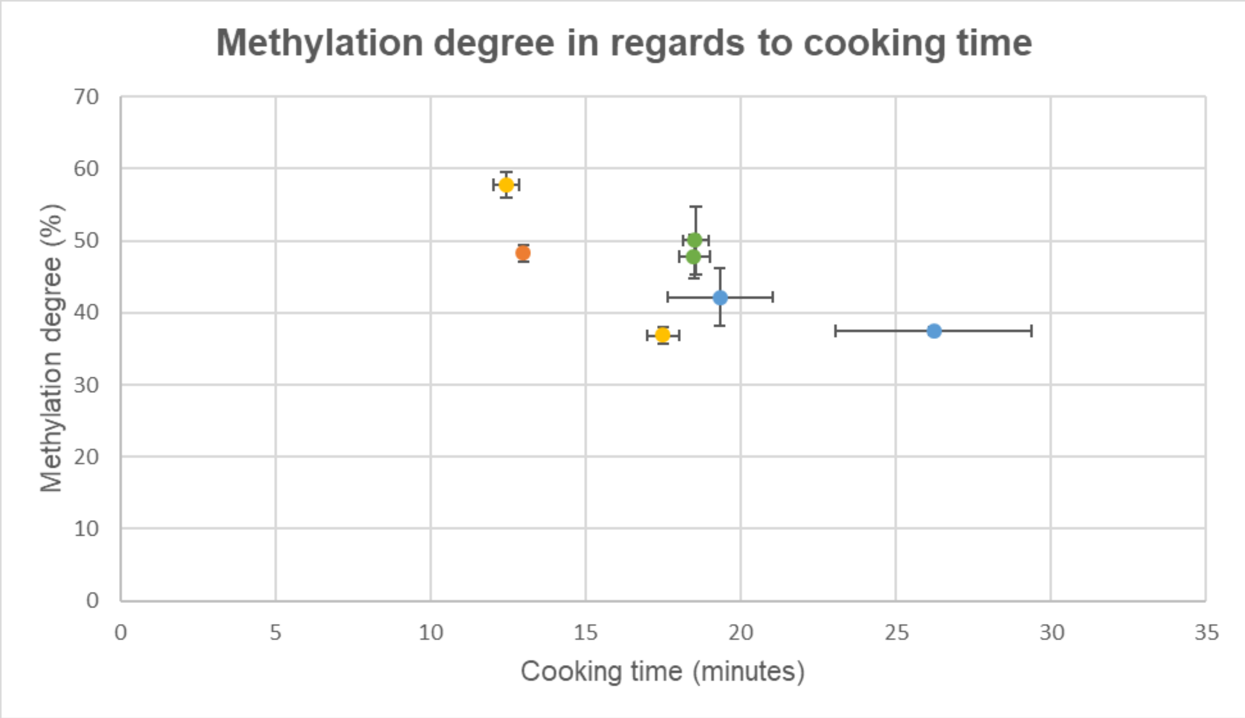


Figure 36 Methylation degree of raw tubers in regards to their cooking time

Regarding the cooking behaviour of Yams, combining enzymatic activity information and CWP composition of steamed samples, three different mechanisms of polysaccharide modifications during cooking were hypothetized depending on the species and the varieties (see report on the Impact of cell wall composition on texture of boiled Yams, currently under writing).

So far, there is **no correlation between starch, dry matter and textural properties or cooking time** in the studied samples. Nevertheless, this could be partly due to overcooking of samples which produced a reduced range of textural properties in steamed samples.

These results have yet to be confirmed on a larger set of samples. In addition, the steaming time used in the first version of the SOP texture (to produce these samples in P3) was too long for these Yams and produced steamed Yams with similar textures excepted for extremely long cooking tubers. For this reason, it was difficult to find significant differences between textures of steamed Yams and then to build accurate correlations between texture and biophysical traits in P3. This will be done in P5.

At UAC-FSA-Benin, 15 Yam samples that differed by the variety (Ala, Dèba, Gnidou, Kpètè and Laboko) and the cutting sections (proximal, central and distal) were used for biochemical evaluation of raw and boiled Yam through measurement of the contents of dry matter, sugar and total phenols at UAC/FSA lab. Raw and boiled Yam samples were used for the determination of dry matter content while flours (obtained after oven-drying and blinding of samples) were used to measure total phenols, and sugar contents.

The expected relationships between quality trait of boiled Yam and biochemical components of raw Yam were established (Table 12) although they need to be consolidated.

Table 12 Pearson correlation matrix of boiled Yam quality traits and raw Yam biochemical properties (with additional correlations between biochemical components of raw and boiled)

		Biochemical components of raw Yam		
		Total phenols (uM/g, db)	**Sugar (%, db)**	**Dry matter (%)**
Quality traits of boiled Yam	Brown	0.039	-0.099	-0.025
	Crumbly	0.374	-0.092	0.168
	Easy to chew	0.303	0.227	0.131
	Sweet	0.440	0.280	0.333
	Bitter	0.045	-0.039	0.274
Biochemical components of boiled Yam	Total phenols (uM/g, db)	0.307		
	Sugar (%, db)		0.256	
	Dry matter (%)			0.746

At IITA-Nigeria, biochemical analyses (DM and Starch) were performed in period 4 on 16 Yam genotypes. The dry matter(DM) and starch content ranged from 25.11 to 44.21% and 42.46 to 55.26%, respectively, on a fresh weight basis (Figure 37). TDr 1100055 had the lowest DM, while TDr 1100128 had the highest. TDr Meccakusa had the highest and TDr 1400158 the lowest starch content amongst the Yam genotypes.

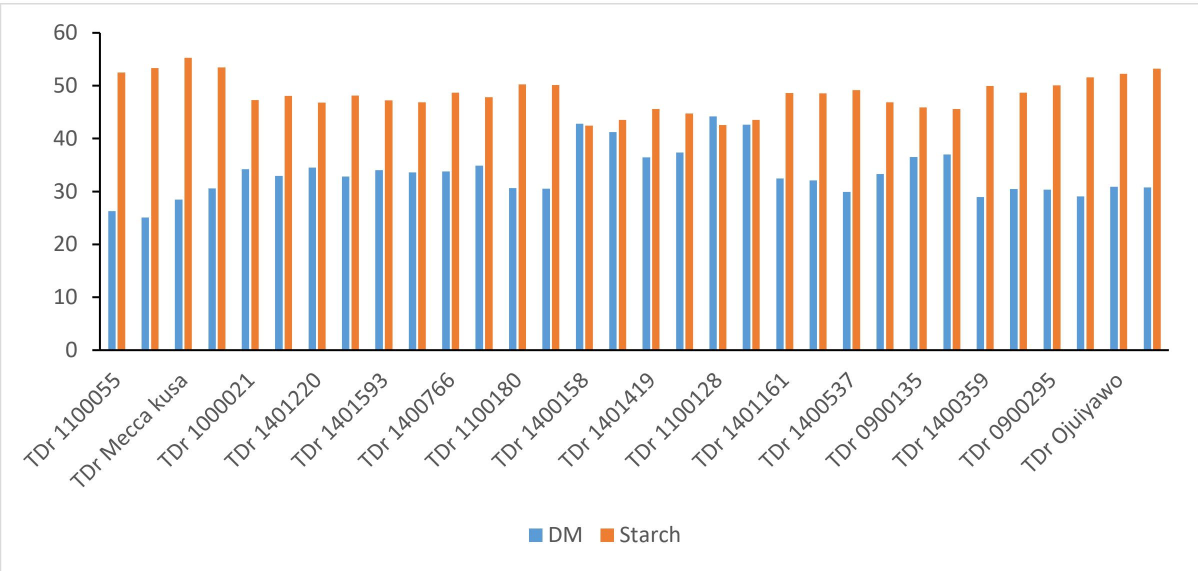


Figure 37 Dry matter and Starch content (% wet basis) of selected Yam genotypes

<u>*Synthesis on progress achieved:*</u>

A SOP for the assessment of total pectins has been written and the role of pectins on the texture has been demonstrated. A significant correlation between total galacturonic content and texture was evidenced; firmness increases with total galacturonic content. The hardness decreases after cooking for 5 minutes while the gelatinization is already achieved and appears linked to the degradation of pectins. A negative correlation between cooking time and methylation degree of raw Yam tubers has also been found.

The other major components such as starch, amylose and water contents are not directly linked to the texture but can contribute to the texture of boiled Yam.

Institute-Country	Targeted Quality Trait / Sensory Descriptor	Suspected Responsible Biochemical Attributes of Raw Material				
		Attribute	**Analysis Method**	**Proof of Concept established? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**SOP available ? (Yes/No/ Pending)**
CIRAD-France& Guade-loupe	Firmness (penetrometry or compression)	Dry matter	Drying at 105°C	Pending	468	Yes
		Pectin content	Colorimetric/flux	Yes	24	Yes
			Colorimetric/manual	Yes	6	Pending
		Amylose content	DSC	Pending	6	Yes
		Starch content	Enzymatic	Pending	6	Pending
INRAe-France & Guade-loupe	Texture/ Cooking time	pectin	SOP cell wall extraction	pending	16	yes
INRAe-France & Guade-loupe	Texture/ Cooking time	Cell wall	SOP cell wall analysis	pending	16	yes
INRAe-France & Guade-loupe	Texture/ Cooking time	procyanidin	Menthofuranolysis + HPLC-DAD and UPLC-MS	pending	32	no
INRAe-France & Guade-loupe	Texture/ Cooking time	lignin	Metaxas et al, 2004	pending	32	no
INRAe-France & Guadeloupe	Texture/ Cooking time	methylation	SOP cell wall analysis	pending	16	yes
INRAe-France & Guade-loupe	Texture/ Cooking time	PME/PG	PME: method adapted from Ribas-Agusti et al. (2017); PG : adapted from Gross (1982),	pending	12	Yes/no
INRAe-France & Guade-loupe	Texture	Starch	Megazyme kit	No	16	no
INRAe-France & Guadel-oupe	Texture	Amylose	SOP-amylose by iodine staining	No	210 (111 Yam tuber flour, 45 amylose validation, 54 starch validation)	Pending
INRAe-France & Guade-loupe	Nutritional	Protein	MOP-INRAE based on ISO 16634-2, 2016	No	67	No
UAC-FSA-Benin	Brown colour	Colour	Polyphenols content	Yes	15 samples * 3 cooking batches * 2 replicates	Yes

Institute-Country	Targeted Quality Trait / Sensory Descriptor	Suspected Responsible Biochemical Attributes of Raw Material				
		Attribute	Analysis Method	Proof of Concept established? (Yes/No/ Pending)	Nb of Analyses performed in 2021	SOP available ? (Yes/No/ Pending)
	Crumbly	Texture	Starch content	No	15 samples * 3 cooking batches * 2 replicates	No
	Easy to chew	Texture	Dry matter content	Yes	15 samples * 3 cooking batches * 2 replicates	Yes
	Sweet taste	Taste	Sugar content	Yes	15 samples * 3 cooking batches * 2 replicates	Yes
	Bitter taste	Taste	Polyphenols content	Yes	15 samples * 3 cooking batches * 2 replicates	Yes
IITA-Nigeria	Hardness	Texture	Starch content	No	16 genotypes	No
	Easy to chew	Texture	Dry matter content	No	16 genotypes	Yes

Next steps & activities planned on the establishment of proofs of concept:

At CIRAD-France, the perspectives are to analyze all the 52 varieties from Guadeloupe for pectin, amylose and starch contents. At CIRAD-France, Yam samples from Benin already characterized for texture will also be analysed. CIRAD shall also train RTBfoods partners on the SOP for pectin analysis.

At INRAe-France, in Period 5, reproducibility will be done for these biochemical attributes of some alata and esculenta Yam samples in order to have the biochemical signature of a second harvest year and with a more adapted steaming time on the one hand, and these biochemical attributes will be determined on other Yam species and varieties on the other hand, in order to reach more robust conclusions concerning the mechanisms during cooking. Moreover, MIRS analysis will be done in parallel on the same samples.

At UAC-FSA Benin, in Period 5, 4 additional varieties will be characterized regarding the biochemical properties targeted. In addition, starch content, amylose contents and sugar profile will be determined on all Yam varieties using enzymatic, DSC and HPLC methods, respectively.

At IITA, Nigeria, the perspectives for period 5 are to analyze all the varieties provided by WP4 for cooking time, water absorption, and biochemical parameters. SOPs for textural analysis will be validated by working closely with the UAC-FSA and the RTBfoods focal person for texture analysis. The correlation between boiled Yam's sensory and instrumental texture attributes will be revalidated in period 5.

4.5.5 Scientific collaborations between partners

There was an effective interaction between IITA and UAC-FSA, especially in the area of development and sharing of protocols. There was information sharing during the RTBfoods-Africa Yam workshop held in Cotonou, Benin.

NRCRI, CIRAD and UAC-FSA have worked on protocols for priority traits of boiled Yam and shared the protocol for sensory evaluation and texture evaluation of boiled Yam.

In addition, CIRAD and INRAe are in touch about the SOP for amylose determination and analysis of pectins.

4.5.6 Next steps on Boiled Yam

The focal points (on sensory and texture) recommend to consolidate / validate the relationships between sensory hardness and instrumental hardness (3 partners); penetrometry test should be promoted as it allows to discriminate samples and giving measures correlated with sensory hardness. In addition, new (or improved; with a better control of temperature, higher number of replicates etc) instrumental textural measurements should be developed to find relationships with PQT (crumbly, chewiness).

It is also recommended to enlarge the sampling and the analysed chemical components (soluble sugars and polyphenols, in particular) to identify the chemical bases of the colour and taste of boiled Yam (all partners).

Concerning the biochemical bases of texture, a better collaboration between partners will be favoured in period 5 to promote exchanges on procedures for pectins and methylation, starch, and polyphenols determinations and exchanges of samples. SOPs are available (pectins) and will be shared, others should be finalized (starch) or written (polyphenols) after exchanges between partners.

An increased number of samples will be analysed in period 5 to confirm/improve the correlations between biophysical results (pectin level, methylation degree, starch and amylose contents etc) and sensory or texture evaluations. Samples will be shared between partners to check analytical results. In addition, the kinetic evolution of starch (gelatinization) and of pectin (degradation) during cooking will be studied (CIRAD) in order to predict the cooking behaviour and texture form these biophysical characteristics, and a characterization of cell wall components on a larger set of samples will be perform to try to identify other cell wall components that can contribute to the texture of boiled Yam (INRAe)

Concerning rapid kitchen tests, a larger set of samples will be tested to validate the WAB procedure (at IITA) and the hand-held penetrometer (at NRCRI) for predicting the cooking behaviour and texture of boiled Yam.

4.6 Pounded Yam

4.6.1 Sensory evaluation in Nigeria

One SOP is available for the sensory characterization of pounded Yam: Bolanle OTEGBAYO, Abiola TANIMOLA, Oroniran OLUINKA, (2021). Sensory Characterization of Pounded Yam. Biophysical Characterization of Quality Traits, WP2. Iwo, Nigeria: RTBfoods Laboratory Standard Operating Procedure, 15 p. This SOP is used by NRCRI and Bowen University, in Nigeria.

The sensory descriptors used for sensory evaluation of pounded Yam are:

- Colour
- Stretchability
- Mouldability
- Softness/hardness
- Stickiness

- Smoothness

Key priority Quality Traits identified by Bowen University-Nigeria and that must absolutely be passed on to breeders for integration as breeding priorities are **color, and textural attributes** (**Soft, Smooth/no lumps, Stretchable, Mouldable/cohesive, Not-Sticky/adhesive)**

Nb of samples & Nb of genotypes characterized by the sensory panel in 2021

At **Bowen University-Nigeria**, 13 WP4 samples from IITA breeding population have been characterized in 2021, in addition to 4 varieties previously used for 'Consumer Testing' within WP1 step 4, both fresh and stored samples. In total, 21 different samples have been characterized by the sensory panel at Bowen University in 2021.

At **NRCRI-Nigeria,** this experiment is to be conducted in period 5 after the SOP for texture evaluation of pounded Yam is validated.

Key results & graphs:

Sensory data analysis is on-going at Bowen University-Nigeria.

4.6.2 Textural evaluation & correlation with sensory descriptors in Nigeria

A first draft of SOP for instrumental textural characterization of pounded Yam is available, developed by Bowen University and reviewed by the CIRAD focal person for texture.

Nb of samples & Nb of genotypes characterized for texture in 2021

At **Bowen University-Nigeria**, the same 21 samples characterized by the sensory panel have been used to develop the pending SOP for instrumental texture, in 2021.

List of key sensory descriptors for texture (generated & used by trained panellists) to be measured instrumentally:

At **Bowen University-Nigeria**, the key sensory descriptors for texture to be measured instrumentally are softness/hardness, stretchability, cohesiveness, stickiness/adhesiveness

Level of progress in the establishment of a correlation with instrumentally measured parameters:

At **Bowen University-Nigeria,** 13 samples (7 *rotundata* and 6 *alata*) have been completely analysed using TPA. The result of Pearson correlation analysis and correlation probabilities between Instrumental texture parameters and descriptive sensory evaluation parameters for pounded Yam made from *D. alata* and D. *rotundata* varieties combined are presented in Table 13

Table 13 Correlation analysis between instrumental texture parameters and descriptive sensory evaluation parameters for pounded Yam

Instrument	Sensory				Instrumental						
	Adhesiveness	Moudability	Stretchability	Hardness	Hardness	Adhesiveness	Stickiness	Stringiness	Resilience	Cohesiveness	Springiness
Hardness	-0.498	0.507	0.257	0.165	1						
Adhesiveness	0.173	-0.347	-0.471	-0.048	0.065	1					
Stickiness	0.337	-0.193	-0.304	-0.318	-0.044	0.863	1				
Stringiness	-0.320	-0.390	-0.522	0.423	0.202	-0.065	-0.147	1			
Resilience	-0.094	0.213	-0.022	-0.138	0.665	0.735	0.593	-0.006	1		
Cohesiveness	0.010	0.839	0.907	-0.315	0.307	-0.645	-0.451	-0.302	-0.124	1	
Springiness	-0.320	-0.390	-0.522	0.423	0.202	-0.065	-0.147	1	-0.006	-0.302	1

The probabilities highlighted in red are significant at 5 % level

The only significant correlations between sensory and instrumental textural attributes are (figure 38):

- Instrumental cohesiveness and sensory mouldability (r = 0.839, P = 0.0003)
- Instrumental cohesiveness and sensory stretchability (r = 0.907, P < 0.0001)

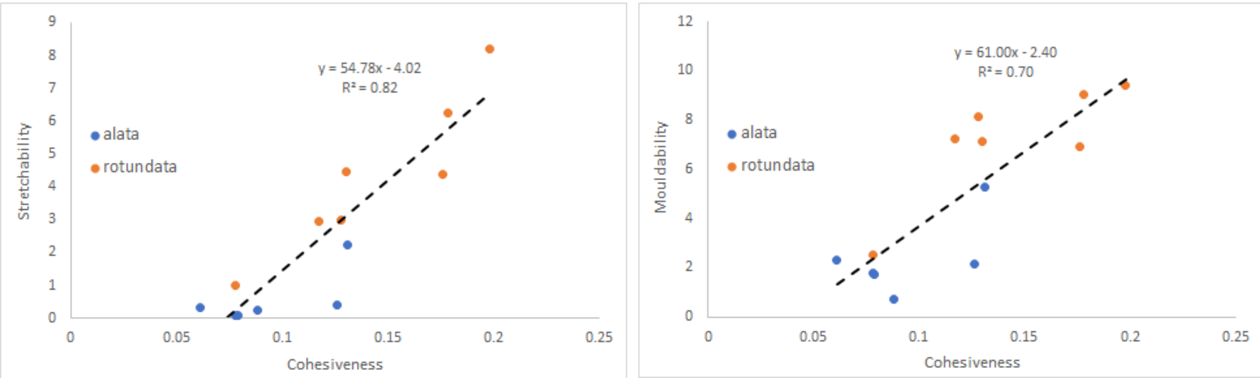


Figure 38 Correlations between instrumental cohesiveness and sensory stretchability and mouldability

Also, a validation exercise was conducted for instrumental textural characterization by the focal person in CIRAD, for which four genotypes of contrasting textural attributes were considered. The experiment included 2 replicates per genotypes and 14 measurements per replicate. Results (Figure 38) showed good repeatability between replicates within the genotypes except for one genotype; good discrimination between the genotypes regarding the textural attributes; and significant differences between the genotypes by ANOVA. Generally, adhesiveness and stickiness were poor discriminating textural quality attributes between the genotypes. The more discriminatory textural attributes were stiffness/hardness, chewiness, springiness, gumminess, and cohesiveness.

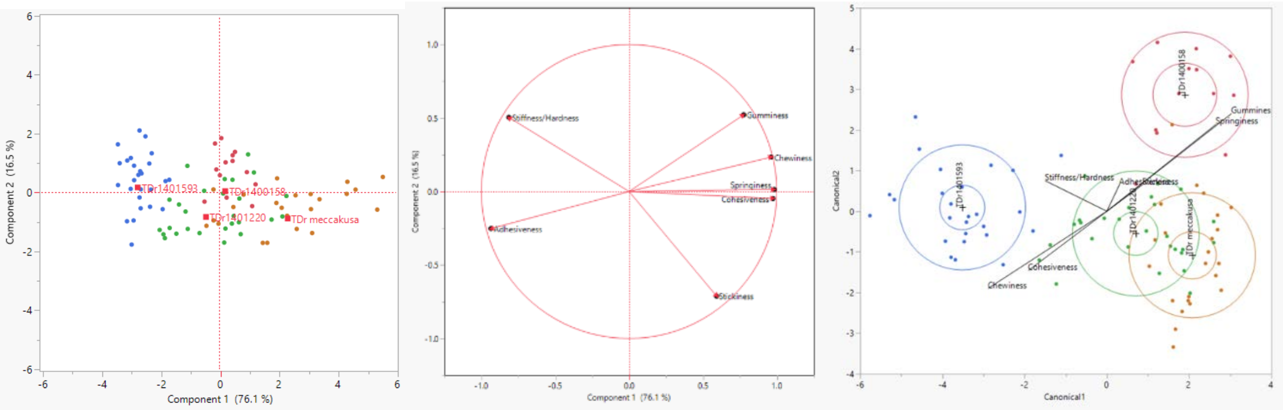


Figure 39 Validation results of PCA, discriminant and hierarchical analyses for textural attributes of pounded Yam made from 4 contrasting Yam genotypes.

Synthesis on progress achieved:

NRCRI and Bowen University in Nigeria share the same SOP for sensory analysis.

A SOP for instrumental texture analysis being validated at Bowen University in early Period 5 (a first draft is available). The focal person for texture at CIRAD conducted a validation exercise of the instrumental textural characterization SOP where four genotypes of contrasting textural attributes were considered. For predictions of sensory attributes with instrumental texture, so far only one correlation between instrumental cohesiveness and sensory stretchability and mouldability was observed.

Fill-in this summary table:

Partner Institute	**Targeted Quality Trait / Sensory Descriptor**	**Instrumental Method for Measurement on Final product**				
		Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
Bowen University -Nigeria	Softness/ hardness	Texture analyzer and sensory evaluation	Yes	Yes	21	13
	Stretchability	Sensory evaluation	Yes	No, but other tests will be performed to evaluate stretchability instrumentally	21	13
	Cohesiveness	Texture analzyer and sensory evaluation	Yes	Yes	21	13
	Stickiness&adhesiveness	Texture analyzer & sensory evaluation	Yes	Yes	21	13
	Instrumental Texture characterization (hardness, chewiness, gumminess,	Texture analyzer TPA	Pending. First draft available, reviewed.Final validation awaited.	No	4 genotypes, 2 replicates per genotype. 14 measurements per	4

Partner Institute	Targeted Quality Trait / Sensory Descriptor	Instrumental Method for Measurement on Final product				
		Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
	cohesiveness and springiness)				measurement	
NRCRI-Nigeria	Colour	hromameter	YES	none	none	none
	Texture	Texturometer TPA test	Pending	none	none	none

Next steps & activities planned to measure key sensory descriptors for texture instrumentally:

The SOP for textural characterization will be validated during period 5, based on the validation exercise performed during period 4.

At **NRCRI-Nigeria**, sensory and texture of pounded Yam will be conducted using 15 genotypes (7 alata, 7 rotundata from African Yam project and a preferred land race) in period 5. Data generated will be used to establish a correlation between the sensory properties of pounded Yam (especially the prioritized traits) and related texture parameters.

Since stretchability is a very important or key attribute in pounded Yam, **Bowen University-Nigeria** is to develop an objective (instrumental) MTTP or HTTP method to measure it in pounded Yam. Rheological protocol will be planned and conducted at **CIRAD-France** to evaluate the viscoelastic properties of this pasty product.

4.6.3 Rapid / intermediate kitchen tests to assess the processing & the cooking ability of pounded Yam

Rapid kitchen test or intermediate assessment methods investigated:

At NRCRI-Nigeria, the rapid kitchen test investigated is meant to assess the **pounding ability** of Yam. A preliminary assessment of the pounding ability of 13 Yam genotypes harvested from African Yam project multilocation trial cited in two locations in Nigeria was carried out at NRCRI in period 4. 3 tubers per genotype were selected and their length measured. Each of the tubers was cut into proximal, central and distal and thereafter peeled and washed. From the proximal and distal parts, 1/10 and 3/10 was cut off at the bottom and head section and discarded while for the central part, 2/10 was cut off from both ends of the central section and discarded. Each part (6/10) is divided following the length into two subsections. 820ml of water was used to boil the Yam samples, remnant of the boiling water was measured to ascertain the quantity absorbed by the Yam sample. Different sections of boiled Yam samples were pounded subsequently using the Yam pounder following the protocol developed by Bowen University. Processing parameters of the Yam samples were taken before, during cooking and pounding. Dry matter content of the samples was also evaluated using standard methods. Data obtained showed that head section had higher dry matter content and took longer pounding time compared to the middle section, however no significant statistical difference was observed between the pounding time of the head region and the tail region. This study will however be repeated in period 5.

Synthesis on progress achieved:

Experiments conducted in Period 4 at NRCRI-Nigeria indicate differences in dry matter and pounding time among the 13 genotypes characterized. A possible correlation between dry matter and pounding time was also identified. This needs to be repeated and confirmed in Period 5.

Next Steps in the development of rapid / intermediate kitchen assays for pounded Yam

At NRCRI-Nigeria, Further experiments of Yam pounding will be conducted using the Yam harvests planned in Period 5, in order to expand the dataset from Period 4 and verify the possible correlation between dry matter and pounding time. Such correlation may enable to predict pounding time, a key indicator of pounding quality of Yam, with simple dry matter measurements.

RVA experiments with mashed fresh Yam tubers and with Yam flour will be conducted, to investigate the possibility to predict ease of dough formation using RVA parameters. If significant correlations are identified, a SOP will be developed.

4.6.4 Dissection and understanding of key quality traits for pounded Yam

Quality trait(s) of focus/investigated:

<table>
<tr><th>PRODUCT Presentation (Stage)</th><th colspan="2">TRAIT</th><th>Biophysical candidate</th><th>Proof of Concept</th></tr>
<tr><td rowspan="2">Raw Yam</td><td colspan="2">Low water content on Yam tuber</td><td>Dry matter</td><td>Established</td></tr>
<tr><td colspan="2">No colour change</td><td>Polyphenol oxidase enzyme activity, Tannin, Soluble sugars , total phenol, browning index, phenolic profile</td><td></td></tr>
<tr><td>Processing</td><td colspan="2">No colour change</td><td>Polyphenol oxidase enzyme activity, Tannin, Soluble sugars thermal degradation during cooking</td><td>Proof of concept on phenolic profile of Yam tubers may be developed</td></tr>
<tr><td>Intermediate product (Boiled Yam)</td><td colspan="2">Easy to pound boiled Yam</td><td>Starch and amylose, crude fiber</td><td>To be developed</td></tr>
<tr><td rowspan="2">End product (Pounded Yam)</td><td colspan="2">Colour in pounded Yam</td><td>thermal degradation during cooking, Polyphenol oxidase enzyme activity, Tannin, Soluble sugars</td><td>Proof of concept on phenolic profile of Yam tubers may be developed</td></tr>
<tr><td>Textural qualities :</td><td>Hardness, smoothness, strectchability, mouldability, stickiness Soft/hardness</td><td>Dry matter, Starch, Amylose, non-starch carbohydrates (Pectin, hemicellulose, cellulose, lignin)
Functional properties of Starch : Swelling capacity, Gelatinization, Water absorption capacity, Water binding Capacity, Pasting characteristics</td><td>To be developed</td></tr>
</table>

Level of progress in the development of biochemical proof of concept / method to measure the biochemical attributes of raw material

At Bowen University-Nigeria, 22 Yam varieties are being analysed; results are presented for the 13 varieties analysed. Several biophysical analyses on raw Yam have been performed in parallel to sensorial evaluation and texture instrumental measurements:

- **Analyses carried out on raw Yam tubers include:**
  - Starch granules morphology (Otegbayo *et al*., 2011).
  - Dry matter content (SOP RTBfoods)
  - PolyPhenol oxidase determination
  - Instrumental colour evaluation

- Pasting characteristics of fresh Yam paste (RVA, Perten Instruments of Australia, 2015)

- **Analyses carried out on Yam starch tubers:**
  - Swelling and solubility
  - Water binding capacity
  - Water absorption capacity
  - Pasting characteristics of Yam starch
- **Chemical Analyses of Yam flour: (Done using NIRS at IITA)**
  - Proximate composition
  - Starch and sugar content
  - Amylose and amylopectin
  - Anti-nutritional factors (Tannin and Phytate)

Few correlations between sensorial (or instrumental texture measurements) and biophysical analyses could be evidenced, but ash content and mouldability appeared correlated (Figure 40): the more ash content, the less the mouldabity. This can be an interesting rapid test for breeding. In addition, this can be linked to the role of pectins; the more the divalent cations, the more the pectins are complexed.

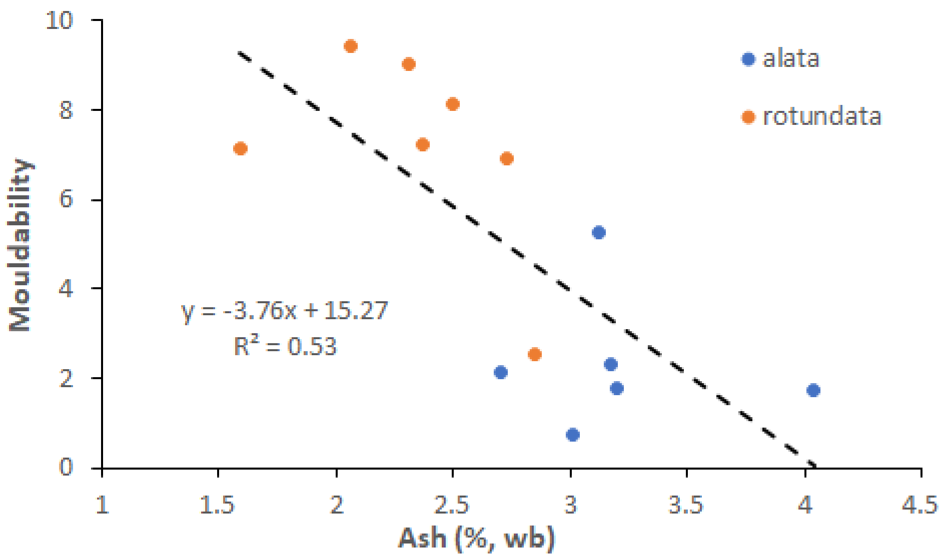


Figure 40 Correlation between ash content and mouldability

Synthesis on progress achieved:

At Bowen University-Nigeria, several biophysical analyses on 14 raw Yams have been performed in parallel to sensorial evaluation and texture instrumental measurements. Additional analyses are on doing on 8 samples. A candidate for predicting texture is ash content

Next steps & activities planned on the establishment of proofs of concept:

At Bowen University-Nigeria, finalizing the analyses on the 8 samples is a first step for identifying biophysical candidates for predicting pounded Yam quality. In addition, other analyses on starch and cell wall components could be an interesting track for the texture while the polyphenols and polyphenol oxidase activity can be used for predicting colour of pounded Yam. A proof of concept on total phenol content as a measure of rate of browning or phenolic profile of the Yam tubers, is considered to be performed in Period 5.

The focal point for texture encourages the partner to perform their analyses with reference procedures (SOPs when available) in place of NIRS prediction when establishing the PoC. NIRS calibration can be performed in parallel, but NIRS determination is not sufficiently precise at this step. A collaboration with other partners working on Yam (boiled and/or pounded) for the analyses will also a good track as samples and analyses could be exchanged and as biophysical bases of quality can be close for both PPs.

4.6.5 Scientific collaborations between partners

NRCRI and Bowen University have interacted extensively on different activities within the work plan for pounded Yam in period 4.

In addition, CIRAD visited Bowen University, tested the SOP for texture and proposed improvements.

4.6.6 Next steps on Pounded Yam

Since stretchability is a priority quality trait (PQT) of pounded Yam, **Bowen University-Nigeria** and **CIRAD** will act to develop an objective (instrumental) MTPP or HTPP method to measure it in pounded Yam in Period 5, by adapting TPA and/or using new textural analysis such as Lubricated Squeezing Flow and/or rheological measurements. Samples will be evaluated by sensory analysis in parallel. This procedure may be used by **NRCRI**, too.

Concerning the biochemical bases of texture of pounded Yam, a better collaboration between partners will be favoured in period 5 to promote exchanges on procedures for pectins and methylation, and starch determinations and exchanges of samples. SOPs are available (pectins) and will be shared, others should be finalized (starch) after exchanges between partners; analysis through NIRS should not be used at this step.

4.7 Boiled Sweetpotato

4.7.1 Sensory evaluation in Uganda

A SOP is available for sensory characterization of boiled sweetpotato: Mariam NAKITTO (2020). SOP for Sensory Evaluation on Boiled Sweetpotato. Kampala, Uganda: RTBfoods Project Report, 17 p. The sensory descriptors used by the panellists to profile sweetpotato samples are shown in Table below.

Category	Attributes
Orthonasal aroma (odor)	Sweetpotato aroma, caramel aroma, pumpkin aroma, off odour
Appearance	Orange color intensity, uniformity of color, degree of translucency, fibrous appearance
Retronasal aroma	Sweetpotato flavor, pumpkin flavor, cooked carrot flavor, floral flavor
Basic taste	Sweet taste, bitter taste
Texture in mouth	Fracturability, firmness in mouth, crunchiness, moisture in mass, crumbliness in mouth, adhesiveness, fibrousness, smoothness, rate of breakdown
Texture by hand	Hardness by hand, mealiness by hand, moisture in mass, cohesiveness

In Uganda, the priority quality traits identified during preference surveys include firmness, mealiness, sweetness and fibrousness. However, other attributes that are important for profiling sweetpotato genotypes by breeders include orange color intensity and aroma attributes.

Nb of samples & Nb of genotypes characterized by the sensory panel in 2021

A total of 112 samples, representing 62 genotypes from the 2020 B and 2021 A seasons' trials were characterised by the sensory panel in 2021. To track the performance of the sensory panel, at least one genotype was served in duplicate during each session. Briefly, the samples for sensory analysis were from WP4 advanced trials for both the 2020B and 2021 harvest across 5 sites (data collection is still ongoing), the Mwanga Diversity Panel (MDP) trial at Namulonge (40 genotypes) and an activity where varieties which had been used in WP1 were analysed for sensory and texture to link WP1 and WP2 (Table 2). Data processing for the last activity is also still ongoing and will be reported later.

Table 14. Number of genotypes characterized by sensory panel in 2021 by type of trial

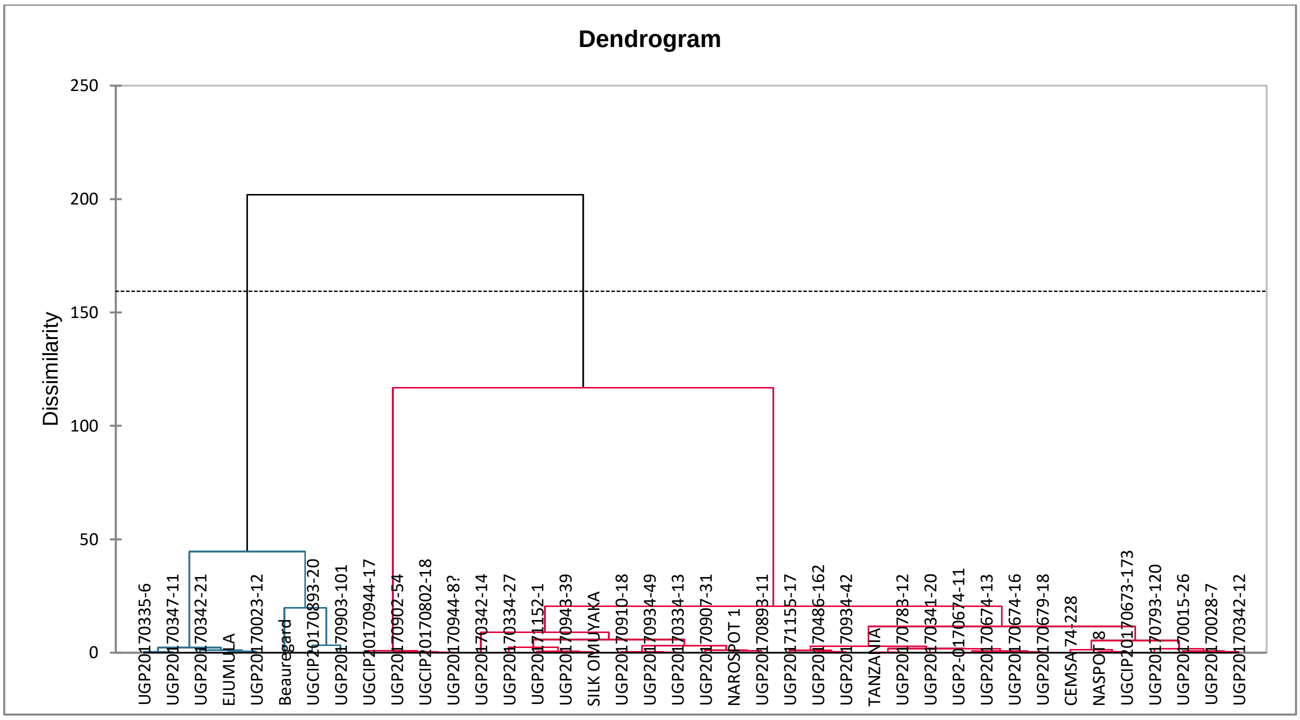
Harvest	Number of genotypes	Objective	Progress
2020B multi-location advanced trial			
Rwebitaba	12	To validate instrumental texture SOP with sensory firmness To pilot methods correlating descriptive sensory profiles to consumer preference	Complete
Serere	5		
2021A MDP trial			
Namulonge	40	To generate sensory profiles of genotypes in MDP trial	Data collection completed, analysis ongoing
2021A multi-location trial of advanced clones			
Namulonge	14	To compare sensory profiles of genotypes planted in different locations (GXE study)	Data collection completed, analysis ongoing
Arua	11		Data collection completed, analysis ongoing
Serere	11		Data collection completed, analysis ongoing
Hoima	At least 11		Data collection ongoing
Rwebitaba	At least 11		Data collection ongoing
WP1 genotypes from farmers			
Namulonge	8	To establish consumer preferred levels/ thresholds of sensory and biophysical measures	Data collection completed, analysis ongoing

Key results & graphs:

2020 B multi-location trial: Results from the activity were used to the validate instrumental texture SOP and presented in a separate report. In brief and a summary will be provided under the text on texture in the following section.

MDP trial Namulonge harvest: During period 4, forty out of the 220 genotypes of the MDP trial at Namulonge were characterised by the sensory panel for 17 attributes. The flesh colour of boiled sweetpotato roots ranged from white to deep orange. The scores for sweet taste ranged from 1.8 to 7.7. The scores for hardness by hand ranged from 1.0 to 8.8 while mealiness by hand ranged from 0.7 to 9.3. Due to variation among the genotypes, the set of samples is expected to effectively support efforts to develop models for the high throughput phenotyping under WP3. A Principal Component Analysis (PCA) was conducted on the number of scores for the four priority quality attributes (firmness, mealiness, sweetness and fibrousness) with sweetpotato varieties as the observation labels, and the mean water absorption and dry matter content of each sample as a supplementary quantitative variable.

The Agglomerative Hierarchical Cluster analysis of the forty genotypes of the Mwanga diversity panel characterised for the priority quality attributes (viz, firmness, sweetness, mealiness and fibrousness) of sweetpotato resulted in 2 distinct groups namely, 'sweet, firm and mealy boiled roots with ' *UGP20170893-11* (80%) and 'sweet, soft and less mealy' boiled sweetpotato roots with '*UGP20170335-6'* (20%) as the central genotype (**Figure 40).**



The PCA plot explained 92.61% of priority quality sensory characteristic variance (F1 76.20% and F2 16.41%). Most of the variance was explained by the first axis (**Figure 41**).

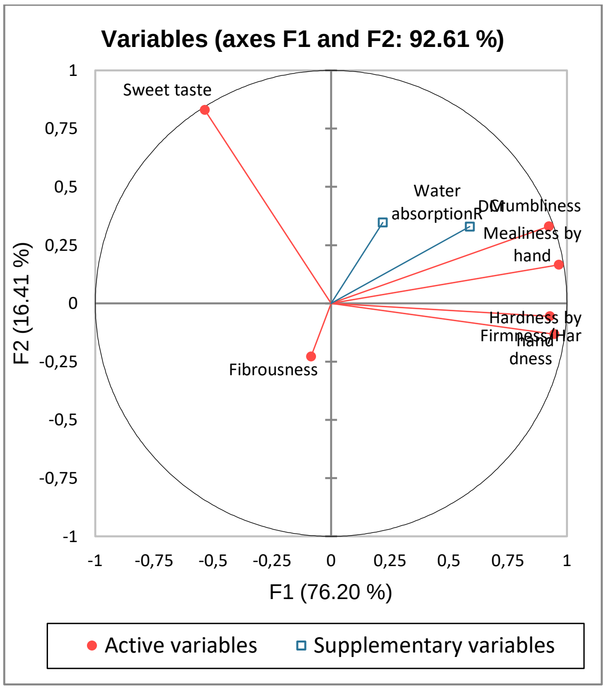


Figure 41 Principal Component Analysis plot for priority quality sensory characteristics of boiled sweetpotato

2021 A multi-location trial of advanced clones: There are 14 genotypes in the trial including 9 test clones (1.44, D11, D15, D20, D26, NKB3, NKB105, S36, S47, and S97) and 5 checks (Ejumula, NASPOT 8, NASPOT 10 O, NASPOT 11, and New Kawogo). These have been grown in 5 locations: Namulonge, Arua, Serere, Hoima and Rwebitaba (Fort Portal). Sweetpotato roots samples have been collected from each of these areas and taken to the lab for evaluation by the panel, depending on the number of roots available. The data will be used to compare the sensory profiles of genotypes from different locations.

4.7.2 Textural evaluation & correlation with sensory descriptors in Uganda

The SOP for instrumental textural characterization of hardness has been under revision at CIP-Uganda, since Period 2. During Period 4, more experiments were conducted to identify a suitable probe and also validate the drafted SOP.

Nb of samples & Nb of genotypes characterized for texture in 2021

At CIP-Uganda, a total of 125 samples, representing 122 genotypes from the 2020 B and 2021 A seasons' trials were characterised for texture in 2021. Briefly, the samples for texture analysis were from WP4 advanced trials for both the 2020B and 2021 harvest across 5 sites (data collection is still ongoing), the MDP trial at Namulonge (110 genotypes) and an activity where varieties which had been used in WP1 were analysed for sensory and texture to link WP1 and WP2 (Table 14). Data processing for the last activity is also still ongoing and will be reported later.

List of key sensory descriptors for texture (generated & used by trained panellists) to be measured instrumentally:

The key sensory descriptor for texture to be measured instrumentally is hardness.

Level of progress in the establishment of a correlation with instrumentally measured parameters:

Dry matter content correlated slightly with sensory hardness (r=0.500) and mealiness (r = 0.717) by hand, but not with the same evaluated in the mouth. A moderately strong correlation was found between water absorption and sensory mealiness by hand and in mouth (r = 0.418 and r = 0.424, respectively) and sensory firmness by hand and in the mouth (r=0.297 and r=0.314). The optimal cooking time (OCT) was not correlated to any of the sensory attributes, or with instrumental texture parameters. Mealiness and hardness by hand and in the mouth were correlated to instrumental texture parameters, in particular Positive force 1 (Table 15). Consequently, a model was developed to predict sensory firmness in the mouth, from the positive force 1 (instrumental texture), water absorption and optimal cooking time (Table 16). The RMSEC and RMSEV were 1.0 and 0.9, respectively. The model was used to predict values of sensory firmness for 89 genotypes from MDP trial analysed by instrumental texture, optimal cooking time and water absorption.

Table 15 Pearson correlation analysis of sensory texture attributes with OCT, water absorption, dry matter and parameters of instrumental texture analysis of the MDP clones.

Variables	OCT	Water absorption	Dry matter	Positive force 1	Positive force 2	Positive area 1	Positive area 2
Hardness by hand	0.164	**0.297**	**0.500**	**0.731**	**0.633**	**0.734**	**0.649**
Moisture release	0.070	**-0.384**	**-0.595**	**-0.270**	**-0.226**	**-0.344**	**-0.218**
Cohesiveness	-0.119	**-0.310**	**-0.517**	**-0.629**	**-0.496**	**-0.646**	**-0.553**
Crumbliness/mealiness by hand	0.047	**0.418**	**0.717**	**0.544**	**0.400**	**0.604**	**0.439**
Fracturability	0.130	**0.362**	**0.643**	**0.637**	**0.521**	**0.676**	**0.551**
Firmness/Hardness	0.142	**0.314**	**0.568**	**0.716**	**0.608**	**0.712**	**0.630**
Crunchiness	0.111	0.153	0.120	**0.565**	**0.472**	**0.500**	**0.477**
Moisture in mass	-0.048	**-0.382**	**-0.683**	**-0.585**	**-0.473**	**-0.626**	**-0.505**
Crumbliness	0.099	**0.424**	**0.717**	**0.554**	**0.420**	**0.612**	**0.463**
Adhesiveness (Stickiness)	-0.080	**-0.216**	**-0.283**	**-0.191**	-0.074	**-0.216**	-0.131

Variables	OCT	Water absorption	Dry matter	Positive force 1	Positive force 2	Positive area 1	Positive area 2
Fibrousness	0.021	-0.071	**-0.327**	-0.172	-0.121	**-0.212**	-0.154
Smoothness	-0.112	**-0.192**	**-0.354**	**-0.660**	**-0.575**	**-0.608**	**-0.600**
Rate of breakdown	-0.093	**-0.236**	**-0.518**	**-0.686**	**-0.581**	**-0.670**	**-0.606**
OCT	**1**	0.087	0.073	**0.270**	**0.311**	**0.275**	**0.344**
Water absorption	0.087	**1**	**0.410**	**0.183**	0.104	**0.207**	0.106
Dry matter	0.073	**0.410**	**1**	**0.461**	**0.371**	**0.539**	**0.394**
Positive force 1	**0.270**	**0.183**	**0.461**	**1**	**0.936**	**0.953**	**0.933**
Positive force 2	**0.311**	0.104	**0.371**	**0.936**	**1**	**0.871**	**0.972**
Positive area 1	**0.275**	**0.207**	**0.539**	**0.953**	**0.871**	**1**	**0.881**
Positive area 2	**0.344**	0.106	**0.394**	**0.933**	**0.972**	**0.881**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Table 16 Model parameters for predicting sensory firmness from OCT, water absorption, parameters of instrumental texture analysis of in the MDP population

<table>
<tr><th>Source</th><th>Value</th><th>Standard error</th><th>t</th><th>Pr > |t|</th><th colspan="4">Model robustness</th></tr>
<tr><td>Intercept</td><td>1.896</td><td>0.418</td><td>4.531</td><td><0.0001</td><td colspan="2">r^2</td><td rowspan="2">RMSEC</td><td rowspan="2">RMSEV</td></tr>
<tr><td>OCT</td><td>-0.030</td><td>0.017</td><td>-1.731</td><td>0.089</td><td>Training</td><td>Validation</td></tr>
<tr><td>Water absorption</td><td>0.084</td><td>0.058</td><td>1.456</td><td>0.151</td><td rowspan="2">0.58</td><td rowspan="2">0.70</td><td rowspan="2">1.0</td><td rowspan="2">0.9</td></tr>
<tr><td>Positive force 1</td><td>0.001</td><td>0.000</td><td>8.152</td><td><0.0001</td></tr>
</table>

This information has been used to select 94 genotypes that will be genotyped by Intertek for validating the KASP markers for firmness in period 5.

Synthesis on progress achieved:

A new texture method has been developed, optimized and approved by CIRAD texture focal point. A new texture SOP has then been made available.

Significant correlation was found between sensory firmness by hand and in mouth with positive area measured by TPA (R=0.748, 0.715, respectively).

A paper has been submitted for publication by CIP on sensory and textural attributes of boiled sweetpotato. Mariam Nakitto, Suzanne D. Johanningsmeier, Mukani Moyo, Christophe Bugaud, Nelly Forestier-Chiron, Layal Dahdouh, Julien Ricci, Christian Mestres, Henriette de Kock, Tawanda Muzhingi, Sensory guided selection criteria for breeding consumer-preferred sweetpotatos in sub-saharan africa, Food Quality and Preference.

Institute-Country	**Targeted Quality Trait / Sensory Descriptor**	**Instrumental Method for Measurement on Final product**				
		Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
CIP-Uganda	Hardness	Instrumental texture	Yes	Yes	>140	>90
		Optimal cooking time	Yes	No	>140	>90

Next steps & activities planned to measure key sensory descriptors for texture instrumentally:

The focal points (on sensory and texture) recommend:

- On texture attributes:
  - Firmness: validate the relationship between firmness perceived by panellists and instrumental parameters.
  - Fibrousness: try to find easy-to-measure method to assess this indicator
  - Mealiness: try to find easy-to-measure method to assess this indicator. Textural parameters are known to be not relevant for predict mealiness.
- On colour attributes: Try to find relationships between pulp colour and chromameters parameters by using relevant indicators as Hue angle, which is a yellow-orange indice
- On taste attributes: Try to find relationships between sweetness and sugar content.

4.7.3 Rapid / intermediate kitchen tests to assess the processing & the cooking ability of boiled sweetpotato

Rapid kitchen test or intermediate assessment methods investigated:

At CIP-Uganda, the rapid kitchen test for boiled sweetpotato investigated in period 4 was to assess cooking ability and mealiness through water absorption. In this method, the pieces sweetpotato prepared following the sample preparation method for instrumental texture analysis are weighed before and after steaming to quantify the amount of water retained or lost during cooking. Water absorption did not show a significant correlation with mealiness (Pearson correlation coefficient = 0.201). Additional work of starch characterisation in South Dakota State University and NaCRRI is expected to contribute to a biochemical proof of concept.

Synthesis on progress achieved:

A tentative procedure for water absorption is under development. The procedure is still not finalized and the correlations with sensory and instrumental texture are not sufficient.

Next Steps in the development of rapid / intermediate kitchen assays for boiled sweetpotato

Further experiments will be organized with the sweetpotato harvests planned during Period 5, so as to expand the datasets of instrumental and sensory characterizations, and/or to adapt the procedure for water absorption. In particular, regression models with a dataset containing more samples will be developed and will help test if water absorption could be an interesting predictor of sensory mealiness. Additionally, starch characterization by RVA to further understand the relationship is planned.

4.7.4 Dissection and understanding of key quality traits for boiled sweetpotato

Quality trait(s) of focus/investigated:

At CIP-Uganda, research activities focused on understanding hardness and mealiness which are two key priority quality traits for boiled sweetpotato, coming from preference surveys. Biochemical attributes investigated at CIP-Uganda re dry matter and starch chemical and physical properties (RVA).

At JHI-UK, research activities focused on boiled sweetpotato texture. The hypothesis is that the texture of boiled sweetpotato correlates with aspects of cell wall structure and/or starch characteristics. This is based on exemplars for other RTB crops such as potatoes where the degree of pectin methylation correlates with cooking time and textural characteristics, and emerging data from sweetpotato which implicates starch composition as an important component of cooking time.

Level of progress in the development of biochemical proof of concept / method to measure the biochemical attributes of raw material

At CIP-Uganda, dry matter was evaluated using the SOP made available through the RTBfoods website. Dry matter was not correlated with sensory results based on the characterization of the 12 genotypes population also used to develop the SOP on instrumental texture analysis. However, when data collected throughout Period 4 were pooled together and analysed using Pearson correlation, dry matter was found to correlate slightly with sensory firmness (0.500) and mealiness (r = 0.717).

During Period 4, we planned to send at least 40 sweetpotato freeze-dried powder samples to the food bioscience's lab at NaCRRI for characterisation of starch gelatinization properties using the SOP for RVA. Unfortunately, due to the frequent breakdown of the freeze dryer the activity could not progress and will be continued in Period 5 in NaCRRI labs. Starch characterization will be performed at South Dakota State University on starch samples from the MDP trial to understand granule size and packing through scanning electron microscopy (SEM) and x-ray diffraction (XRD).

At JHI-UK, the research activities focused on cell walls. To study cell walls, one needs to isolate them. Preferably they should be isolated in pure form with the minimum of structural degradation. A de-starching step is necessary before beginning cell wall analysis. A SOP for the isolation of sweetpotato cell walls has been developed and is under review. The method is relatively small scale and high throughput. The preparation uses 2.5 g of milled freeze-dried sweetpotato root powder and routinely yields ca. 10% dry weight of cell walls that can be used for further analysis.

The monosaccharide composition of the cell walls of sweet potatoes (or their derived polysaccharides) can be informative about the types and amounts of polysaccharides present. This information can be correlated back to different textural properties noted in different genotypes or varieties. CWM and polysaccharide solids are hydrolyzed by heating in 2 M trifluoracetic acid at 120 ^{0}C for 1 h. Liquid chromatography-based methods can be used to detect both neutral and acidic sugars in hydrolyzed cell wall samples. High-performance anion-exchange chromatography (HPAEC) coupled with electrochemical detection (ECD) allows for direct analysis of monosaccharides and oligosaccharides without derivatization or labeling. It uses high pH (pH 12–13) to partially deprotonate the sugar hydroxyl groups, yielding sugar anions that can be separated on anion-exchange columns designed to function at high pH (Dionex Ltd). A SOP for this method has been prepared and is under review. Freeze dried sweetpotato root samples were provided by CIP Uganda and cell walls were prepared from these. The monosaccharide composition of these cell wall samples was determined as described above. In Uganda, the cooking time for these samples had been established previously. No correlation was observed between cooking time and the level of any cell wall monosaccharides or indeed any grouping of monosaccharides that could be associated with a particular cell wall polysaccharide. Indeed, the focus was on mixes that could be associated with pectic arabinogalactans (see appendix).

Fourier Transform infra-Red (FTiR) spectroscopy is a powerful and rapid technique for analyzing cell wall components and putative cross-links, which is able to non-destructively recognize polymers and functional groups and provide abundant information about their *in muro* organization. FTiR spectroscopy has been reported to be a useful tool for monitoring cell wall changes occurring *in muro* as a result of various factors, such as growth and development processes, mutations or biotic and abiotic stresses. Of particular value in this project, is the use of FTiR spectroscopy to investigate differences in the degree of esterification of pectin, a major cell wall component. We developed a SOP for analysing sweetpotato cell walls by FTiR and analysed cell wall preparations from 18 genotypes for which cooking time data were available (from CIP Uganda). Ratio of signals at 1730 and 1625 cm^{-1} and 1415 and 1235 cm^{-1} have been associated with the degree of pectin methylation in potato cell walls and textural properties of the tubers (Ross et al. (2011) Pectin engineering to modify product quality in potato. Plant Biotechnology Journal **9**, pp. 848–856 - doi: 10.1111/j.1467-7652.2011.00591.x). These can be used to discern the relative level of pectin methylation. Although there was significant variation in this ratio between the samples from the different genotypes, these was no significant correlation of this parameter with cooking time (see appendix). Indeed, further work showed that there were no spectral features that correlated with the pattern of cooking times noted for the sweetpotato root samples (submitted in Period 4)

Synthesis on progress achieved:

In period 4, correlations were identified between dry matter of raw sweet potato and sensory firmness (0.500) and mealiness (r = 0.717) of boiled sweet potato. No significant correlations however were evidenced between cell wall composition of raw sweet potato (either at the monosaccharide content level or the degree of pectin methylation) and cooking time or instrumental texture of cooked sweet potato. This may be partly linked to the fact that texture behavior was not properly determined at this stage, as the texture SOP for boiled sweet potato is still under development.

Institute-Country	**Targeted Quality Trait / Sensory Descriptor**	**Suspected Responsible Biochemical Attributes of Raw Material**				
		Attribute	**Analysis Method**	**Proof of Concept established? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**SOP available? (Yes/No/ Pending)**
CIP-Uganda	Mealiness	Starch gelatinization properties	Rapid Visco Analyser, RVA	Pending	-	No
	Hardness	Dry matter	Hot air oven method	Pending	>60	Yes
JHI-UK	Cooking time	Cell wall monosaccharide content	HPAEC	Pending	36	Pending
	Cooking time	Degree of pectin methylation	FTiR	Pending	36	Pending

Next steps & activities planned on the establishment of proofs of concept:

In Period 5, CIP will perform statistical data analysis with a bigger database to establish the correlation between dry matter (obtained using hot air oven method) and sensory hardness of steamed sweetpotato. Due to the mechanical issues of the freeze-dryer, most of the RVA work planned during Period 4 will be carried over to Period 5.

At JHI-UK, unlike in other RTB crops, cell wall composition either at the monosaccharide level or the degree of pectin methylation appears not to be related to cooking time or other textural parameters. However, some studies suggest that for sweetpotato roots there is correlation with □-amylase activity and/or starch properties. This will be investigated for these samples by CIRAD-France. We shall also investigate whether the total cell wall pectin content in correlated with textural parameters using a method developed by CIRAD-France.

A new set of samples better characterized for texture using the finalized SOP for texture can be used in period 5 to link sweetpotato texture behaviour with RVA data (proposed by CIP-Uganda) and biochemical characteristics (cell wall components but also b-amylase activity) proposed by JHI-UK, in partnership with CIRAD-France. The critical point of this study is the planning, exchange of information/data and of samples that clearly failed in period 4.

4.7.5 Scientific collaborations between partners

Samples of sweetpotato roots have been grown, harvested and assessed for sensory traits by CIP Uganda in period 4, but samples analysed in period 4 by JHI were those harvested and analysed by CIP in period 3.

4.7.6 Next steps on Boiled sweetpotato

Concerning texture, it is recommended to collect further data during Period 5 in order to validate the relationship between firmness perceived by panellists and instrumental parameters. In addition, period 5 will be focused on the prediction model for mealiness which was not robust enough; to this end additional textural parameters and/or use of image analysis from the DigiEye can be tested.

Concerning the physico-chemical bases of texture, CIP plans to focus on starch characterization and RVA analyses, but a better collaboration between partners of the project should be favoured in period 5 to promote exchanges on procedures for pectins and methylation, on starch characterization and exchanges of samples.

4.8 Boiled Potato

4.8.1 Sensory & textural evaluation in Uganda

Two SOPs for sensory and instrumental textural characterization of boiled potato are being developed at CIP-Uganda and should be completed and validated in Period 5; no analysis has been performed so far.

Next steps & activities planned to measure key sensory descriptors for texture instrumentally:

The focal points for sensory and texture at CIRAD recommend the followings:

- On texture SOP: interactions with CIRAD texture team are recommended.
- On firmness: try to find relationships with instrumental parameters.
- Mealiness: try to find easy-to-measure method to assess this indicator.
- On yellow colour: Try to find relationships with chromameters parameters by using relevant indicators as Hue angle, which is a yellow-orange indice
- On potato aroma: proof of concept with very small panel to detect or not potato aroma

4.8.2 Dissection and understanding of key quality traits for boiled potato

Quality trait(s) of focus/investigated:

In Period 4, CIP-Uganda started investigating the contribution of starch gelatinisation properties measured through RVA method to mealiness. No results have been obtained in period 4. The gelatinization properties of freeze-dried potato flour will be evaluated using a Rapid Visco Analyser, in Period 5.

Institute-Country	**Targeted Quality Trait / Sensory Descriptor**	**Suspected Responsible Biochemical Attributes of Raw Material**				
		Attribute	**Analysis Method**	**Proof of Concept established? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**SOP available? (Yes/No/ Pending)**
CIP-Uganda	Mealiness	Starch gelatinisation properties	Rapid Visco Analyser	Pending	-	Pending

Next steps & activities planned on the establishment of proofs of concept:

See section 4.8.3 below.

4.8.3 Next steps on Boiled Potato

It is hypothesized (by CIP-Uganda) that mealiness can be predicted by RVA data; this will be tested in period 5. The focal point encourages CIP to test other alternatives such as biochemical characterization: starch, and/or pectin contents, methylation degree of pectin, in partnership with JHI-UK and/or CIRAD-France.

4.9 Boiled Plantain

4.9.1 Sensory evaluation in Cameroon

One SOP is being written at CARBAP-Cameroon. The methods to prepare and taste samples, and the vocabulary specific to boiled plantain were given in a scientific paper: Kouassi H.A., Assemand E.F., Gibert O., Maraval I., Ricci J., Thiemele D.E.F., Bugaud C. 2020. Textural and physicochemical predictors of sensory texture and sweetness of boiled plantain. International Journal of Food Science and Technology: 28 p.

List of sensory descriptors extracted from Kouassi et al. (2020) are:

- firmness
- chewiness
- stickiness
- mealiness
- moisture
- sweetness.

In Cameroon though, surveys on preferences show that the priority quality traits that must be passed on to breeders are **dry matter content, pulp colour, firmness, chewiness, aroma and odou**r. The new SOP developed by CARBAP-Cameroon should pay attention to include sensory descriptors for those traits.

Nb of samples & Nb of genotypes characterized by the sensory panel in 2021

In Period 4, no sensory analysis was performed at CARBAP-Cameroon. CARBAP decide not to engage in sensory analysis because their texturometer was not operational, which is a necessary condition to allow correlations between instrumental and sensory measurements of texture attributes.

4.9.2 Textural evaluation & correlation with sensory descriptors in Cameroon

Level of progress in the establishment of a correlation with instrumentally measured parameters:

At CARBAP-Cameroon, the texture analyser was not operational in Period 4. Following the training workshop held in Cotonou – Benin, this equipment will be run during Period 5. So correlation between sensory descriptors for texture and instrumental texture parameters is yet to be performed.

Institute-Country	**Targeted Quality Trait / Sensory Descriptor**	**Instrumental Method for Measurement on Final product**				
		Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
CARBAP-Cameroon	Pulp colour	Chromameter	pending	pending	> 10, on clones from WP5 trials in 2 contrasted localities	NA
	Firmness	Texturometer	pending	no	none	NA
	Chewiness	Texturometer	pending	no	none	NA

Institute-Country	Targeted Quality Trait / Sensory Descriptor	Instrumental Method for Measurement on Final product				
		Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
	Plantain aroma	Acceptability values using a Yes and No scale for the absence or presence of aroma	no	no	none	NA
	Plantain odour	Acceptability values using a Yes and No scale for the absence or presence of odour	no	no	none	NA

Next steps & activities planned to measure key sensory descriptors for texture instrumentally:

The next steps at **CARBAP-Cameroon** are, first to carry out sensory analyses with trained panellists and generate sensory vocabulary; secondly, develop a discriminant SOP for instrumental textural characterization and carry out texture analysis on plantain samples (raw and boiled); finally develop SOPs for sensory QDA and off-flavour QDA.

4.9.3 Rapid / intermediate kitchen tests to assess the processing & the cooking ability of boiled plantain

Rapid kitchen test or intermediate assessment methods investigated:

At CARBAP-Cameroon, the rapid kitchen tests developed in period 4 for boiled plantain concern cooking ability and pulp colour. The toothpick or fork test on boiled plantain slice 5 minutes after cooling gives an idea on how cooked is the boiled plantain. This method is processor dependent though, and needs to be standardized. The colour of raw and boiled plantain pulp is measured using a chromameter giving the L*a*b* parameters of boiled plantain slices after 5 minutes of cooling. The chromameter delivers exact colour values, however a correlation with a panel will give an idea on the ideal after-cooking colour, using a range of varieties.

Synthesis on progress achieved:
Experiments and SOP development are at preliminary stages.

Next Steps in the development of rapid / intermediate kitchen assays for boiled plantain

Further experiments depending on availability of materials should be organized in order to develop and finalize SOPs by end of Period 5.

4.9.4 Dissection and understanding of key quality traits for boiled plantain

Quality trait(s) of focus/investigated:

At CARBAP-Cameroon, the quality traits investigated are pulp colour (raw and boiled), pulp firmness (raw and boiled), chewiness and Easiness to peel. The biochemical attributes suspected of being involved are: carotenoids contents for pulp colour, pectin and starch contents for chewiness and firmness, pulp to peel ratio and peel thickness for easiness to peel.

At INRAe-France, the research activities focuses on textural attributes of cooked plantain (sensory and instrumental). Biochemical attributes suspected of being involved are starch, cell wall, pectin and lignin content, methylation degree, galacturonic acid and neutral sugars composition, pectin molar mass distribution, procyanidin composition.

Level of progress in the development of biochemical proof of concept / method to measure the biochemical attributes of raw material

A Proof of Concept (PoC) for the correlation between pulp colour and carotenoids contents is available in the literature.

PoC for the correlation between texture/pectin and starch is available in the literature. However, INRAe-France is currently undergoing some work on PoC for pectins.

At INRAe-France, **the proof of concept about biophysical bases of processing and cooking ability of boiled plantains (INRAe, CIRAD)** was carried-out using the methods and approach developed in Period 3 and Period 4 in order to study the impact of cell wall, lignin, procyanidin and starch on boiled plantain texture. Cooking evaluation and sensory evaluation took place in CIRAD Montpellier in the frame of A. Kouassi phD thesis (under the supervision of C. Bugaud) whereas polysaccharide, lignin and procyanidin analyses were carried out in Avignon. Half-ripe plantains from 7 varieties (2 hybrids, 5 wild types) from Ivory Coast with contrasted textural properties after cooking were send by CIRAD Montpellier (C. Bugaud) in freeze-dried form to INRAE Avignon and then analysed at INRAE Avignon in P4. Each sample was studied raw and after boiling for 20 minutes.

First, cell wall composition of these plantain samples in their raw form was determined thanks to the SOPs developed in P3/P4 and can be compared to other RTBs. Plantains from this collection contain between 4.5 and 10.4 mg/g Galacturonic acid on dry basis. It was found that pectins in plantains are highly linear, i.e. they contain only low rhamnogalacturonan and short ramifications. In addition, their ramifications are mainly composed of arabinose (53 to 67% of the molar sum of galactose and arabinose) which is very different from other RTBs such as Yam, sweet potato or cassava.

Comparisons between sensory properties of boiled plantains and CWP composition allowed to show an inverse correlation between firmness after cooking and total mass of pectin (Galacturonic acid + Galactose + Arabinose + Rhamnose) in raw plantain, as shown in Figure 42. This inverse correlation was not expected as pectin are known to firm up food products, but it might be explained by important degradation of pectin during cooking. A link has been observed between galacturonic acid in raw plantain and firmness but it has to be consolidated by a larger data set.

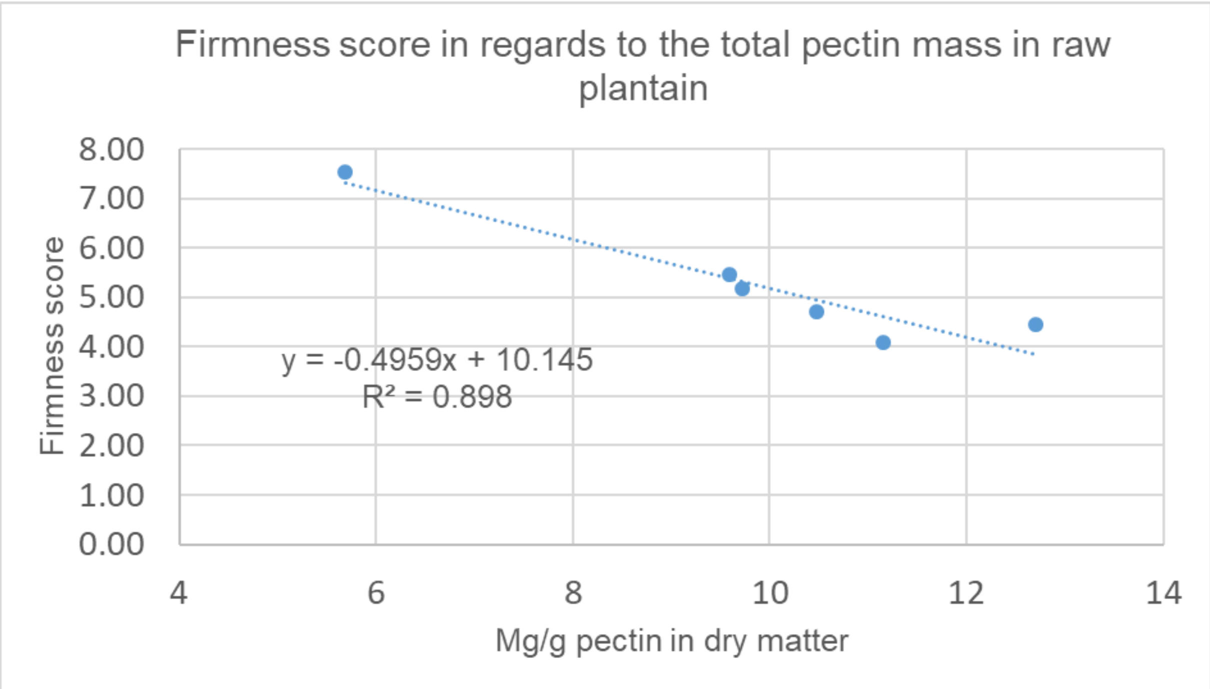


Figure 42 Correlation between firmness and total pectin mass in raw plantain, n = 6

When considering cooked plantain, a negative correlation appeared between firmness and methylation degree (Figure 43). This correlation includes the FHIA 21 hybrid, which is particularly interesting. This link between methylation degree and firmness does not exist in raw plantain, which would be consistent with an action of pectin methylesterase (PME) during the cooking process.

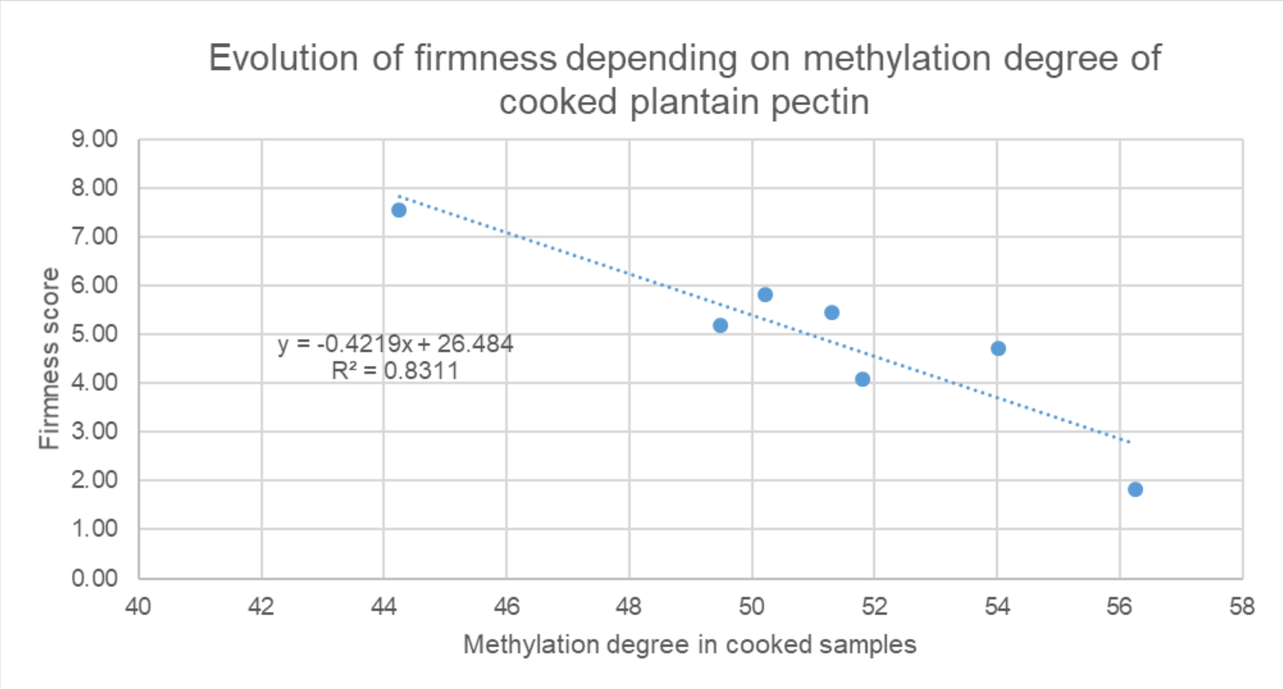


Figure 43 Firmness in regards to methylation degree in cooked plantain (boiling time: 20 minutes)

Firmness was also positively correlated with the Galactose/Arabinose ratio in cooked plantain (R^2 = 0.74, n=6), but not in raw plantain (R^2 = 0.52, n=6). This could be attributed to interactions between galactose and other polysaccharides such as hemicellulose. In non-hybrid cultivars, there is a strong inverse link between the total account of pectin in cooked plantain dry matter and chewiness (R^2=0.91, n=5). This exists also for hybrids between them: more pectin in the cooked product is associated with a lower chewiness score. It has to be noted that chewiness is lower in hybrids that in native cultivars in our sample set.

Concerning the analysis of procyanidins and lignins some trends were found that could explain textural properties but these data analysis are still in progress and these results will be consolidated in P5.

These first results on 7 varieties have to be confirmed on a larger set of plantain samples, this will be done in P5. In particular, cell wall analyses of plantains hybrids from CARBAP (G Ngoh Newilah) have also been planned in P4 but the samples were not available in P4 and this will be delayed to P5.

Synthesis on progress achieved:

Correlation between pectin content or methylation degree of cooked plantain and firmness has been evidenced; but no correlation with raw plantain pectin characteristics could be evidenced. Analyses on raw and cooked samples (cell wall, pectins etc) for a new set of samples on which texture and colour measurements will be performed in parallel (10-12 samples planned). The focal point for biochemistry recommends to increase the number of analysed samples to improve the chance to identify proof of concept and to improve its robustness.

Institute-Country	Targeted Quality Trait / Sensory Descriptor	Suspected Responsible Biochemical Attributes of Raw Material				
		Attribute	**Analysis Method**	**Proof of Concept established? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**SOP available? (Yes/No/ Pending)**
CARBAP-Cameroon	Pulp colour	Carotenoids contents	To be determined	Pending	None	Pending
	Pulp firmness	Pectin/Starch	To be determined	Pending	None	Pending
	Pulp chewiness	Pectin/Starch	To be determined	Pending	None	Pending
INRAe-France	Texture	pectin	SOP cell wall extraction	pending	15	yes
	Texture	Cell wall	SOP cell wall analysis	pending	15	yes
	Texture	procyanidin	Menthofuranolysis + HPLC-DAD and UPLC-MS	pending	45	no
	Texture	lignin	Metaxas et al, 2004	pending	45	no
	Texture	methylation	SOP cell wall analysis	pending	15	yes
	Texture	Starch	Megazyme kit	No	16	No

Next steps & activities planned on the establishment of proofs of concept:

At CARBAP-Cameroon, proofs of concept to be developed include:

- PoC for the correlation between peeling time, pulp to peel ratio and peel thickness;
- PoC for the correlation between cooking time from sensory analyses and biophysicochemical parameter (DSC, pH, TSS, TTA, DMC, Starch, RVA, Conductivity, etc.);
- PoC for the correlation between product quality and dry matter content.

At INRAe-France, investigations will be done on pectinolytic enzymatic activities (PME and PG) on a set of 10-12 varieties of fresh plantains (landraces and hybrids from CNRA, Ivory Coast and CARBAP, Cameroun) in order to confirm the impact of methanol and enzymatic activities on cooking behaviour of plantains. In parallel, cooking, texture and other biochemical analyses (Starch, cell wall, pectin and lignin content, methylation degree, acetylation, galacturonic acid and neutral sugars composition, soluble pectin molar mass distribution, procyanidin composition, ferulic acid content) will be done on these samples. Moreover, divalents ions that could have a great impact on the pectin behaviour, in particular calcium, will be quantified in the different fractions of the samples. Data acquired in P4 and P5 on plantains properties cited above will be put together and statistically analysed in order to extract general tendencies on the determinants of plantain cooking behaviour.

4.9.5 Scientific collaborations between partners

Plantains from CNRA (A Kouassi) were send by CIRAD Montpellier (C. Bugaud) in freeze-dried form to INRAE Avignon and then analysed at INRAE Avignon in P4: meetings for exchanging about the strategy, protocols and the results.
Meetings with G N'Goh Newilah (CARBAP) for fresh samples supplying to INRAE.

4.9.6 Next steps on Boiled Plantain

At CARBAP-Cameroon, the focal points (on sensory and texture) at CIRAD recommend:

- On texture attributes: Write and update the SOP and validate the good relationships (Kouassi et al., 2020) observed between sensory attributes (firmness and chewiness) and instrumental parameters (puncture force / hardness by TPA) (CARBAP)
- On colour: Try to find relationships between pulp colour and chromameters parameters (CARBAP)
- On odour and aroma plantain: proof of concept with very small panel to detect or not odour / aroma of plantain (CARBAP with support of CIRAD)

At INRAE, concerning cell wall components, the study will be focused on PME activity in period 5. A set of 10-12 varieties of fresh plantains will be analyzed in order to confirm the impact of methanol and enzymatic activities on cooking behaviour of plantains and establish the proof of concept (PoC) on the role of pectins and PME. With this aim, INRAE-France will collaborate with CNRA and CARBAP to obtain fresh plantain samples with a controlled origin and maturation stage for texture characterization. Maturation, sample preparation and stabilisation, cooking and texture are planned to be carried by A. Kouassi (CNRA) at CIRAD Montpellier, whereas other biochemical analyses will be done at INRAE.

4.10 Matooke

4.10.1 Sensory evaluation in Uganda

A SOP for sensory characterization is available: Kephas NOWAKUNDA (2019). SOP for Sensory Evaluation on Matooke. Kampala, Uganda: RTBfoods Project Report, 18 p. The sensory descriptors used for sensory evaluation are:

- Appearance: yellow, homogeneity of colour
- Texture in mouth: firmness, moisture, smoothness
- Texture by touch: hardness, moldability, stickiness
- Taste and impression: sweetness, sourness, astringency
- Aroma: Matooke, pumpkin, grassy

Key priority quality traits for Matooke are: texture (smoothness, firmness), colour (yellowness, uniformity of the yellowness) and Matooke taste and aroma (determined by sensory descriptors).

Nb of samples & Nb of genotypes characterized by the sensory panel in 2021

At NARL-Uganda, 30 Genotypes were characterized by the sensory panel in 2021.

Key results & graphs:

At NARL-Uganda, results obtained in period 3 on 40 genotypes showed that local cultivars were characterized by higher yellow colour, homogeneous colour, and matooke aroma. Texture attributes are not relevant to differentiate local and hybrids cultivars. The hybrids were divided in 3 groups: a first group with local cultivars, a second group of firm products, and a third group of products with lower yellow colour and matooke aroma. Products repeated 2 or more times were quite close to each other, which proves that panel was well trained.

4.10.2 Textural evaluation & correlation with sensory descriptors in Uganda

A SOP for textural characterization of Matooke is already available since Period 3, developed by NARL-Uganda. This is based on double compression however; we plan to develop a SOP based on rheometry.

Nb of samples & Nb of genotypes characterized for texture in 2021

At NARL-Uganda, 30 Genotypes were characterized for texture in 2021.

List of key sensory descriptors for texture (generated & used by trained panellists) to be measured instrumentally:

At NARL-Uganda, the key sensory descriptors for texture to be measured instrumentally are texture in mouth: firmness, moisture, smoothness and texture by touch: hardness, moldability, stickiness.

Level of progress in the establishment of a correlation with instrumentally measured parameters:

At NARL-Uganda, correlations between sensory descriptors of texture and instrumentally measured parameters were explored by principal component analysis (figure 44). All the landraces fell in the first and second quadrants and were associated with sensory attributes of moistness, moldability, and stickiness. All the landraces were negatively correlated with both instrumental parameters and sensory attributes of hardness and firmness (figure 44).

Pearson correlations analysis identified weak correlations between TPA parameters and firmness by mouth and hardness by touch of matooke (table 17, maximum r = 0.611). Similarly, no significant correlation was identified between the sensory perception of yellow and colorimeter parameters L* abd b* (table 18).

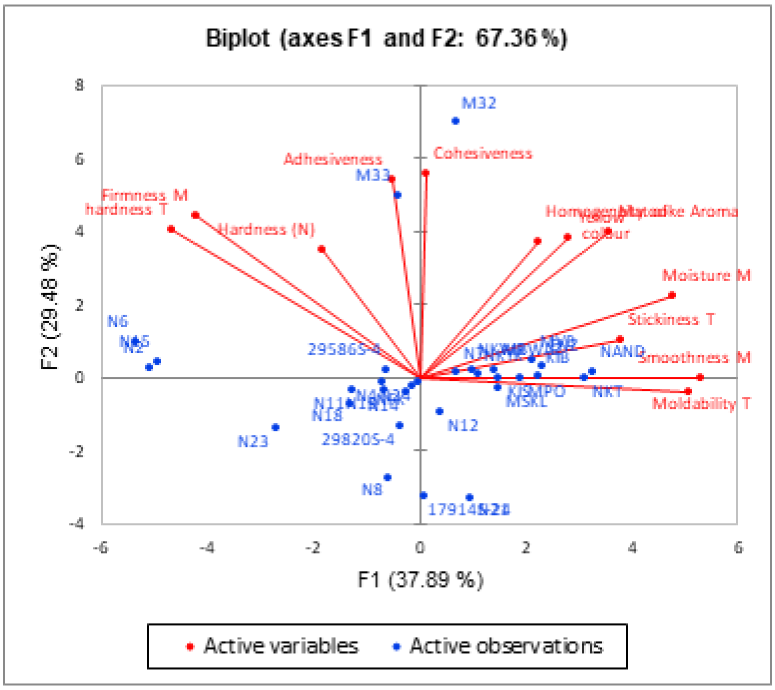


Figure 44 Biplot showing relationships between sensory attributes, instrumental textural parameters and the different matooke genotypes

Table 17 Correlation matrix of sensory and instrumental texture attributes of matoke

	Hardness (N)	Cohesiveness	Adhesiveness
Hard. (N)	**1**	0.328	0.295
Cohesiv.	0.328	**1**	0.970
Adhesi.	0.295	0.970	**1**
Firm. M	**0.479**	**0.560**	**0.611**
Moist. M	-0.136	0.388	0.298
Smooth. M	-0.202	0.134	0.002
Hard. T	**0.526**	**0.490**	**0.558**
Molda. T	-0.176	0.097	-0.009
Stick. T	-0.187	**0.431**	0.338

NB: M = evaluation in mouth; T = evaluation by touch

Table 18 Correlation matrix of sensory attribute yellow and instrumental color parameters of matoke

	L*	b*	Yellow
L*	**1**		
b*	-0.225	**1**	
Yellow	-0.143	0.453	**1**
Homogeneity of colour	-0.099	0.371	0.920

Synthesis on progress achieved:

At NARL-Uganda, cohesiveness measured by TPA helped to predict sensory attributes but with a very low determination coefficient (R^2=0.31 for firmness in mouth and R^2=0.24 for hardness by hand): that means that the three textural parameters used are not so relevant to predict the sensory texture of Matoke (remark already made in period 3). A surprising result is the lack of correlation between colour attributes and chromameters Lab.

Institute-Country	**Targeted Quality Trait / Sensory Descriptor**	**Instrumental Method for Measurement on Final product**				
		Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
NARL-Uganda	Hardness H / firmness M	TPA	yes	yes	30	
	Other texture attributes	TPA	yes	no	30	
	Colour attributes	Chromameter	pending	no	30	

Next steps & activities planned to measure key sensory descriptors for texture instrumentally:

The team is focusing on measuring textural characteristics of the raw sample and will be used to predict textural characteristics of the cooked sample.

The focal points for sensory and texture at CIRAD recommend:

- On texture attributes: the poor correlation found between texture attributes and TPA parameters suggest to look for other textural parameters, in particular rheological parameters that could be more relevant to describe pasty products such as Matooke.
- On colour attributes: Try to find relationships between pulp colour and chromameters parameters by using other combinations (for example Hue angle calculated from a and b).
- On taste attributes: Try to find relationships between sweetness and sugar content.

4.10.3 Dissection and understanding of key quality traits for Matooke

Quality trait(s) of focus/investigated:

At NARL-Uganda, research activities focused on the colour (yellowness), softness and Matooke taste.

Level of progress in the development of biochemical proof of concept / method to measure the biochemical attributes of raw material

During 2021, a total of 30 genotypes were characterized using descriptive sensory tests at NARL Uganda. Also, key biophysical analyses including amylose and amylopectin content, total starch, polyphenols were carried out on the same genotypes. Exploratory correlation of these components with sensory attributes were calculated.

Principal Component Analysis (PCA) of the sensory and biochemical dataset was performed on 23 Matooke genotypes. Ten (10) were landraces: Enzirabahima, Kibuzi, Kisansa, Mbazirume, Mpologoma, Musakala, Nakinyika, Nakitembe, Nandigobe and Nfuuka;, and thirteen (13) were hybrids: NARITA 2, NARITA 4, NARITA 6, NARITA 8, NARITA 11, NARITA 12, NARITA 14, NARITA 15, NARITA 17, NARITA 18, NARITA 19, NARITA 21, and NARITA 23.

Accordind to the PCA, stickiness by touch and moldability by touch were significantly correlated (Figure 45); however this was not confirmed by Pearson correlation analysis (r = 0.585). A correlation matrix between the biochemical components and sensory textural parameters revealed negative but non-significant correlations between DMC (raw) and stickiness by touch, DMC (cooked) and moisture, DMC (cooked) and stickiness by touch, and amylopectin and firmness in the mouth (Table 46).

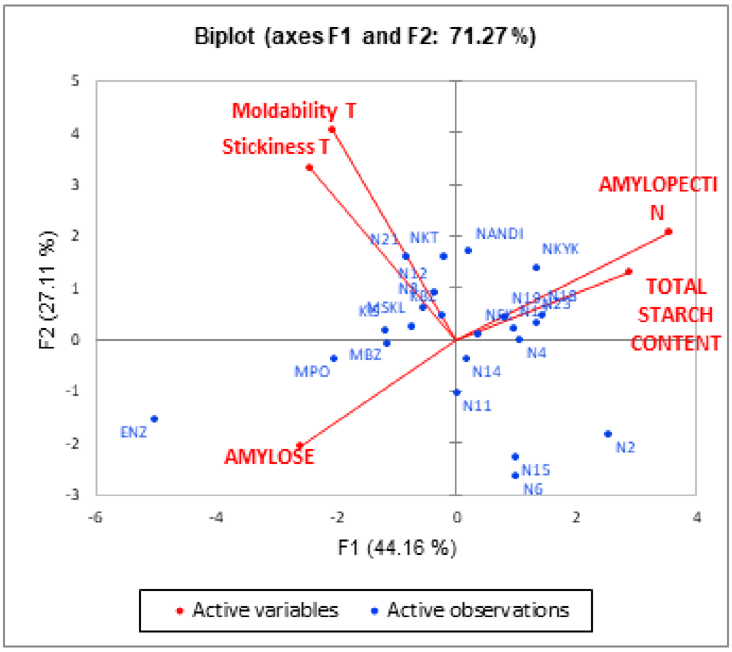


Figure 45 Biplot showing relationships between sensory attributes (moldability and stickiness), biochemical components (amylose, amylopectin and total starch) and the different matooke genotypes

Table 19 Correlation matrix between sensory texture attributes and biochemical components (dry matter, total starch, amylose, amylopectin)

	Firmness M	Moisture M	Smoothness M	hardness T	Moldability T	Stickiness T
DMC (Raw)	-0.404	-0.477	-0.280	-0.316	-0.221	-0.560
DMC (Cooked)	-0.391	-0.505	-0.156	-0.332	-0.141	-0.577
Total starch content	-0.486	-0.346	-0.225	-0.405	-0.191	-0.341
Amylose	-0.316	-0.093	-0.070	-0.336	-0.119	-0.029
Amylopectin	-0.539	-0.404	-0.131	-0.463	-0.125	-0.463

Genotypes were distributed among the four quadrants of the PCA space (figure 45). The first quadrant was characterized by high amylopectin and total starch content, and grouped three landraces (Nakinyika, Nandigobe, Nfuuka) and four hybrids (NARITA 18, NARITA 19, NARITA23, NARITA 17). The second quadrant was characterized by being less sticky and less moldable, and grouped only hybrids (NARITA14, NARITA11, NARITA 2, NARITA 15, NARITA 6).

Correlations between instrumental colour and some biochemical components were assessed by PCA (Figure 46). The results showed that the L* (lightness) by the chromameter was negatively correlated with total phenolics content (TPC). The analysis of carotenoids was done on flour of 30 matooke genotypes using iodometric method, as shown in earlier sections of this report, exploratory correlations with instrumental and sensory colour were performed. Also, total polyphenols were quantified in the flour of the same genotypes also by the Folin-Ciocateau. Exploratory correlations with instrumental and sensory colour have also been performed. Genotypes with high polyphenol content tend to develop dark and unappealing appearance leading to consumer rejection. Most hybrids are characterized with high total phenolics content. The SOPs for carotenoids, polyphenols and starch are under development. They have been identified among deliverables for period 5.

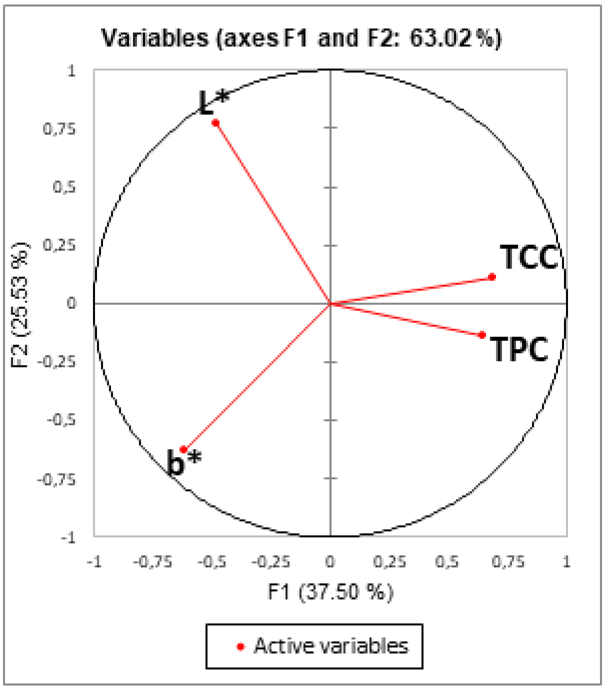


Figure 46 Correlation circle showing relationships between instrumental colour (L and b*) and biochemical parameters total carotenoids content (TCC) and total phenolics content (TPC).*

Synthesis on progress achieved:

At NARL-Uganda, L* (lightness) by the chromameter was negatively correlated with total phenolics content (TPC). Further measurements with more genotypes and samples are needed to strengthen the results obtained in Period 4.

Institute-Country	**Targeted Quality Trait / Sensory Descriptor**	**Instrumental Method for Measurement on Final product**				
		Measurement method	**SOP available? (Yes/No/ Pending)**	**Correlation established with sensory? (Yes/No/ Pending)**	**Nb of Analyses performed in 2021**	**Nb of WP4 clones characterized in 2021**
NARL-Uganda	Starch	Iodometric	Pending	Pending	30	30
	Amylopectin	Iodometric	Pending	Pending	30	30
	Total phenols	Folin-Ciocateau method	Pending	Pending	30	30
	Dry matter	Oven drying	Yes	Pending	23	23
	Colour	Chroma	Pending	Pending	23	23

Next steps & activities planned on the establishment of proofs of concept:

Planned activities of NARL for period 5 include biophysical analyses focusing on polyphenols, total starch, amylopectins, chroma and dry matter.

The focal point encourages NARL to harmonize their analytical procedures (starch, polyphenol, amylose/amylopectin) with the other partners and to use the SOPs for these procedures as soon as available.

SOPs for color on raw samples will also be finalised during period 5.

4.10.4 Next steps on Matooke

In Period 5, NARL plans analysing additional samples to increase the dataset of matooke and improve the correlation analyses between sensory, instrumental analysis (texture - TPA and colour - chromameter) and biochemical analyses focusing on polyphenols, total starch, amylopectin, and dry matter.

The WP2 management team recommends:

- On texture attributes: To look for other textural parameters, in particular rheological parameters that could be more relevant to describe pasty products such as Matooke.
- On colour attributes: Try to find relationships between pulp colour and chromameters parameters by using other combinations (for example Hue angle calculated from a and b).
- On taste attributes: Try to find relationships between sweetness and sugar content,
- On biochemical characteristics: to harmonize the analytical procedures (starch, polyphenol, amylose/amylopectin) with the other partners and to use the SOPs for these procedures as soon as available.
- On data analysis: Include all data in the PCA analyses for better consistency and interpretations with Pearson correlation analyses.

5 DATA MANAGEMENT & ONTOLOGIES

5.1 Laboratory data management

Narrative on Data Management at WP2 level - What has been done so far to ensure that WP2 teams will be reporting their data in a harmonized manner to facilitate future comparisons of results between labs, especially between labs working on the same product profile? Emphasize on Gaps observed [10 lines].

A template for collecting both WP2 and WP3 datasets was developed during Periods 2 and 3 (referred to as “WP3 template”). During Period 4, most WP2 and WP3 partners started to use this template to store and submit their laboratory biophysical data and NIRS data. Some inconsistencies in the use of the template remain to be solved among partners, before a fully harmonized data collection can be achieved, in particular regarding the completeness of the metadata and the use of consistent samples codes. The hiring of Amos Asiimwe at the end of 2021 to be responsible for Data management within RTBfoods should help to verify data quality and streamline the use of the WP3 template.

Next steps in Period 5 for the storage of lab data onto BreedBase for the 5 RTB crops. Mention if you consider using the WP3 database template, or not. Describe how will be organised the coordination of this sub-set of activities in the last project period (who to supervise at WP2 coordination level?)– Mention expected contribution & implication of partner institutes [15 lines].

Three RTBfoods partners (NaCRRI, IITA, CIAT) are already uploading their datasets to Breedbase, using the WP3 template. In Period 5, other RTBfoods partners need to start using the WP3 template, in order to deliver the datasets of the five RTBcrops in a standardized format. One of the tasks of the data manager (Amos Asiimwe) will be to support partners to improve their use of the WP3 template for consistent formatting of their datasets including meta-information. For uploading the laboratory datasets to Breedbase, the responsibility may go to Amos Asiimwe, as he receives completed WP3 templates from partners.

5.2 Ontology development for quality traits

Summary of activities performed in Period 4 to develop ontologies for quality traits for RTB products – Mention partner institutes involved + RTB product concerned. Emphasize on scientific findings & lessons learnt from challenges faced in Period 4 [15 lines].

In Period 4, **Trait Dictionaries & lexicon were generated for sensory traits** of the following food products: steamed matooke, boiled/steamed cassava, boiled sweet potato, boiled Yam, pounded Yam, Fufu, Gari-Eba, and attieke. Extensive review of the lexicons for each product was conducted to correct inconsistencies and ambiguities, led by the Alliance Bioversity-CIAT. The following RTBfoods partners were involved in this undertaking: NARL (matooke), NaCRRI (boiled cassava), CIP (boiled sweetpotato), UAC-FSA (boiled Yam), Bowen University (pounded Yam), IITA (Fufu, Gari-Eba). Junior consultants from Makerere University (Uganda) and Wageningen University (Netherlands) also supported the work.

Similarly, **ontologies were developed to describe post-harvest processing techniques** of different food products, and to harmonize the way post-harvest data are collected and described. The Trait Dictionary template was adapted to the Food processing techniques. The definition of post-harvest processing traits was complicated as the team had to start from reports on processing each food product, and not from a lexicon. The following RTBfoods partners were involved in this undertaking: UAC-FSA (boiled Yam), CIP (boiled sweet potato), boiled cassava (UAC-FSA, NaCRRI), Bowen University (pounded Yam).

Results of ontology developments in Period 4 are presented in details in the report “Creating Food Product Quality Traits in the Crop Ontology for Roots, Tubers and Bananas” (Asimwe et al., 2021).

The trait dictionaries for all the food products mentioned above were uploaded on the CIRAD RTBFoods platform.

Next steps in Period 5 for the development of ontologies for laboratory quality traits. Describe how will be organised the coordination of this sub-set of activities in the last project period (who to supervise at WP2 coordination level?) – Mention expected contribution & implication of partner institutes [15 lines].

To be accessible and usable by breeders, the ontologies and trait dictionaries developed in Period 4 need to be made available in Breedbase, so that breeders can define variables for post-harvest quality, and upload data in the same way as they already do for agronomic data. To this end, some adaptations are needed in Breedbase, because currently the forms to fill in do not correspond to the elements of a sensory panel data set (e.g. panel size, members, randomization of samples, repetition, etc.). Of particular importance is to define the meta-data to include in trait dictionaries and ontologies (e.g. plot-name, plot ID, accession name, Plot_number, block_number, replication_number, etc.), so that the datasets can be linked to the plant in the field and therefore to the field trials in Breedbase.

Recommendations for Period 5:

1. The Template for Food Product Trait Dictionaries will have to be promoted for extending the Ontologies with Sensory traits and processing techniques.
2. A Food Product section needs to be created in the Crop Ontology.
3. All Trait Dictionaries developed will be uploaded into Crop Ontology.
4. Expert group for cassava and Yam needs to be created.
5. Data sets produced from sensory panels must include the information on the material provenance (field trial, plant number, population, etc.) and the ontology will have to be used for the attributes' and variables' names.
6. Breedbase will have to be adapted for the entry of sensory panel results.
7. RTBfoods partners to enter their datasets in the WP3 template, and share them with WP2 co-leaders and Amos Asiimwe, who will verify the quality of the formatting and proceed to upload to Breedbase.
8. Ontologies for biophysical measurements like texture will be developed.

5.3 Ring tests

Brief introduction on the importance of this sub-set of activities & their contribution to WP overall objectives [10 lines].

A ring test is practical way for the training and validation of the SOP by all partners. This is a necessary step to ensure the reliability and exchangeability of data between partners.

Narrative activities performed in Period 4 regarding ring testing – Mention institutes involved & challenges faced [10 lines].

Seven samples (flours) have been prepared by UAC/FSA from the different RTBs (cassava, Yam and sweet potato) and have been sent to CIRAD. These samples will be distributed to each partner with the SOPs that will be used for analysis: a first list of 7 procedures of interest and shared by most partners (dry matter, starch, amylose, free sugars, polyphenols, pectins, cell wall material) has been listed.

Next steps to be implemented & draft timeline for Period 5; partner institutes to be involved & type of implication/contribution [10 lines].

Some SOPs must be finalized, and the inventory of procedures that can be used by each partner will be rapidly updated before distribution of the samples. This will be performed in the first part of period 5, in order to analyse the data and propose practical adaptations of the SOPs (if necessary) for routine using by the partners.

6 COVID-19 IMPACT ON WP WORKPLAN IN PERIOD 4

*In the table below, summarize the consequences of Covid-19 crisis on the implementation of activities planned at the beginning of the period, for each team. For impacted activities, mention the **level of impact** (i.e. postponed field or lab work, missing harvests, support gaps, delays in data analysis, etc.), the **consequences** on sub-sequent activities (including activities performed in other WPs) & the impact on the **capacity to deliver** in Period 4.*

NB: This section aims to provide elements to the PMU to justify eventual deficiencies in reaching Period 4 milestones/targets & to request a budget reallocation for Period 5 (if applicable).

Lab	Product Profile	WP2 Focal Point	Sensory Characterization	Development of instrumental protocols to measure sensory traits (including texture)	Biochemical Proof of Concepts to understand/ predict processing/ cooking ability & other sensory quality traits
IITA-Nigeria	**Eba**	M. Adesokan	Experienced a limited COVID-19 impact, except the effect of drought that delayed harvesting and a few of the trained panellist could not participate in the exercise. Despite the setback, all planned activities for Period 4 were done.	Experienced a limited COVID-19 impact, except the effect of drought that delayed harvesting. Despite the setback, all planned activities for Period 4 were done.	Experienced a limited COVID-19 impact, except the effect of drought that delayed harvesting. Despite the setback, all planned activities for Period 4 were done
NRCRI-Nigeria	**Eba**	U. Chijioke	No impact.	No impact.	No impact.
	Fufu		No sensory activity.	No impact.	No impact.
	Boiled Yam		No impact.	No impact.	No impact.
	Pounded Yam		No impact.		No impact.
Bowen University-Nigeria	**Pounded Yam**	B. Otegbayo	No impact. It was done as proposed	No impact. It was done as proposed	Still on it
NARL-Uganda	**Matooke**	K. Nowakunda	Limitation of gatherings led to suspension of sensory characterisation	Progress slowed down due to limitations on number of staff at laboratory and transportation of staff since public transport was initially suspended	Progress on all the activities was slowed down by national lockdown due to COVID-19. Some of the lockdown measures were later eased by strictness on number of staff at work (20%) remain in force.
NaCRRI-Uganda	**Boiled Cassava**	E. Nuwamanya	Given the prevailing SOPs, no sensory	This too was impacted negatively. Genotypes	Attempts were made to undertake some analyses although limitations on the

Lab	Product Profile	WP2 Focal Point	Sensory Characterization	Development of instrumental protocols to measure sensory traits (including texture)	Biochemical Proof of Concepts to understand/ predict processing/ cooking ability & other sensory quality traits
			characterisation was carried out in Period 4	harvested were not characterised for all traits to allow for comparisons	number of samples analysed limited meaningful development of proofs of concepts
CIP-Uganda	**Boiled Sweetpotato**	M. Moyo			
	Boiled Potato				
JHI	**Boiled Sweetpotato**	M. Taylor			Missing harvests by partners in Uganda have delayed the availability of samples for biochemical analysis at JHI.
CNRA- Côte d'Ivoire	**Attiéké**	C. Ebah			
	Pounded Yam				
	Fried Sweetpotato				
	Fried Plantain				
UAC-FSA	**Boiled Yam**	N. Akissoe	Little impact (delay in data analyses)	None	A member of UAC-FSA team should go to CIRAD to carry out some lab analyses. Due to Covid-19 crisis, this journey was delayed and scheduled for Period 5. Chemicals needed for lab analyses take time to be delivered.
	Boiled Cassava		Little impact (delay in data analyses)	None	A member of UAC-FSA team should go to CIRAD to carry out some lab analyses. Due to Covid-19 crisis, this journey was delayed and scheduled for Period 5. Chemicals needed for lab analyses take time to be delivered.
CARBAP-Cameroon	**Boiled Plantain**	G. Ngoh	Sensory analyses of boiled plantain samples with trained panellists in order to generate a vocabulary was delayed by COVID19. Hence a reactivation of the preselected panel had to be done recently before undergoing a	The delay of sensory analyses directly affected texture measurements since these measurements had to be correlated with data obtained from sensory analyses to better understand consumer preferred traits.	Sensory characterization and development of instrumental protocols to measure sensory traits were the prerequisite for biochemical proof of concepts.

Lab	Product Profile	WP2 Focal Point	Sensory Characterization	Development of instrumental protocols to measure sensory traits (including texture)	Biochemical Proof of Concepts to understand/ predict processing/ cooking ability & other sensory quality traits
			proper sensory evaluation.		
CIAT-Colombia	**Boiled Cassava**	T. Tran	No major impact of covid on the RTBfoods activities at CIAT in 2021, except for the sensory analysis experiments, which were significantly delayed (to June 2021 instead of March as initially planned). Contact cases happen regularly among the team, which forces the person concerned to isolate for 7 to 10 days. Nevertheless, it has so far been possible to adjust to this constraint and maintain most of the activities as scheduled. Fortunately the covid safety protocols put in place by CIAT after the initial lockdown in 2020 (and some trial-and-error) have allowed to avoid any full lab closure in 2021.		
INRAe-Guadeloupe	**Boiled Yam**	D. Rinaldo		Texture (penetrometry) was measured at 45 °C as expected. But less samples were analyzed compared to the previsions because of a bad harvest caused by a late planting because of covid crisis (shutdowns).	Biochemical characterization will be postponed to P5. Only dry matter and ash contents could be determined in P4.
INRAe - Avignon	**Boiled Yam**	A. Rolland-Sabaté			Biochemical characterization were done as expected in P4 but on P3 harvest only because of late reception of Yam samples from Guadeloupe caused by shutdowns due to covid crisis and strikes. The biochemical characterization of P4 samples will be postponed to P5. Only enzymatic activities were measured on P4 samples.
	Boiled Plantain	A. Rolland-Sabaté			Biochemical characterization were done as expected in P4, except on fresh samples from CARBAP because no samples were send to INRAE in P4 due to covid crisis. The biochemical characterization of fresh samples will be postponed to P5.
CIRAD-Montpellier	**Boiled Yam**	C. Mestres			
	Boiled Cassava	C. Mestres			

7 WP2 COORDINATION

Describe briefly how has been organized the coordination of the WP in Period 4 and shared support responsibilities between WP coordinators & activity focal points – Highlight Strengths, Complementarities & eventual Gaps [± 0.5 page].

In period 4, the CIRAD Sensory team (Christophe Bugaud, Nelly Forestier-Chiron and Isabelle Maraval) made the partners aware of the preparation and analysis of sensory data by offering them 2 tutorials accessible on the RTBfoods platform.

1. Christophe BUGAUD, Isabelle MARAVAL, Nelly FORESTIER-CHIRON, (2021). RTBfoods Manual - Part 2 – Tutorial. Monitoring Panel Performance and Cleaning Data from Descriptive Sensory Panels for Statistical Analysis. Biophysical Characterization of Quality Traits, WP2. Montpellier, France: RTBfoods Methodological Report, 13 p.
2. Christophe BUGAUD, Isabelle MARAVAL, Karima MEGHAR, (2021). RTBfoods Manual - Tutorial: Statistical Analyses (PCA and multiple regression) to Visualise the Sensory Analysis Data and Relate it to the Instrumental Data. Montpellier, France: RTBfoods Methodological Report, 24 p.

The CIRAD Sensory team also sensitized the RTBfoods partners, as well as those of the AfricaYam project, to the identification of acceptability thresholds for key quality traits. The interest in identifying these acceptability thresholds for breeding programs was highlighted by the advisory committee during the virtual annual meeting in April 2021. There were 2 presentations on the methods to identify acceptability thresholds for the key quality traits, which were dEbated with the partners during the RTBfoods Annual Meeting (April 2021) and during the AfricaYam / RTBfoods workshop on Yam Quality evaluation in Benin (November 2021).

During the AfricaYam / RTBFoods workshop (Benin, November 2021), all partners involved in quality assessment of Yam products participated to practical exercises, which were organized by the CIRAD and UAC-FSA teams. The objectives were to sensitize the partners to the implementation of sensory tasting sessions, to the difficulty and importance of having an efficient panel, to the evaluation of their performance and to the statistical processing of sensory data in relation with physico-chemical characteristics.

In period 4, the CIRAD Texture team (Layal DAHDOUH, Julien RICCI and Toyin Ayetigbo) provided many technical and scientific supports to the partners:

1- Julien RICCI: January-February 2021 CIRAD Guadeloupe: Implement the texture measurements of boiled Yam at CIRAD Guadeloupe.
2- Toyin Ayetigbo: November-December 2021 Nigeria: Provide support for IITA, Bowen University, NRCRI to overcome technical issues in relation with texture measurements. Perform texture analyses on different sample to evaluation the robustness and the discriminating ability of texture analyses for pasty products (Eba, Pounded Yam and Fufu). Analyse raw textural data provided by partners and improve the texture measurements accordingly. Implement and evaluate the texture method at IITA for boiled Yam using UAC/FSA SOP and provide suggestions according to the obtained results.
3- Layal DAHDOUH: Remote support for CIP to improve the texture SOP for Boiled sweet Potato.

During the AfricaYam / RTBFoods workshop (Benin, November 2021), all partners involved in quality assessment of Yam products participated to practical texture sessions, which were organized by the CIRAD (Julien RICCI) and UAC-FSA teams. The objectives were to initiate the partners to the different steps required to develop a robust and discriminating texture test. These steps were explained during a theoretical presentation made by Layal DAHDOUH.

During the AfricaYam / RTBFoods workshop (Benin, November 2021), the partners were reminded the approach and critical points for defining a Proof of Concept based on biophysical analysis for predicting quality. Unfortunately, the Ring test that has been planned for the validation of biophysical analyses could not be finalized in period 4.

Which are the gaps observed in Period 4 (ex: key traits not yet measured instrumentally, missing proof of concepts for some product profiles, etc.)? Which are the possible strategies/action points to be implemented in the last project period to make sure that the WP2 reaches its overall objectives for the 11 RTB food products? Mention institutes that should be involved/supported more or differently. [0.5 to 1page].

Several SOPs of texture need to pass final validation. In the case of several product profiles, only composition data are available so far (i.e. dry matter, starch content, sugar content), and more complete datasets including instrumental texture and/or sensory need to be collected in Period 5.

8 INTERACTION MECHANISMS BETWEEN WPS

Fill-in the table below with a brief description or bullet-point lists of interactions with other WPs (successful ones & gaps) & propositions for risk mitigation.

<table>
<tr><th></th><th>Successful Interactions/ Coordination with other WPs (specific actions concerned, frequency, tool sharing)</th><th>Gaps in Interactions/Coordination with other WPs: What is needed from other WPs ?
(NR = not relevant)</th><th>Risk mitigation : How to Improve (specific actions to be taken, frequency, tool sharing?)</th></tr>
<tr><td>WP1</td><td>Two common presentations on the methods to identify acceptability thresholds for the key quality traits, which were dEbated with the partners during the RTBfoods Annual Meeting (April 2021) and during the AfricaYam / RTBfoods workshop on Yam Quality evaluation in Benin (November 2021).</td><td>Improvement in exchanges of data for identifying acceptability thresholds</td><td>Better sharing data; propose sharing methods to identify acceptability thresholds.</td></tr>
<tr><td>WP3</td><td>NIRS analysis (WP3) on raw Yam for developing prediction models of dry matter determination (IITA, CIRAD),
NIRS analysis (WP3) on raw Yam flour from the colziyanm collection (INRAE Guadeloupe, CIRAD) and amylose, starch, protein, sugars, dry matter determination for prediction models, collaboration to corresponding SOPs</td><td rowspan="2">Improvement of sample exchange and data between WPs and between partners</td><td rowspan="2">Increase the communication between the labs to share the new information and sampling strategies, increase cross sampling, for a better efficiency (between WP2, 3 and 4 and research institutes): eg. Better sharing of meeting minutes, or more cross meetings at the beginning of the period, etc…</td></tr>
<tr><td>WP4</td><td rowspan="2">Analysis of Yam and cassava samples common to WP2, 3, 4 and/or 5 (CIRAD, IITA, INRAe)</td></tr>
<tr><td>WP5</td><td></td><td></td></tr>
<tr><td>WP6/PMU</td><td>Training for sensory and texture analysis in UAC (Benin)</td><td></td><td></td></tr>
</table>

9 COMPLEMENTARITY WITH OTHER PROJECTS OR BREEDING PROGRAMS ON RTB CROPS

*<u>In the table below</u>: Summarize complementarities with other projects or breeding programs (e.g. NextGen, AfricaYam, SweetGAINS, CRP RTB, ABBB, HarvestPlus, other national breeding programs) on WP3 activities. For each WP team concerned, list the **points of complementarity** (students, equipment share, facilities, co-funding of activities, participation in partner projects, events or initiatives on RTB crops).*

Country	Institute	Product Profile	Complementary Projects/Initiatives	Points of complementarity / Activities performed &/or funded jointly
Uganda	NaCRRI	Boiled Cassava	Nextgen project	• Use of SOPs from RTB foods for characterisation of traits of interest • Similar trial areas for the same genotypes • Incorporation of RTB selected traits in the selection indices for other traits of priority in the complimetary projects
Colombia	CIAT	Boiled Cassava	RTB flagship FP4, cluster CC4.1 RTB post-harvest quality and CA4.2 Cassava processing	Joint funding of the RTBfoods WP2, WP3 and WP4 activities at CIAT
Nigeria	NRCRI	Gari-Eba	Nextgen project	Provision of genotypes used for WP 1, 2 and 3 activities. Provided project vehicles for WP1 participatory processing activities, determination of biophysical properties and acquisition of spectral data on freshly harvested cassava genotypes by WP2 and 3 in multi-locational trials. Facilitated the installation of the lypholyzer hence enabling us to freeze dry cassava roots for determination of cell wall components
Nigeria	NRCRI	Fufu	Nextgen project	Provision of genotypes used for WP 1, 2 and 3 activities. Provided project vehicles for WP1 participatory processing activities, determination of biophysical properties and acquisition of spectral data on freshly harvested cassava genotypes by WP2 and 3 in multi-locational trials. Facilitated the installation of the lypholyzer hence enabling us to freeze dry cassava roots for determination of cell wall components
Nigeria	NRCRI	Boiled & Pounded Yam	African Yam	Provision of genotypes used for WP 1, 2 and 3 activities.
Nigeria	IITA	Gari-Eba	NextGen	Provision of genetic materials and sharing of SOPs
Nigeria	IITA	Fufu	Not applicable	Not applicable
Nigeria	IITA	Boiled	African Yam	Provision of genetic materials and sharing of SOPs
Nigeria	Bowen University	Pounded Yam	PEARLs	Equipments and facilities share
Benin	UAC-FSA	Boiled Yam	AfricaYam	AfricaYam center (Benin) supports the WP2 RTBfoods activities by accepting to plant the five Yam varieties, identified during WP1, and which constituted the raw material for WP2.

Country	Institute	Product Profile	Complementary Projects/Initiatives	Points of complementarity / Activities performed &/or funded jointly
				"Training on Yam Quality Evaluation". International Workshop organized by AfricaYam & RTBfoods projects. November 22-26, 2021 – UAC-FSA, Benin
France	CIRAD	Boiled Yam		
Guadeloupe	CIRAD	Boiled Yam	AfricaYam	44 samples of raw Yam flour of the popB of D. alata were send to INRAE Guadeloupe for NIRS analysis and prediction of DM, Starch, Protein, Sugar, Hardness… using calibration models developed by Ehounou and al. in 2021
France	INRAe	Boiled Yam	CRB	Biophysical traits of samples from: Harvest 2021: 12 fresh raw samples from D. alata, D. trifida and D. esculenta; Harvest 2020: 8 freeze-dried raw samples and 8 freeze-dried boiled samples from D. alata and D. esculenta
France	INRAe	Boiled Plantain		
Guadeloupe	INRAe	Boiled Yam	Africa-Yam	44 samples of raw Yam flour of the popB of D. alata were analyzed by NIRS and prediction of DM, Starch, Protein, Sugar, Hardness…were made using calibration models developed by Ehounou and al. in 2021 and were send to CIRAD (G. Arnau). NIRS acquisition spectra on 132 raw Yam flours, harvested at Roujol and Godet in 2020 and 147 at Roujol in 2021, corresponding to 47 genotypes for,CIRAD-Montpellier breeding program Texture and processing ability of samples from harvest 2021: 66 fresh raw and 66 boiled samples from *D. alata*, *D. trifida* and *D. esculenta*
Uganda	NARL	Matooke		NARL Matooke RTBFoods team work closely with Accelerated Breeding Better Bananas, a sister project supported by Bill and Melinda Gates. The project benefits from the RTBFoods data and have used WP1 data to improve the selection criteria of hybrids. ABBB project has also procured a Chromameter to support the RTBFoods activities
Uganda	CIP	Boiled Sweepotato	SweetGAINS	SweetGAINS project has supported the remodelling and refurbishment of a modern Kitchen at Namulonge SweetGAINS has also awarded the Artificial intelligent lab at Makerere University a subgrant of $50,000 to develop prediction models for mealiness from image analysis. RTB CRP-breeding community of practice assigned an additional $15000 to support the consultancy of Ephraim Nuwamanya (potato and sweetpotato starch characterisation).
Uganda	CIP	Boiled Potato		
UK	JHI	Boiled Sweepotato		CIRAD/ CIP Uganda – samples from Uganda – complementary cell wall starch analysis/ sensory trait data
Cameroon	CARBAP	Boiled Plantain	Africa-Yam	CARBAP participated to the joint AfricaYam/RTBfoods workshop on Yam Quality Evaluation from the 22nd to 26th November 2021.

10 Conclusion & perspectives

10.1 WP2 progress & key scientific achievements in period 4

Summary narrative on Key Scientific Achievements at WP level in Period 4: Synthesis on main highlights and major results at WP level.

WP level

- ✓ SOP for sensory analysis (one of the main challenges of the RTBfoods project) and texture have been defined (partners trained for texture analysis and interpretation of results) for almost all the products

Table 20 List of SOPs for sensory evaluation and instrumental texture (drafted procedures are highlithed in yellow)

Intermediate or final ready to eat products characterization	Boiled Cassava		Gari/EBA	Attieke	Fufu	Boiled yam	Pounded Yam	Boiled Plantain	Fried Plantain	Matooke	Boiled sweetpotato	Fried sweetpotato	Boiled potato
Preparation & Sensorial Evaluation													
UAC/FSA	E.6.5 :SOP_Sensory Characterization_Boiled Cassava.pdf					E.6.3_SOP: Sensory Characterization_Boiled Yam.pdf							
IITA			E.6.7_SOP: Sensory Characterization_Eba.pdf										
Bowen U.							E.6.6_SOP: Sensory Characterization_Pounded Yam_2020.pdf						
NaCRRI	E.6.4 : SOP_Sensory Characterization_Boiled Cassava.pdf												
CIP											E.6.2_SOP: Sensory Characterization_Boiled Sweetpotato.pdf		
NARL										E.6.1_SOP: Sensory Characterization_Matooke.pdf			
NRCRI					E.6.7_SOP: Sensory Characterization_Fufu.pdf								
CARBAP								Procedure from CIRAD needs writing and updating if necessary (Kouassi et al, 2021)					
CNRA				E 6.9_SOP: Sensory_Attikeke									
Textural analysis	CIAT/NaCRRI	UAC/FSA											
TPA		Cassava sample_preparation_for_texture.doc (under revision)	E.5.5 SOP: Lab Processing & Textural Characterization_Eba.pdf		E 5.7_SOP: Textural Characterization of Fufu	E.5.4_SOP: Lab Processing & Textural Characterization_Boiled Yam.pdf	E 5.8_SOP: Textural Characterization of Pounded Yam	Procedure from CIRAD needs writing and updating if necessary (Kouassi et al, 2021)		E.5.2_SOP: Lab Processing & Textural Characterization_Matooke.pdf	E 5.3_SOP: sample_preparation_for_texture_sweetpotato (under revision)		
Penetration test													
Extrusion	E.5.1_SOP_Lab Processing & Textural Characterization_Boiled Cassava.pdf												

- ✓ Relationship between instrumental texture measurements and texture sensory evaluation has been evidenced for several products

- ✓ Rapid preparation procedures and/or kitchen tests have been proposed for testing processing ability for several products (Table 21).

Table 21 List of SOPs for kitchen tests (drafted procedures are highlithed in yellow)

Intermediate or final ready to eat products characterization	Boiled Cassava	Gari/EBA	Attieke	Fufu	Boiled yam	Pounded Yam	Boiled Plantain	Fried Plantain	Matooke	Boiled sweetpotato	Fried sweetpotato	Boiled potato
Processing ability												
Peeling ability												
Fermentation			Friability test during boiling (draft procedure under	Clarity and foamability of fermenting liquor (draft procedure under writing)								
				Penetrometry during fermentation (draft procedure under writing)								
Cooking ability												
Swelling power and texture	E 5.6_SOP: characterization of water absorption, cooking time and closing angle of boiled cassava	Volume increase with hot water (draft procedure under writing)			Water absorption, cooking time (draft procedure under writing)	RVA measurements (draft procedure)	Cooking hability (toothpick or fork test under development)			Water absorption (draft procedure under writing)		
Colour & imaging		Colour parameters using Hunter Lab (draft procedure under writing)					Colour parameters using chromameter (draft procedure under writing)		Colour parameters using chromameter (draft procedure under writing)	Image analysis from the DigiEye for predicting mealiness (procedure under		

✓ Candidates for biophysical analysis (cell wall content and pectin contents, methylation degree of pectins, polyphenols etc) have been identified; SOPs have been proposed (Table 22) and Proof of concepts demonstrated for some of them.

Table 22 List of SOPs for biophysical analyses of RTBs (drafted procedures are highlithed in yellow)

RTB raw material characterization	Cassava	Yam	Bananas	Sweetpotato	Potato
Dry Matter	E.4.1_SOP_Dry Matter_2019.pdf				
Sugar content & composition					
Colorimetry	E.4.2_SOP_Starch & Sugars through Acid Hydrolysis_2019.pdf				
HPLC	E.4.3_SOP_Sugars by HPLC_2019.pdf				
Starch					
Colorimetry	E.4.2_SOP_Starch & Sugars through Acid Hydrolysis_2019.pdf				
Enzymatic (under revision)	Starch-analysis-enzymatic (under revision)				
Amylose					
DSC	E.4.3_SOP_Sugars by HPLC_2019.pdf				
Colorimetry (under revision)	Complexation with iodine (under revision)				
Total Pectin					
Colorimetry-manual	E 4.9_SOP: Determination of galacturonic content				
Colorimetry-automatic (writing)	Extraction & coloration with MHDP (under writing)				
Methylation level		Internal procedure (INRAe)		E 4.13_SOP: Fourier Transform Infra-Red Spectroscopy analysis of cell walls from sweetpotato roots	
CW extraction	Draft procedure (CIAT)	E 4.10_SOP: Sample preparation for cell wall polysaccharides analysis of raw and boiled yam		E 4.11_SOP: Preparation of cell wall material from sweetpotato roots	
CW characterization		E.4.5_SOP: Analysis of Cell Wall Polysaccharides from Yam & Plantain_2020.pdf		E 4.12_SOP: Analysis of monosaccharide composition of sweetpotato cell wall material/ polysaccharides after acid hydrolysis by high performance anion exchange chromatography (HPAEC)	
PME		Internal procedure (INRAe)		E.4.6_SOP_Determination of Pectin Methylesterase Activity in Sweetpotato Roots_2020.pdf	
Polyphenols	Extraction, colorimetry with Folin-Ciocalteu (writing, IITA)				
PPO	Draft procedure in IITA				
Cyanide content	Draft SOP in NaCRRI				
RVA	Internal procedure (CIAT)				

Success Story: Predicting mealiness of boiled cassava by biophysical analyses

Mealiness is a key sensory attribute of boiled cassava, identified consistently during consumer surveys as essential for varietal adoption (NaCRRI, Uganda and UAC-FSA Benin). In Period 4, boiled cassava mealiness was evaluated by sensory panellists at CIAT, Colombia, and for the first time significant correlations were found with Water Absorption at 30' (WA30, R2 = 0.64). The ratio of the final (FF) and maximum forces (MF) measured by the texturometer using the extrusion protocol developed at CIAT, also correlates very well with the friability (mealiness) (FF/MF, R2 = 0.67). The link with FF/MF ratio confirms that mealiness is related to the perception of breaking behaviour during chewing, as samples that do not break during texture-extrusion are perceived as more mealy, and vice-versa. Thus, mealiness can be predicted instrumentally, which is key to increase phenotyping throughput and integrate product quality criteria in the screening and selection process.

As NIRS predictions are strengthened on the basis of Water absorption and texture parameters, it may become possible in Period 5 to predict mealiness of up to 100-200 samples per day, and achieve high-throughput screening of a sensory attribute. These results illustrate how RTBfoods through the integrated interdisciplinary work of food scientists and breeders develops medium- and high-throughput phenotyping tools that predict the sensory quality of RTB products, for the benefits of RTB breeding programs. The development of these new tools will reduce breeding costs and should lead to better varietal adoption rates by cassava farmers.

Success Story: **Sensory firmness well predicted on structured product by instrumental analysis**

At the end of period 4, nine RTB foods product profiles were sensorially characterized, based on the Standard Operating Protocols (SOPs) and using the previously validated descriptors for boiled cassava, Gari-Eba, attiéké, Fufu, boiled Yam, pounded Yam, boiled plantain, matooke, and boiled sweet potato. The capacity of the partners has been strengthened to organize their own sensory panel, but also to control and analyse the results produced. The organization of sensory panels, in complete autonomy, is now possible by adapting the RTBfoods methodology for other products to be tested. For the first time, we have extensive and specific descriptors for each of these product profiles (PP) where texture plays a major role. Among sensory attributes considered as priority quality traits, firmness (or hardness or softness), whether measured in the mouth or by hand, was the sensory attribute that best correlated with instrumental measures of texture (penetrometry, TPA, extrusion) on products that did not undergo destructuring during preparation (boiled cassava, Yam, plantain and sweet potato). Typical correlation coefficients R^2 between sensory and instrumental firmness were greater than 0.70. This means that it is now possible to assess the firmness of these PPs by instrumental measurements, which are more rapid and objective than sensory analyses, thus opening the door to screening larger numbers of hybrids for firmness, earlier in the breeding pipeline.

10.2 Perspectives for period 5

Please note that this section requires that WP coordinators take time to discuss with each WP team and agree on priorities that are aligned on WP overall expected outputs by the end of the project (see hereunder).

*The table below lists **WP2 outcomes & outputs** due for Period 5 and the **committed targets** that should be reached by the end the last project period (January 2022-January 2023). It is the responsibility of WP2 coordinators and partners to plan activities that will allow the entire WP to keep these commitments.*

Outcome Output	**Expected/Committed Targets** at end of Period 5
1.3	**10 SOPs on Cirad Dataverse &/or BTI repositories on open access** **Proven use of SOPs by breeders** and food scientists
1.3.2	**22 quality traits** *(minimum 2 traits per product profile)* for RTB food/processed products defined with lexicon and objective **attribute goals** *(= to reflect end-user acceptability)*
1.5.2	*(In total)* **32 quality traits for which quantitative information is available** in a database
1.5.4	*(with WP4 breeders)* **100% of genotyped clones** *(= populations selected in RTBfoods WP4)* from partner breeding programs **attached/informed with laboratory data in BreedBase**

*<u>In the table below</u>: Ask each WP team to inform the table below with their **Priority Activities** planned for the last project period (January 2022 - January 2023).*
NB: Obviously, additional rows can be inserted.

<u>Column in red</u>: It is crucial to have in mind that we committed to characterize 100% of WP4 clones by the end of Period 5 – all partner activities must now be aimed at this goal.

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
Uganda	NaCRRI	Boiled Cassava	Development of proof of concepts for starch, amylose and hydrogen cyanide	740	140	28	3 proofs of concepts for starch, amylose and HCN	Ephraim Nuwamanya
Uganda	NaCRRI	Boiled Cassava	Sensory characterisation of advanced clones			28	Genotypes with good sensory traits selected	Micheal Kaaabi
Uganda	NaCRRI	Boiled Cassava	Characterisation of cassava genotypes for starch, amylose and HCN	740	140	28	Reference information for WP3 Characteristics of genotypes	Ephraim Nuwamanya
Colombia	CIAT	Boiled Cassava	1. Harvests of the RTBfoods progenitors collection at 9, 10 and 11 months after planting (December 2021 to February 2022). Characterization of dry matter (fresh and 30' boiling), cooking time (fork method), water absorption at 30' boiling, texture-extrusion at 18' boiling, NIRS (fresh	31 x 3 harvests = 93	31 x 3 harvests = 93	n/a	1. Dataset of NIRS spectra and reference data of the progenitors collection uploaded to Cassavabase 3. Report on NIRS calibrations and prediction of cooking quality of boiled cassava	Thierry Tran

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
			and 30' boiling), sensory analysis at 20' boiling.				4. Peer-reviewed article on correlations between instrumental texture and cooking quality (cooking time, water absorption, sensory traits) of boiled cassava.	
Colombia	CIAT	Boiled Cassava	2. Harvest of the RTBfoods progeny collection at 10 and 12 months after planting (January 2022 and March 2022). Characterization of water absorption at 30' boiling, NIRS (fresh).	237 x 2 harvests = 474	237 x 2 harvests = 474	n/a	2. Dataset of NIRS spectra and reference data of the progeny collection uploaded to Cassavabase	Thierry Tran
Colombia	CIAT	Boiled Cassava	3. Proof of concept: Investigate if there is a level of Ca2+ saturation of pectins, above which the hardness of boiled cassava no longer increases. Check if this saturation level is different depending on genotype.		5 to 10	n/a	5. Report on effect of Ca^{2+} on water absorption and texture of boiled cassava.	Thierry Tran
Nigeria	NRCRI	Gari-Eba	1.Sensory profile analysis will be	22 génotypes	30 génotypes	5 genotypes	1. Reports of correlation	Ugo Chijioke

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
			conducted using 10 trained panellist and samples will be simultaneously evaluated for Instrumental texture profile analysis. 2. Proof of Concept of the effects of Polyphenol oxidase on enzymatic and non-enzymatic browning of Gari 3. Proof of concept on biophysical properties of cassava roots influencing ease of peeling and sieving during Gari processing	22 génotypes 54 Nextgen AYT clonesx2locations	30 génotypes 54 Nextgen AYT clonesx2locations		between Sensory profile analysis and instrumental textural analysis of Eba. Progress report on the development of the PoC To obtain threshold for good, intermediate and poor cassava genotypes with respect to productivity	
Nigeria	NRCRI	Fufu	1 Validate result Sensory characterisation of Nextgen cassava advanced clones 2. Validate result of relationship between sensory properties and instrumental texture attributes of Fufu 3. Characterize retting ability of Nextgen	18 18 43clones x 2 location (40 improved genotypes and 3 checks)	18 18 43clones x 2 location (40 improved genotypes and 3 checks)	5 5	Genotypes with good sensory traits selected Establish relationship between sensory and instrumental textural properties of cooked Fufu Classify the different	Ugo Chijioke

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
			cassava genotypes at early breeding stage 4. Proof of concept between colour of cooked Fufu ,tannin con tent of fresh cassava roots and colour of fresh cassava roots				genotypes into 3 categories namely; **Fast retting, intermediate retting and slow retting**. To obtain threshold of tannin for good, intermediate and poor cassava genotypes with respect to colour Fufu mash and cooked Fufu	
Nigeria	NRCRI	Boiled Yam	1. Sensory characterisation of African Yam advanced 2. Texture characterisation of African Yam advanced clones 3. Characterization of dry matter, crude fiber, starch, sugar, amylose amylopectin and total phenol content of fresh and 20' steam, cooking time (fork and	18 18 18 x 4 locations	18 18 18 x 4 locations	5 5 5	Genotypes with good sensory traits selected Establish relationship between sensory and instrumental textural properties of cooked Fufu Prediction of relationship between texture, colour (cooking quality) of	Ugo chijioke

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
			handheld penetrometer method) chromamoter reading and NIRs spectra acquisition				boiled Yam and biophysical properties of fresh Yam	
Nigeria	NRCRI	Pounded Yam	Conduct sensory analysis of pounded Yam Conduct instrumental texture analysis of pounded Yam Relate the dry matter, total properties of Yam (fresh and stored) to pounding time and colour of pounded Yam	18 18 54	5 5		Genotypes with good sensory traits selected Establish relationship between sensory and instrumental textural of pounded Yam and biophysical properties of fresh and stored Yam tubers	Ugo Chijioke
Nigeria	IITA	Gari-Eba	1.Sensory profile analysis was conducted using 14 trained panellist and samples were simultaneously evaluated for Instrumental texture profile analysis. 2. Proof of Concept of the effects of Polyphenol oxidase on enzymatic and non-enzymatic browning of Gari	26 génotypes	30 génotypes	10 génotypes	1. Reports of correlation between Sensory profile analysis and instrumental textural analysis of Eba. Progress report on the development of the PoC	

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
Nigeria	IITA	Fufu						
Nigeria	IITA	Boiled Yam	To harmonize the SOP amongst partners for the détermination of cooking time and water absorption of boiled Yam	16 genotypes	16 genotypes	16 genotypes	Reports of correlation between Sensory profile analysis and Intrumental textural analysis of boiled Yam	
Nigeria	IITA	Pounded Yam						
Nigeria	Bowen University	Pounded Yam	1)Complete biochemical analyses on Yam used fo consumer studies in WP1 both fresh and stored 2) Establish instrumental method to evaluate stretchability		13		-Biochemical composition of Fresh and Stored Yams used for consumer testing in WP1 -instrumental method to evaluate stretchability -Proof of concept to use pasting characteristics of raw Yam to indicate stretchability	
Benin	UAC-FSA	Boiled Yam	Complete biophysical characterization (starch, sugars, amylose and pectin) at Montpellier on the	Not applicable	Not applicable	Not applicable	Writing report on biophysical and biochemical characterization of boiled Yam	L. ADINSI

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
			samples collected during sensory profiling in period 3					
			Extend/expand four additional Yam varieties harvested from farmer field + all biophysical and biochemical characterization	Not applicable	Not applicable	Not applicable		
France	CIRAD	Boiled Yam	Pectin and amylose déterminations		54		PoC about the relationship between pectin and/or amylose content and texture of boiled Yam	C. Mestres
France	CIRAD	Boiled Cassava			20		PoC about the relationship between pectin and/or amylose content and texture of boiled cassava	
		Boiled sweet potato			20		PoC about the relationship between pectin and/or amylose content and texture of boiled sweet potato	
		Gari/Eba	Develop rheometric and/or viscosimetric procedure to		10 (?)	10 (?)	New SOP for measuring the textural	S. Arufe

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
			discriminate cultivars and rely instrumental measures with sensory evaluation				properties of pasted product (Eba)	
		Pounded Yam	Develop rheometric and/or viscosimetric procedure to discriminate cultivars and rely instrumental measures with sensory evaluation Pectin and amylose déterminations		10 (?)	10 (?)	New SOP for measuring the textural properties of pasted product (pounded Yam) PoC about the relationship between pectin and/or amylose content and texture of pounded Yam	S. Arufe
France	INRAe	Boiled Yam	Proof of concept cell wall Texture, cooking ability	5	None			
France	INRAe	Boiled Plantain	Proof of concept cell wall		10			
Cameroon	CARBAP	Boiled Plantain	Finalize proof of concept for the relation between pulp colour of raw and boiled plantain pulps, amend SOP on colour measurement.				Proof of concept, SOP on colour measurement	G. Ngoh
			Finalize SOPs for pectin and starch determination; elaborate proof of				Proof of concept for the correlation between	G. Ngoh

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
			concept for the correlation between texture/pectin and starch				texture/pectin and starch; SOPs for pectin and starch determination	
			Sensory analyses of boiled plantain				Boiled plantain sensory vocabulary	G. Ngoh
			Laboratory physicochemical characterization			10 clones from Njombe and * clones from Bansoa	Data sheet	G. Ngoh
Uganda	NARL	Matooke	Sensory characterisations, biochemical analyses- starch, amylose/amylopectins polyphenols, pectins, carotenoids, Cooling behaviour	82	181	4	3 SOPs (Texture on raw samples, polyphenols and colour)	Kephas NOWAKUNDA
Uganda	CIP	Boiled Sweepotato	Sensory characterisaion for the parental genotypes+ Advanced clones Instrumental Textural analysis Starch characterisation	40 220	20 220	9 9	Textural and sensory profiles for priority quality traits for the SPVD hybrid breeding training population POC-establish correlations for boiled sweetpotato	Mukani, Mariam & Reuben

Country	Institute	Product Profile	Activity Description (i.e. sensory characterization, dvpt of instrumental methd for., proof of concept on…, etc.)	Nb of Genotpyes Characterized			Deliverable	Accountable Person for Delivery
				Also sent to WP3 for scanning	From WP4 RTBfoods population	From WP5 Elite Clones (assessed in PVS)		
							sensory attributes with starch content composition (amylose and amylopectin proportion), RVA parameters and maltose.	
Uganda	CIP	Boiled Potato	Sensory characterisaion for the Advanced clones Instrumental Textural analysis	40 40	40 40		POC-establish correlations for boiled potato sensory attributes with starch content composition (amylose and amylopectin proportion), RVA parameters and maltose.	Mukani, Mariam & Reuben
UK	JHI	Boiled Sweetpotato	Biochemical analysis - cell wall preparation , cell wall monosaccharide composition, cell wall FTiR analysis	18				Taylor
All	WP2 coordinators	All						

*Narrative on Draft Period 5 Roadmap & Expected Outputs: From discussions with WP2 partner teams and priority activities listed here above per team, consolidate the Period 5 roadmap at WP level, first – This roadmap should **clearly show how the WP team consider reaching the expected outputs** reported in the table, at the beginning of section 9.2 [± 3 pages].*

NB: special attention should be paid to planning:

1- Laboratory characterization of 100% of WP4 breeding clones;
2- the transfer onto BreedBase of all laboratory data on the quality of WP4 clones

WP level

For Sensory evaluation and Texture:

- Three SOPs will be rapidly finalized and missing SOPs for boiled potato and fried sweetpotato will be adapted from the existing SOPs (see Table C1),
- In addition, SOPs will be developed for PQT of pasted PPs such as stretchabiity for pounded Yam using adapted texture measurements and/or rheology mesurments
- Evaluation of sensory and textural characterisation of expanded numbers of varieties by RTB partners wil thus be performed in period 5,
- A data base of sensory evaluation and texture results will be built, and
- Models for predicting quality will be proposed based on the outstanding relationships between the sensory and textural attributes of product profiles

For kitchen tests:

- numerous candidates of kitchen tests identified in period 4 will be developed
- They will be tested in period 5 (see Table C2) on a large set of samples,
- A data base of kitchen test results will be built, and
- Models for predicting quality and/or processing ability will be proposed based on the outstanding relationships between kitchen test results and quality and/or processing ability

For biophysical analyses:

- Several SOPs will be rapidly finalized and shared with partners (see Table C3),
- On-line training will be organized, when necessary, and a ring test will be organized to validate the use of the procedures by all the partners,
- Biophysical analyses will then be performed on expanded numbers of varieties by RTBfoods partners in period 5, in parallel to sensory evaluation and texture analyses,
- A data base of biophysical analyses will be built, and
- Models for predicting quality will be proposed based on the outstanding relationships between the biophysical results and sensory and/or textural attributes of product profiles

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