Laboratory Standard Operating Procedure

SOP for Sample Preparation, Determination of Instrumental Texture of Steam-cooked Cassava

Biophysical Characterization of Quality Traits, WP2

Cotonou, Benin, November 2022

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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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WP2: Biophysical Characterization of Quality Traits

SOP: Sample preparation, determination of instrumental texture of steam-cooked cassava

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ABSTRACT

Steam-cooked cassava preparation show variations related to the cassava piece size and the cooking mode; this presumably affect the sensory descriptors and texture of final product. In this context, a robust methodology for steam-cooked cassava preparation and texture analyses was established. After washing and cutting the roots in about 5 cm long, each piece is divided in the shape of half cylinder prior to peel, and then washed in tap water before cooking. Ideal cooking time (ICT) defined as fixed cooking duration (45 min) of cassava varieties was determined. The ICT is used to steam the samples which were tested at 45 °C by penetration test, simple compression and extrusion test using conical probe P/40C, compression plate (P/75) and Ottawa cell with 5-blade grid respectively. All methods (penetrometry, compression, extrusion) exhibited clear discrimination between cassava varieties regarding texture parameters.

Keywords: Steam-cooked cassava, cooking time, penetration test, compression test, extrusion test

1 SCOPE AND APPLICATION

This SOP describes the protocol for the preparation of cassava samples for steam-cooking for ideal cooking time (ICT) prior to performing texture and sensory analyses of steam-cooked cassava. In comparison to cooking in water, steam cooking is preferably used to avoid disintegration of cooked cassava.

2 REFERENCES

Laurent ADINSI, Laurenda HONFOZO, Noël AKISSOE, (2021). *Sample Preparation and Cooking Time for Texture Analysis of Steam-cooked Yam. Biophysical Characterization of Quality Traits, WP2.* Cotonou, Benin: RTBfoods Laboratory Standard Operating Procedure, 15 p.

Thierry TRAN, Andres ESCOBAR (2019). *SOP for Protocol for Characterization of Cooking Time and Texture of Steam-cooked Cassava: Texture-extrusion.* Cali, Colombia, RTBfoods Project Report, 19 p.

3 DEFINITIONS

Cassava roots are washed, cut longitudinally to length of about 5 cm and peeled. Thereafter, each piece is cut in the shape of half cylinder and then steam-cooked up to 30 minutes, before being evaluated for the change in texture every 5 minutes until optimum cooking time (as determined by probing the pieces with a fork) is reached. The resulting steam-cooked cassava pieces are evaluated by assessors in the point of view of texture, mainly the hardness (firmness).

Optimum cooking time (OCT): Duration of the steam-cooking, necessary to obtain adequate texture acceptable for consumption. It corresponds to the time when all panellists assessed that the cassava pieces are at least fairly soft using a 3 scale points.

Ideal cooking time (ICT): Fixed duration determined from OCT of range of cultivars. It is used to prepare samples for both sensory and texture analyses. The ICT was chosen as the cooking time for optimal cooking and used to generate final texture data.

Max Force (Hardness) for penetromety test (N or g): Defined as the maximum force at max displacement of the probe in the steam-cooked cassava piece as captured in Figure 1.

Area (work done for penetration) (N.s or g.s): Defined as the full area under the curve, i.e. between 0 and 10 mm travel distance or 0 and 20 s travel time into the sample as captured in Figure 1. This area represents the work (energy) required to carry out the penetration test.

Hardness for simple compression (N or g): Defined as the maximum force required by the probe to compress the steam-cooked cassava piece as captured in Figure 2.

Slope for extrusion test (N/mm): Slope of the texture curve at the beginning of the extrusion that is recorded between 0 and 1 mm travel distance (based on preliminary experiments).

Max force (Hardness for extrusion test) (N or g): Mean maximum force recorded during the extrusion experiment as captured in Figure 3.

Distance at Max force for extrusion test (mm): Distance at which the maximum force occurs during the extrusion experiment

Area (work done for extrusion) (N.s or g.s): Full area under the curve, i.e. between 0 and 20 mm travel distance into the sample. This area represents the work (energy) required to carry out the extrusion test.

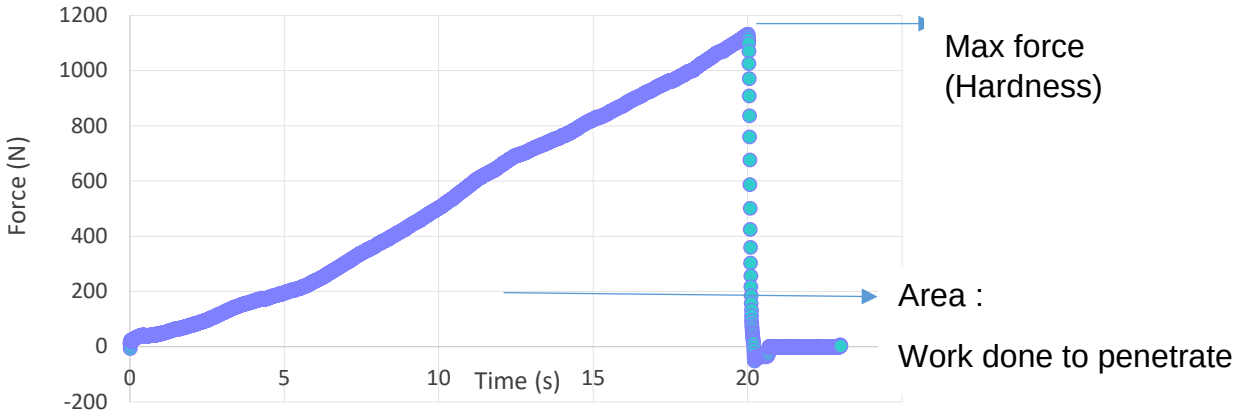


Figure 1: Penetrometry curve of steam-cooked cassava

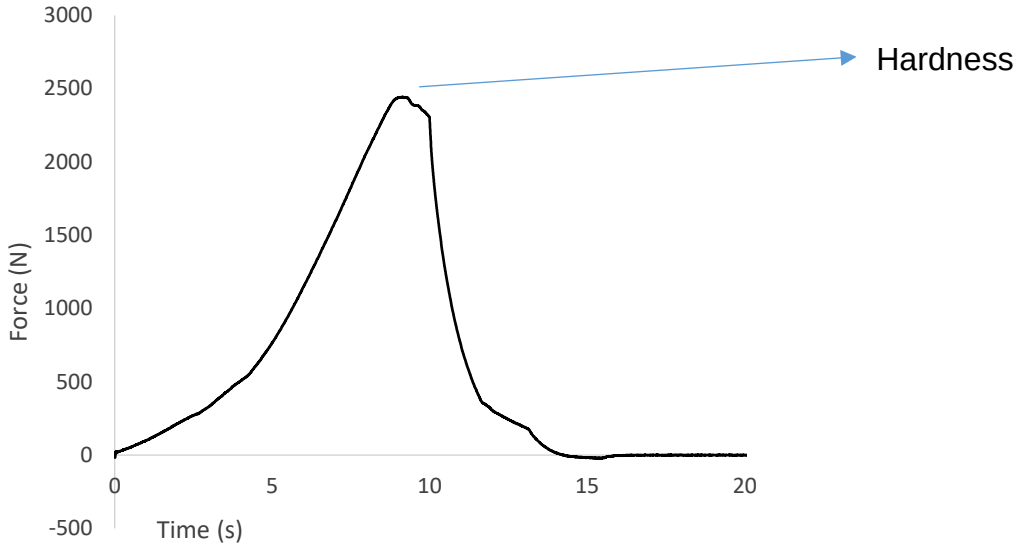


Figure 2: Simple compression curve of steam-cooked cassava

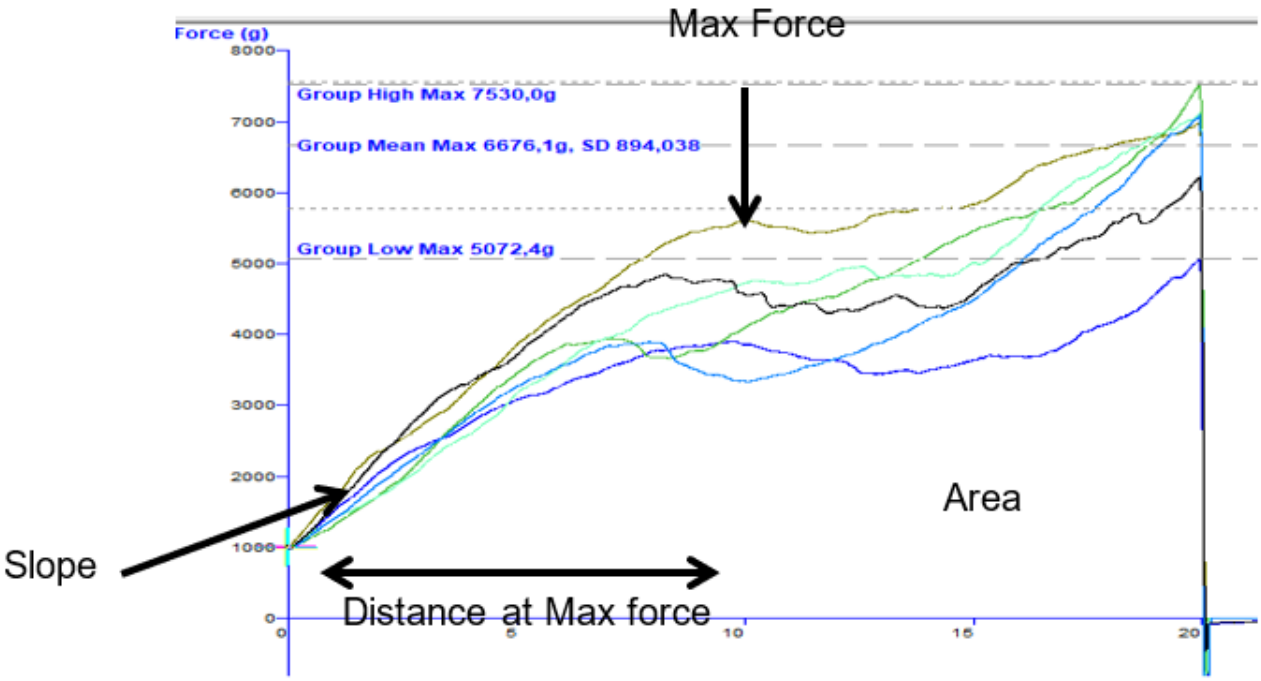


Figure 3: Extrusion curve of steam-cooked cassava

4 PRINCIPLE

The standard operating protocol (SOP) for determination of cooking time and texture of steam-cooked cassava is based on the ability of an instrumental device to break/compress or to penetrate or to extrude a sample during a displacement of the probe at a known speed over a distance.

5 REAGENTS

Not applicable

6 APPARATUS

- Texture analyser (the model used for the development of this SOP is a TA-XTPlus (Stable Microsystem, UK), equipped with a load module of 50 Kg.
- Ottawa extrusion (118 mm*79.9 mm*11.9 mm) cell with 5-blade grid (distance between blade 10.1 mm) (Figure 4)
- Conical probe (P/40C) – Perpex (Figure 5)
- Compression plate (P/75) – Aluminium (Figure 6)
- Steam cooker with three trays was used Only the first tray was used in our experiment.
- Fork to assess optimum cooking time.
- Chronometer
- Meter/rule
- Oven (45 °C)
- Thermometer

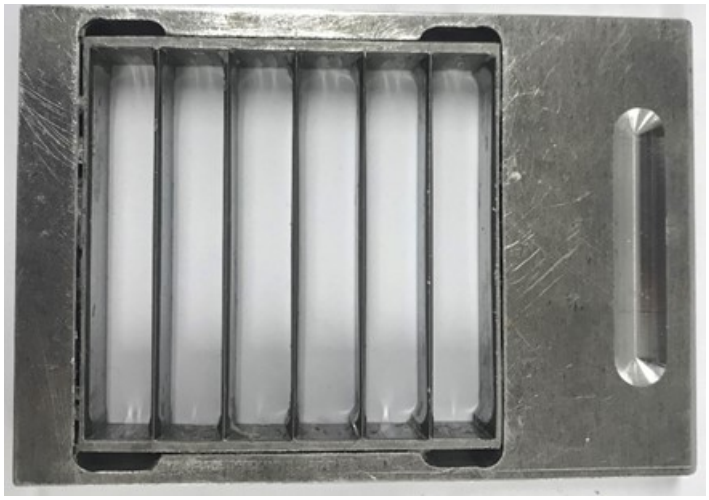


Figure 4: Ad-hoc 5-blade grid used for texture-extrusion analysis

Figure 5: Conical probe (P/40C) used for penetration test

Figure 6: Compression plate (P/75) used compression test

7 PROCEDURE

7.1 Product preparation

7.1.1 Sampling and preparation before steam-cooking

- Select two to three similar-sized cassava roots per variety
- After washing, cut off the proximal and distal ends of the roots (3 cm from both ends), thereafter, cut the roots into pieces of about 5 cm length (Figure 7)
- Cut each piece in the shape of half cylinders

Peel the half-cylinders, wash and rinse them in tap water

figure 7: Cassava roots cut into pieces of about 5 cm length

7.1.2 Determination of optimal cooking time (OCT)

To record optimum cooking time (duration), pieces of half-cylinders from one root per cultivar were steam-cooked up to minimum of 30 min (Figure 8), and thereafter, the softness (as indicator of extent of cooking) is checked every 5 min using a fork probing method. In addition, for each cultivar, softness is assessed by trained assessors using a 3 scale points, where 1=not soft, 2=fairly soft and 3=very soft. Each assessor received one piece. The time when all panellists reported that the pieces are at least fairly soft is defined as OCT.
The ideal cooking time/duration calculated as the average value of OCT of five cassava cultivars is close to, and fixed at at, 45 min.

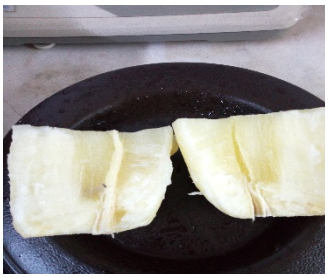


Figure 8: Cooked half cylinders

7.2 Texture measurement

7.2.1 Calibration of texture analyser

The probe height and force are calibrated following the calibration instructions provided in the operation manual. Calibrations of probe distance are done at 40 mm for penetrometry and compression tests, and at 70 mm for extrusion test. The load module used for the three tests is 50 Kg; the force calibration is done using a 2 kg standard weight.

7.2.2 Steam-cooking

Two to three roots were used per cultivar for each steam cooking batch and six to eight half cylinders pieces were collected per root. Each cultivar batch was steam-cooked separately and in triplicate. In total, nine cultivars were studied. After 45 min of cooking, all the pieces are removed and packed in insulated container before storage at 45 °C during 30 min in an oven/incubator before performing the tests.

7.2.3 Penetrometry test

For each cooked and stabilized half cylinder, two points of measure were carried out from both extremity. The penetration test was performed with the conical probe P/40C and the settings collected from Adinsi et al. (2021) are shown below:

Pre-Test Speed	10 mm/s
Test speed	0,5 mm/s
Trigger force	5 g
Target distance	10 mm
Temperature of test	45 °C

18 half cylinders (6 in triplicate) were tested to characterize one cultivar.

Two main variables were measured:

- Max Force (Hardness) (N or g): Defined as the maximum force at max displacement of the probe in the steam-cooked cassava piece.
- Area (work done for penetration) (N.s or g.s): Defined as the full area under the curve (energy) required to carry out the penetration test.

7.2.4 Simple compression

The simple compression was performed on cooked and stabilized half-cylinders with the compression plate (P/75) and the settings shown below:

Pre-Test Speed	10 mm/s
Test speed	0,5 mm/s
Trigger force	10 g
Strain	25%
Temperature of test	45 °C

18 half cylinders (6 in triplicate) were tested to characterize one cultivar.

One variable was measured:

Hardness (N or g): Defined as the maximum force required by the probe to compress the steam-cooked cassava piece

7.2.5 Extrusion test

The SOP on cooking time and texture of steam-cooked cassava developed by Tran and Escobar (2019) was adapted to define the extrusion protocol. After 45 min of cooking half-cylinder samples, the cassava pieces stabilized at 45 °C were analysed with fibers positioned perpendicular to the blades of the extrusion grid. The settings are shown below:

Pre-Test Speed	10 mm/s
Test speed	0,5 mm/s
Trigger force	1000 g
Target distance	20 mm
Temperature of test	45 °C

18 half cylinders (6 in triplicate) were tested to characterize one cultivar.

Three variables are measured:

- Max force (Hardness for extrusion test) (N or g): Maximum force recorded during the extrusion experiment.
- Distance at Max force for extrusion test (mm): Distance at which the maximum force occurs during the extrusion experiment
- Area (work done for extrusion) (N.s or g.s): Full area under the curve, i.e. between 0 and 20 mm travel distance into the sample.

8 EXPRESSION OF RESULTS

The significant differences between mean instrumental texture measurements of the varieties are determined as shown in Annex 1.

9 CRITICAL POINTS OR NOTE ON THE PROCEDURE

Sample temperature and size /shape sample should be controlled as close as possible for each measurement.

10 TEST REPORT

Exemplary test results are discussed in the appendices.

11 APPENDICES

11.1 Annex 1

Cassava Varieties used (var_harvesting month): Agri_9, Agric 12, Attinwéwé_6, Dossi_9, Dossi_12, Hombété_8, Ofèguè_8, Alanmandou_6, and Koléhaomè_6

Penetrometry	Simple compression	Extrusion
Agric_9	Agric_9	Agric_9
Agric_12	Agric_12	Agric_12
Attinwéwé_6	Alanmandou_6	Dossi_9
Dossi_9	Attinwéwé_6	Dossi_12
Dossi_12	Dossi_9	Hombètè_8
Hombètè_8	Dossi_12	Ofégué_8
Ofégué_8	Hombètè_8	
	Koléahonmè_6	
	Ofégué_8	

Statistical accuracy of texture attributes measured

Penetrometry

Parameter	Variety	N	Mean	Std Dev	Std Err	CV, mean (%)
Max force (N)	Agric_9	3	0.657	0.202	0.117	18
	Agric_12	3	0.410	0.139	0.080	20
	Attinwéwé_6	1	1.000	.	.	
	Dossi_9	3	0.507	0.049	0.028	6
	Dossi_12	3	0.227	0.072	0.042	19
	Hombètè_8	3	0.540	0.101	0.059	11
	Ofégué_8	3	0.793	0.146	0.085	11
Area (N.s)	Agric_9	3	6.273	1.563	0.902	14
	Agric_12	3	4.063	1.418	0.819	20
	Attinwéwé_6	1	10.770	.	.	
	Dossi_9	3	4.823	0.853	0.492	10
	Dossi_12	3	2.243	0.879	0.507	23
	Hombètè_8	3	5.340	1.359	0.785	15
	Ofégué_8	3	7.503	1.739	1.004	13

Simple compression

	variety	N	Mean	Std Dev	Std Err	CV, mean (%)
Hardness (N)	Agric_9	3	5.21	1.30	0.75	14
	Agric_12	3	2.61	1.14	0.66	25
	Alanmandou_6	3	10.71	2.40	1.38	13
	Attinwéwé_6	3	6.07	2.38	1.37	23
	Dossi_9	3	2.34	0.31	0.18	8
	Dossi_12	3	1.29	0.57	0.33	26
	Hombètè_8	3	4.95	1.08	0.63	13
	Koléahonmè_6	3	7.07	1.48	0.86	12
	Ofégué_8	3	6.38	4.19	2.42	38

Extrusion

	Variety	N	Mean	Std Dev	Std Err	CV , mean (%)
Slope (N/s)	Agric_9	3	0.470	0.087	0.050	11
	Agric_12	3	0.290	0.106	0.061	21
	Dossi_9	3	0.167	0.096	0.055	33
	Dossi_12	2	0.115	0.049	0.035	30
	Hombètè_8	3	0.393	0.049	0.028	7
	Ofégué_8	2	0.660	0.071	0.050	8
Max force (N)	Agric_9	3	3.777	0.980	0.566	15
	Agric_12	3	2.400	0.250	0.144	6
	Dossi_9	3	2.277	2.078	1.199	53
	Dossi_12	2	1.330	1.824	1.290	97
	Hombètè_8	3	4.357	0.492	0.284	7
	Ofégué_8	2	3.880	1.131	0.800	21
Distance at Max force (mm)	Agric_9	3	0.530	0.139	0.080	15
	Agric_12	3	0.760	0.053	0.031	4
	Dossi_9	3	0.537	0.398	0.230	43
	Dossi_12	2	0.435	0.488	0.345	79
	Hombètè_8	3	0.803	0.025	0.015	2
	Ofégué_8	2	0.585	0.021	0.015	3
Area (N)	Agric_9	3	49.593	14.379	8.301	17
	Agric_12	3	28.083	2.585	1.493	5
	Dossi_9	3	23.187	22.720	13.118	57
	Dossi_12	2	13.205	9.412	6.655	50
	Hombètè_8	3	37.490	7.637	4.409	12
	Ofégué_8	2	51.350	10.706	7.570	15

NB: data outliers were not removed. Outliers can be removed by statistical analysis

Examplary ANOVA of textural attribute Hardness (Max force) for the protocols

Penetrometry – Max force

One way Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Variety	6	0.79	0.13	7.96	1.258e-3
Error	12	0.19	0.016		
C. Total	18	0.99			

Connecting Letters Report

Level				Mean
Attinwéwé_6	A			1.00
Ofégué_8	A			0.79
Agric_9	A	B		0.65
Hombètè_8	A	B	C	0.54
Dossi_9	A	B	C	0.50
Agric_12		B	C	0.41
Dossi_12			C	0.22

Levels not connected by same letter are significantly different.

Simple compression - Hardness

One way Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Variety	8	198.63	24.82	6.25	6.171e-4
Error	18	71.40	3.96		
C. Total	26	270.04			

Connecting Letters Report

Level				Mean
Alanmandou_6	A			10.71
Koléahonmè_6	A	B		7.07
Ofégué_8	A	B	C	6.37
Attinwéwé_6	A	B	C	6.07
Agric_9	A	B	C	5.21
Hombètè_8		B	C	4.95
Agric_12		B	C	2.61
Dossi_9		B	C	2.34
Dossi_12			C	1.29

Levels not connected by same letter are significantly different.

Extrusion – Max force

Oneway Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Variety	5	17.06	3.41	2.16	1.4e-1
Error	10	15.77	1.57		
C. Total	15	32.83			

Connecting Letters Report

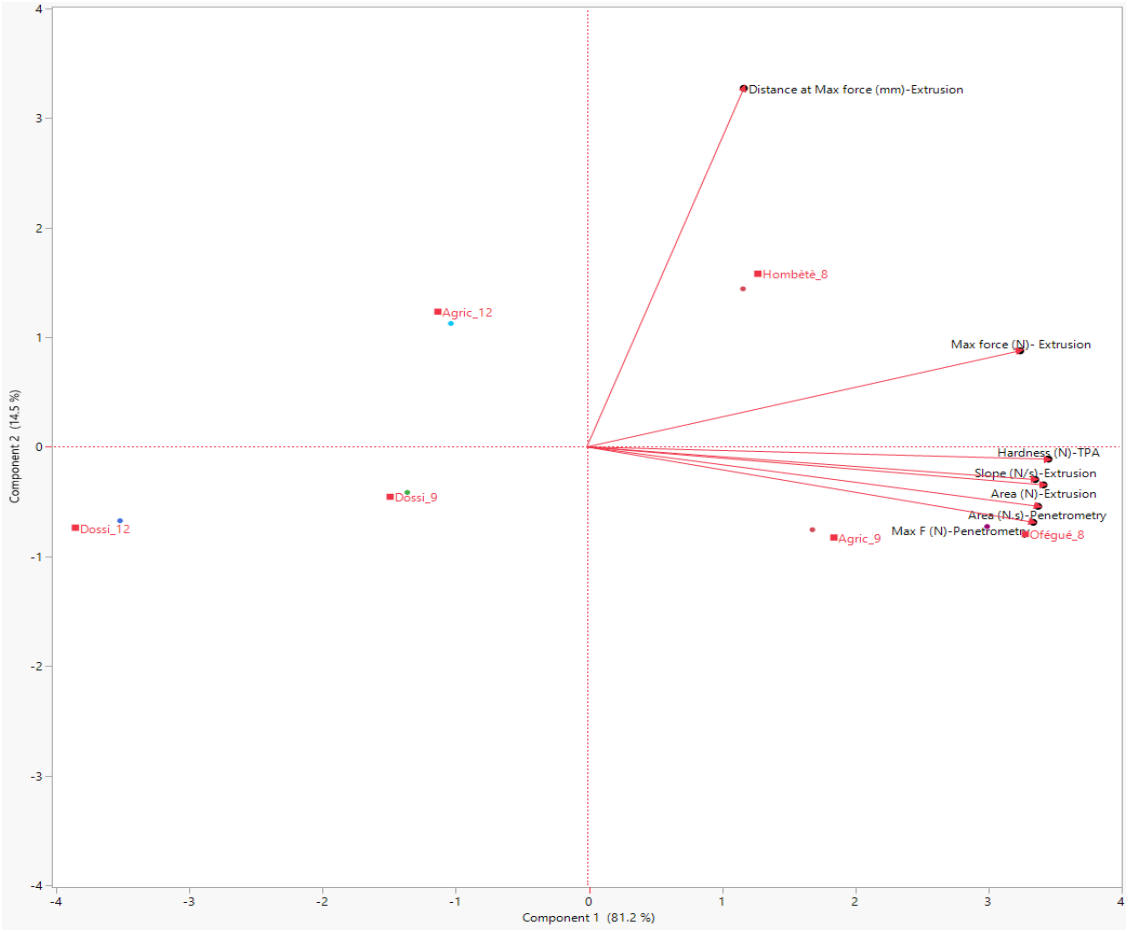
Level		Mean
Hombètè_8	A	4.35
Ofégué_8	A	3.88
Agric_9	A	3.77
Agric_12	A	2.40
Dossi_9	A	2.27
Dossi_12	A	1.33

Levels not connected by same letter are significantly different.

Discriminance between varieties based on textural attributes

PCA

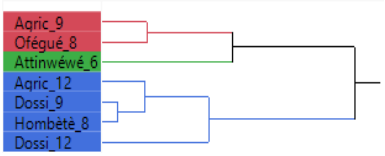
The PCA collating all the textural methods data show that most textural measures are grouped on the first axis. The correlations between the attributes of all the instrumental methods indeed showed high significant correlations. The varieties Agric_9 and Ofegue_8 were closely associated. Most of the other varieties were grouped in different components.

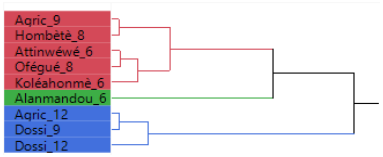


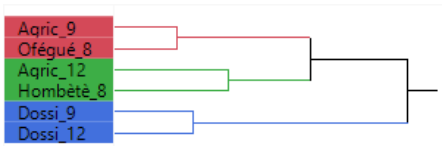
PCA of steam-cooked cassava with collated penetrometry, compression and Extrusion

Hierarchical classes

The varieties were classified into 3 hierarchical classes for each texture protocol in order to cluster the varieties into steam-cooked cassava quality (good, poor, intermediate) groups. The varieties Agric_9 and Ofegue were consistently clustered in same class (with high firmness and poor quality), while at the opposite Dossi_9 and Dossi_12 were classified together consistently regardless of the textural protocol (with low firmness and high quality).







Hierarchical - penetrometry Hierarchical – Compression Hierarchical – Extrusion

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