

Training on Instrumental Textural Characterization of Extensibility of Eba and Pounded Yam

Biophysical Characterization of Quality Traits, WP2

26/09/2022 - 30/09/2022, Ibadan, Nigeria

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<u>Ethics</u>: The activities, which led to the production of this document, 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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ABSTRACT

The stretchability of Eba and pounded yam is a key textural attribute preferred as an acceptable quality parameter by consumers of Eba and pounded yam, respectively. The two new SOPs for determination of uniaxial extensibility and bi-extensional viscosity developed for pounded yam was adapted to determine the extensibility of pounded yam and Eba, respectively, as measured by the texture analyser instrument. The instrumental extensibility parameters will represent the sensory stretchability of Eba and pounded yam.

The mission was conducted at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria between 26-30 September 2022, to train the IITA and the Faculty of Agricultural Sciences, University of Abomey Calavi (FSA-UAC), Benin teams to set up the texture analyser and measure biextensional viscosity of Eba using the lubricated squeezing flow (LSF) protocol; and to measure the extensibility of pounded yam using the uniaxial extensibility protocol.

Some modifications were introduced in the protocols, specific to the food product profile of Eba, by changing test speed to 12mm/min and biaxial strain to 1.0. Statistical evaluations were presented to inform of the accuracy, repeatability, discriminability and best instrument measurement parameters of the protocols.

Key Words: Eba, Pounded yam, Textural attributes, Extensibility, Discriminant analysis, Texture analyser





1 GENERAL OVERVIEW

1.1 Interest of this support mission in RTBfoods framework

- Training for the partners to understand the methodology to determine the extensibility of Eba and pounded yam by instrumental textural protocols. Instrumental extensibility represents sensory stretchability which is a key textural quality parameter preferred by consumers of Eba and pounded yam
- The training is to equip the partners in preparation for determining extensibility of Eba and pounded yam product profiles for the upcoming harvests of yams in 2022 and cassava in 2022 & 2023.

1.2 Specific objectives

- To train partners in the setting up of the texture analyser and determination of extensibility of Eba and pounded yam.
- To evaluate the accuracy, repeatability, and discriminability of extensibility textural protocols used to measure instrumental extensibility parameters.
- To select the best instrument measurement parameters of the protocols

1.3 Organizing committee

- Busie MAZIYA-DIXON, Food Scientist, International Institute of Tropical Agriculture, IITA
- Akissoé NOËL, Food Scientist, FSA- University of Abomey Calavi (FSA-UAC) BENIN
- Emmanuel ALAMU, Food Scientist, International Institute of Tropical Agriculture, IITA
- Michael ADESOKAN, Chemist, International Institute of Tropical Agriculture, IITA





1.4 Support team

NAME First name	Gender (F/M)	External OR Position / Responsibilities within RTBfoods (ex: WP leader, Product Champion)	Background – Expertise (ex: Biochemistry)	Institute / Company + COUNTRY	Email Contact	Consent to Picture use (YES/NO)
AYETIGBO Oluwatoyin	Μ	Focal Point, Texture	Food Science & Physical measurements	CIRAD, FRANCE	oluwatoyin.ayetigbo@cirad.fr	YES

1.5 Targeted audience(s) & staff supported / trained

#	NAME First name	Gender (F/M)	Position	Education - Background (ex: Biochemistry)	Institute + COUNTRY	WP	Email Contact	Consent to Picture use (YES/NO)
1	ADESOKAN Michael	М	Lab manager	Chemistry	IITA, NIGERIA	2	m.adesokan@cgiar.org	YES
2	FAWOLE Segun	М	Lab technologist	Food science	IITA, NIGERIA	2	Segunfawole11@gmail. com	YES
3	ADINSI Laurent	М	Researcher	Biochemistry	FSA-UAC, BENIN	2	adinsil2003@yahoo.fr	YES
4	HOTEGNI Francis	М	Research assistant	Food science	FSA-UAC, BENIN	2	francismedehot@gmail. com	YES

1.6 Experience level of staff supported / trained

Michael Adesokan is the manager of the Food Science and crop utilization lab of IITA. He manages the lab, and is well knowledgeable on the texture measurement procedures.

Segun Fawole is skilled in the use of the texture analyser, and is a primary handler of the texture analyser.

Laurent Adinsi is an assisting lecturer & researcher with the FSA-UAC, Benin, and is knowledgeable on the texture measurement procedures.

Francis Hotegni works as research assistant at FSA-UAC, Benin, and is the principal handler of the texture analyser at FSA-UAC.





2 SUPPORT IMPLEMENTATION

2.1 Support mission agenda

26 September	27 September	28 September	29 September	30 September
 Arrival and familiarisation with staff, lab protocol and materials Collection of gari samples from 7 contrasting varieties of cassava to make Eba Review of new SOP on determination of biextensional viscosity (BEV) by lubricated squeezing flow (LSF) Discussion with team and work plan breakdown Setting up, calibration, and test run of texture analyser Preparation of Eba following established SOP for Eba preparation and first measurements of BEV of 1 genotype WP5 302 at three test speeds (6, 12, 120 mm/min) and two replications Data collection 	 Discussion with team and work plan breakdown Calibration and setting up of texture analyser Sample preparation of Eba from gari following established SOP for Eba preparation Measurements of BEV of 4 genotypes (WP5 303, WP5 304, WP5 305, WP5 306) at three test speeds (6, 12, 120 mm/min) and two replications Data collection 	 Discussion with team and work plan breakdown Setting up, calibration, and test run of texture analyser Sample preparation of Eba Measurements of BEV of 2 genotypes (WP5 307, WP5 308) at three test speeds (6, 12, 120 mm/min) and two replications Data collection Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of BEV 	 Selection of the best test speed and biaxial strain of Eba Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of BEV 	 Setting up, calibration, and test run of texture analyser Review of new SOP on determination of uniaxial extensibility with trainees Sample preparation of pounded yam from 2 yam varieties purchased in market following established SOP for pounded yam preparation Measurements of uniaxial extensibility of pounded yam from 2 varieties Statistical evaluation of data with trainees to determine the uniaxial extensibility parameters, and to determine accuracy, repeatability and discriminant power of uniaxial extensibility protocol





2.2 Daily progress of the support mission

DAY 1

Who: Michael Adesokan, Segun Fawole, Laurent Adinsi, Francis Hotegni

Where: Texture lab

What:

- Arrival and familiarisation with staff, trainees, lab protocol and materials
- Collection of gari samples from 7 contrasting varieties of cassava to make Eba
- Review of new SOP on determination of bi-extensional viscosity (BEV) by lubricated squeezing flow (LSF)
- Discussion with team and work plan breakdown
- Setting up, calibration, and test run of texture analyser
- Preparation of Eba following established SOP for Eba preparation
- First set of measurements of BEV of 1 genotype WP5 302 at three test speeds (6, 12, 120 mm/min), and two replications
- Data download/collection

Specific Methods & Tools Used:

- Discussions
- Demonstrations and hands-on activities by trainees
- Reviewing of validated SOP on BEV by LSF to explain the merits and demerits of the protocol
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface

Challenges Faced:

• In order to adapt the LSF SOP to Eba food product profile, a minor modification of the SOP was made by using the standard sample preparation procedure for Eba.

Output(s) – Result(s):

- Each trainee engaged in hands-on measurements
- BEV measurements raw data for Eba made from genotype WP5 302
- The repeatability of the measurements of the BEV was indicated by the insignificant differences between cooking replicate means by one-way ANOVA.
- Detailed results are shown in the Appendix 1

DAY 2

- <u>Who:</u> Michael Adesokan, Segun Fawole, Laurent Adinsi, Francis Hotegni
- <u>Where:</u> Texture lab
- <u>What:</u> Discussion with team and work plan breakdown
 - Calibration and setting up of texture analyser
 - Sample preparation of Eba from gari following established SOP for Eba preparation
 - Measurements of BEV of 4 genotypes (WP5 303, WP5 304, WP5 305, WP5 306) at three test speeds (6, 12, 120 mm/min) and two replications





- Data download/collection

Specific Methods & Tools Used:

- Discussions
- Validated SOP
- Demonstrations and hands-on activities by trainees
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface

Challenges Faced:

No challenge was encountered

Output(s) – Result(s):

- BEV measurements raw data for Eba made from genotypes WP5 303, WP5 304, WP5 305, and WP5 306
- The repeatability of the measurements of the BEV was indicated by the insignificant differences between cooking replicate means by one-way ANOVA.
- Detailed results are shown in the Appendix 1

DAY 3

- <u>Who:</u> Michael Adesokan, Segun Fawole, Laurent Adinsi, Francis Hotegni
- Where: Texture lab
- What: Discussion with team and work plan breakdown
 - Setting up, calibration, and test run of texture analyser
 - Sample preparation of Eba
 - Measurements of BEV of 2 genotypes (WP5 307, WP5 308) at three test speeds (6, 12, 120 mm/min) and two replications
 - Data download/collection
 - Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of BEV data.
 - A statistical data analysis software (JMP 16) was shared with trainees, and a brief tutorial on basic statistics was conducted.

Specific Methods & Tools Used:

- Discussions
- Validated SOP
- Demonstrations and hands-on activities by trainees
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface

Challenges Faced:

Since trainees were new to the statistical analysis software, they needed a short tutorial to show



how it is used, thereby slowing down the activities for the day

Output(s) – Result(s):

- BEV measurements raw data for Eba made from genotypes WP5 307 and WP5 308
- The repeatability of the measurements of the BEV was indicated by the insignificant differences between cooking replicate means by one-way ANOVA.
- Detailed results are shown in the Appendix 1

DAY 4

Who: Michael Adesokan, Segun Fawole, Laurent Adinsi, Francis Hotegni

<u>Where:</u> Texture lab

- What:
- Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of BEV
 - Selection of the best test speed and biaxial strain of Eba

Specific Methods & Tools Used:

- Discussions
- Validated SOP
- Demonstrations and hands-on activities by trainees
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface
- Statistical software (JMP 16)

Challenges Faced:

Statistical training and data analyses took a considerable period of time

Output(s) – Result(s):

- Statistical analyses showed that the repeatability of the measurements of the BEV was indicated by the insignificant differences between cooking replicate means by one-way ANOVA.
- The best strain rate and test speed for evaluation of BEV was analysed
- Detailed results are shown in the Appendix 1

DAY 5

<u>Who:</u> Michael Adesokan, Segun Fawole, Laurent Adinsi, Francis Hotegni

Where: Texture lab

- <u>What:</u> Setting up, calibration, and test run of texture analyser
 - Review of new SOP on determination of uniaxial extensibility of pounded yam
 - Sample preparation of pounded yam from 2 yam varieties purchased in local





market following established SOP for pounded yam preparation

- Measurements of uniaxial extensibility of pounded yam from 2 varieties
- Statistical evaluation of data with trainees to determine the uniaxial extensibility parameters, and to determine accuracy, repeatability and discriminant of uniaxial extensibility

Specific Methods & Tools Used:

- Discussions
- Validated SOP
- Demonstrations and hands-on activities by trainees
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface
- Statistical software (JMP 16)

Challenges Faced:

- Market yam varieties were used due to unavailability of well-known yam clones in IITA for evaluation. Yam harvest of well-documented clones was planned for later in the year (December 2022).
- Partner does not have some of the instrument's accessories to conduct uniaxial extensibility tests. Accessories used were brought in from CIRAD.

Output(s) – Result(s):

- Data on uniaxial extensibility parameters of pounded yam was collected
- Statistical analyses showed that the repeatability of the measurements of the uniaxial extensibility parameters was indicated by the insignificant differences between cooking replicate means by one-way ANOVA.
- Detailed results are shown in the Appendix 2

2.3 List of material / documents distributed

- Standard Operating Protocol for Determination of Bi-extensional viscosity of Pounded yam by Lubricated Squeezing Flow (LSF) Method. This SOP was adapted to Eba food product profile.
- Validated SOP for the Instrumental Determination of Extensibility of Pounded yam.

2.4 General approach - methods applied

- Open discussion and demonstrations with trainees.
- Hands-on activities by each trainee

3 MISSION OUTPUTS & FEEDBACKS

3.1 Specific outputs of the support mission

 Trainees were able to conduct hands-on demonstration of the SOPs and measurements of the textural parameters, as well as calculations related to the SOPs.





- Textural data on instrumental extensibility of Eba and pounded yam was generated and statistically analysed, and found to be accurate, repeatable and discriminant between genotypes. The data generated may be useful for correlation with sensory data on stretchability of Eba and pounded yam, and the protocols may be useful for screening large populations of cassava and yam genotypes, respectively, for consumer-preferred quality.
- The best operating conditions for measurements were obtained for bi-extensional viscosity of Eba.
- A quick understanding of how to use the JMP 16 statistical software for basic statistical evaluations by trainees

3.2 Challenges faced – paths for improvement

- Market yam varieties were used for the demonstration of uniaxial extensibility protocol for pounded yam due to unavailability of well-known yam clones in IITA. Yam harvest of known clones was planned for later in the year (December 2022).
- Partner does not have some of the instrument's accessories to conduct uniaxial extensibility tests. Accessories used were brought in from CIRAD.
- Among the two protocols demonstrated on the mission, the uniaxial extensibility protocol is limited in use for Eba, which is sticky or adhesive in texture and cannot be rolled into a homogeneous smooth sheet, contrary to pounded yam texture. This disadvantage is not applicable to the LSF protocol which can be used for both food products.

3.3 Feedbacks from staff trained - general remarks from support team

- Request for further statistical training in cleaning textural data and statistical analyses
- Trainees satisfactorily understand the protocols as practicable hands-on sessions were held for each trainee.

3.4 Next steps

- Sensory analyses of the stretchability of Eba and pounded yam should be conducted for the same genotypes in order to correlate them with the instrumental textural parameters (uniaxial extensibility and bi-extensional viscosity) measured during the training.
- The tests need to be conducted for Eba made from a wider range of populations of cassava genotypes.
- Planning to purchase instrument accessories to conduct uniaxial extensibility tests.
- Yam harvests is expected in December, therefore, the protocols can be used in next evaluations.

List of documents attached to the report

1.	Review and statistical analyses of data for uniaxial extensibility and bi-extensional viscosity (Appendices 1 & 2)	Yes
2.	Pictures	Yes





4 APPENDICES

4.1 Annex 1: Review of data on instrumental biaxial extensional viscosity (BEV) of Eba on mission2 in IITA, Ibadan, Nigeria

Genotype

	Genotype	Quality of Eba
1	WP5 302	?
2	WP5 303	?
3	WP5 304	?
4	WP5 305	?
5	WP5 306	?
6	WP5 307	?
7	WP5 308	?

Procedure

Texture measurements of biaxial extensional viscosity of Eba made from seven genotypes of cassava using the texture analyser was carried out by preparing Eba based on the validated RTBfoods SOP for Eba textural analysis in IITA, Nigeria (Maziya-Dixon, 2022), and adapting the biaxial extensional viscosity protocol for pounded yam (Santiago Arufe Vilas et al. 2022) to Eba. Two preparations or cooking replicates per genotype were considered. About 3 measurements per cooking replicate was collected due to time restriction. Measurements were made at three test speeds (6, 12, 120 mm/min) and six biaxial strains (0.1, 0.25, 0.4, 0.5, 0.75, 1.0). The measurements were made at temperature of 30 °C.



Gari samples used to prepare Eba





Statistical accuracy of bi-extensional viscosity of Eba

	Genotype	Ν	Mean	Std Dev	Std Err	CV (%)	CV, mean (%)
Bi-extensional viscosity, ηb [Pa⋅s]	WP5 302	6	159682	8015	3272	5	2.0
	WP5 303	6	161212	12003	4900	7	3.0
	WP5 304	6	149804	19789	8079	13	5.4
	WP5 305	6	136578	11063	4516	8	3.3
	WP5 306	6	137754	10459	4270	8	3.1
	WP5 307	6	145930	1456	594	1	0.4
	WP5 308	6	140738	15534	6342	11	4.5

Statistics to select best biaxial strain and test speed in measuring bi-extensional viscosity (BEV)

For genotype WP5 302 as example

For test speed

For 6mm/min

Oneway Anova of Extensional viscosity (Pa.s) By Biaxial strain (eb)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Biaxial strain (eb)	5	8.124e+11	1.625e+11	388.3554	1.912e-7

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.10	2	1070253	41570.808	29395	696754.11	1443751.9
0.25	2	794250	4777.9205	3378.5	751322.59	837178.41
0.40	2	645790	10231.835	7235	553860.61	737719.39
0.50	2	561333	13632.312	9639.5	438852.04	683814.96
0.75	2	396024	18748.936	13257.5	227571.99	564477.01
1.00	2	275144	10829.34	7657.5	177846.74	372442.26

Oneway Anova of Extensional viscosity (Pa.s) By Replicate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	1659712323	1.6597e+9	0.0204	8.892e-1

For 12mm/min

Oneway Anova of Extensional viscosity (Pa.s) By Biaxial strain (eb)

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Biaxial strain (eb)	5	1.7846e+11	3.569e+10	32.8710	2.754e-4





Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.10	2	536731.5	62340.655	44081.5	-23377.06	1096840.1
0.25	2	405299.5	37007.848	26168.5	72797.181	737801.82
0.40	2	339398	24659.642	17437	117839.91	560956.09
0.50	2	304162.5	21090.874	14913.5	114668.52	493656.48
0.75	2	224756.5	12994.501	9188.5	108005.54	341507.46
1.00	2	159682.5	6108.6955	4319.5	104798.05	214566.95

Oneway Anova of Extensional viscosity (Pa.s) By Replicate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	4493727924	4.4937e+9	0.2490	6.286e-1

For 120mm/min

Oneway Anova of Extensional viscosity (Pa.s) By Biaxial strain (eb)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Biaxial strain (eb)	5	3178504010	635700802	30.9209	3.279e-4

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.10	2	74433.5	9345.8303	6608.5	-9535.454	158402.45
0.25	2	56639.5	4975.9104	3518.5	11932.719	101346.28
0.40	2	48007.5	2689.1271	1901.5	23846.652	72168.348
0.50	2	43689	1790.3944	1266	27602.945	59775.055
0.75	2	33083	393.15137	278	29550.675	36615.325
1.00	2	23783.5	811.05148	573.5	16496.492	31070.508

Oneway Anova of Extensional viscosity (Pa.s) By Replicate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	56324667	56324667	0.1735	6.858e-1





For biaxial strain

Biaxial strain = 0.1

Oneway Anova of Extensional viscosity [Pa·s] By Test speed (mm/min)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Test speed (mm/min)	2	2.98e+12	1.49e+12	262.0736	2.15e-12

Means and Std Deviations

Biaxial strain	Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.1	6	6	1070252.8	120612.28	49239.758	943677.93	1196827.6
0.1	12	6	536731.28	49447.536	20186.872	484839.28	588623.29
0.1	120	6	74433.015	8009.1095	3269.7053	66027.97	82838.06

Oneway Anova of Extensional viscosity [Pa-s] By Replicate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	1.2827e+10	1.283e+10	0.0672	7.987e-1

Biaxial strain = 0.25

Oneway Anova of Extensional viscosity [Pa·s] By Test speed (mm/min)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Test speed (mm/min)	2	1.6338e+12	8.169e+11	222.2050	7.15e-12

Means and Std Deviations

Biaxial strain	Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.25	6	6	794250.14	100451.07	41008.976	688833.22	899667.07
0.25	12	6	405299.61	30216.541	12335.851	373589.29	437009.92
0.25	120	6	56639.318	5076.2123	2072.355	51312.16	61966.476

Oneway Anova of Extensional viscosity [Pa-s] By Replicate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	2186697210	2.1867e+9	0.0207	8.873e-1





Biaxial strain = 0.4

Oneway Anova of Extensional viscosity [Pa·s] By Test speed (mm/min)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Test speed (mm/min)	2	1.0723e+12	5.361e+11	244.4122	3.58e-12

Means and Std Deviations

Biaxial strain	Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.4	6	6	645789.9	78265.253	31951.656	563655.56	727924.25
0.4	12	6	339397.99	20915.94	8538.8969	317448.06	361347.92
0.4	120	6	48007.425	4206.89	1717.4557	43592.565	52422.286

Oneway Anova of Extensional viscosity [Pa-s] By Replicate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	
Replicate	1	1412341887	1.4123e+9	0.0205	8.88e-1	

Biaxial strain = 0.5

Oneway Anova of Extensional viscosity [Pa·s] By Test speed (mm/min)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Test speed (mm/min)	2	8.0388e+11	4.019e+11	271.0314	1.68e-12

Means and Std Deviations

Biaxial strain	Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.5	6	6	561333.1	64216.786	26216.393	493941.71	628724.48
0.5	12	6	304162.63	17622.132	7194.2054	285669.34	322655.93
0.5	120	6	43689.435	3827.1412	1562.4238	39673.097	47705.774

Oneway Anova of Extensional viscosity [Pa-s] By Replicate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	
Replicate	1	1333246472	1.3332e+9	0.0259	8.742e-1	





Biaxial strain = 0.75

Oneway Anova of Extensional viscosity [Pa-s] By Test speed (mm/min)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Test speed (mm/min)	2	3.956e+11	1.978e+11	290.7099	1.01e-12

Means and Std Deviations

Biaxial strain	Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.75	6	6	396024.6	43284.149	17670.68	350600.67	441448.53
0.75	12	6	224756.45	12653.914	5165.9387	211476.98	238035.91
0.75	120	6	33082.648	2748.6926	1122.149	30198.072	35967.224

Oneway Anova of Extensional viscosity [Pa-s] By Replicate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	1032751958	1.0328e+9	0.0408	8.424e-1

Biaxial strain = 1.0

Oneway Anova of Extensional viscosity [Pa·s] By Test speed (mm/min)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Test speed (mm/min)	2	1.8996e+11	9.498e+10	389.4912	1.18e-13

Means and Std Deviations

Biaxial strain	Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
1.0	6	6	275144.28	25728.781	10503.731	248143.58	302144.98
1.0	12	6	159682.47	8015.2771	3272.2232	151270.95	168093.99
1.0	120	6	23783.61	2317.5961	946.15466	21351.442	26215.778

Oneway Anova of Extensional viscosity [Pa-s] By Replicate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	
Replicate	1	260107439	260107439	0.0215	8.852e-1	





Biaxial strain	Test speed (mm/min)	N	Mean BEV	Std Dev	Std Err Mean	Lower 95%	Upper 95%	CV (%)	CV, mean (%)
0.1	6	6	1070253	120612	49240	943678	1196828	11.3	4.6
0.1	12	6	536731	49448	20187	484839	588623	9.2	3.8
0.1	120	6	74433	8009	3270	66028	82838	10.8	4.4
0.25	6	6	794250	100451	41009	688833	899667	12.6	5.2
0.25	12	6	405300	30217	12336	373589	437010	7.5	3.0
0.25	120	6	56639	5076	2072	51312	61966	9.0	3.7
0.4	6	6	645790	78265	31952	563656	727924	12.1	4.9
0.4	12	6	339398	20916	8539	317448	361348	6.2	2.5
0.4	120	6	48007	4207	1717	43593	52422	8.8	3.6
0.5	6	6	561333	64217	26216	493942	628724	11.4	4.7
0.5	12	6	304163	17622	7194	285669	322656	5.8	2.4
0.5	120	6	43689	3827	1562	39673	47706	8.8	3.6
0.75	6	6	396025	43284	17671	350601	441449	10.9	4.5
0.75	12	6	224756	12654	5166	211477	238036	5.6	2.3
0.75	120	6	33083	2749	1122	30198	35967	8.3	3.4
1.0	6	6	275144	25729	10504	248144	302145	9.4	3.8
1.0	12	6	159682	8015	3272	151271	168094	5.0	2.0
1.0	120	6	23784	2318	946	21351	26216	9.7	4.0

Compilation of mean bi-extensional viscosity at different biaxial strains and test speeds

To select the best biaxial strain (i.e. the strain with the most repeatable and accurate bi-extensional viscosity), we selected one genotype WP5302 and then, we determined the ANOVA of the bi-extensional viscosity at each test speed (6, 12, 120 mm/min) and we selected the biaxial strain with the least standard error of mean and CV (%), which is at biaxial strain of 1.0. To select the best test speed, the test speed with the least standard error of mean and CV (%) between the biaxial strains was selected, which is at 12 mm/min. Therefore, the selected biaxial strain and test speed were used for the other genotypes. The ANOVA showed no significant differences between the BEV of the replicates at the test speeds and biaxial strains.

ANOVA and repeatability of bi-extensional viscosity (test speed = 12mm/min, biaxial strain = 1.0) of Eba

Oneway Anova of Bi-extensional viscosity, ηb [Pa·s] By Genotype

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
<mark>Genotype</mark>	<mark>6</mark>	<mark>3624680040</mark>	<mark>604113340</mark>	<mark>3.9334</mark>	<mark>4.152e-3</mark>
Error	<mark>35</mark>	<mark>5375461122</mark>	<mark>153584603</mark>		
C. Total	<mark>41</mark>	9000141162			



Effect Tests



Source	<mark>Nparm</mark>	DF	Sum of Squares	F Ratio	Prob > F
Genotype	6	6	3624680040	<mark>4.1769</mark>	0.0040*
Replicate	1	1	49919124.6	0.3451	<mark>0.5616</mark>
Genotype*Replicate	6	6	1275802487	<mark>1.4702</mark>	0.2244

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
WP5302	6	159682.47	8015.2771	3272.2232	151270.95	168093.99
WP5303	6	161212.18	12002.583	4900.0341	148616.24	173808.11
WP5304	6	149803.7	19788.808	8078.7472	129036.62	170570.78
WP5305	6	136578.07	11062.564	4516.2727	124968.62	148187.52
WP5306	6	137754	10458.978	4269.86	126777.97	148730.02
WP5307	6	145930.19	1456.0262	594.42022	144402.18	147458.19
WP5308	6	140738.03	15533.771	6341.6357	124436.34	157039.72

Means Comparisons

Connecting Letters Report

Level				Mean
WP5303	А			161212
WP5302	А	В		159682
WP5304	А	В	С	149803
WP5307	А	В	С	145930
WP5308	А	В	С	140738
WP5306		В	С	137754
WP5305			С	136578

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value				
WP5303	WP5305	24634.10	7155.059	2267.8	47000.40	0.0230*		1		1
WP5303	WP5306	23458.18	7155.059	1091.9	45824.47	0.0347*				
WP5302	WP5305	23104.40	7155.059	738.1	45470.69	0.0391*				
WP5302	WP5306	21928.47	7155.059	-437.8	44294.77	0.0577				
WP5303	WP5308	20474.15	7155.059	-1892.1	42840.44	0.0911		<u>/</u>		
WP5302	WP5308	18944.44	7155.059	-3421.9	41310.73	0.1427				/
WP5303	WP5307	15281.99	7155.059	-7084.3	37648.28	0.3552	/			/





Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
WP5302	WP5307	13752.28	7155.059	-8614.0	36118.57	0.4800	
WP5304	WP5305	13225.63	7155.059	-9140.7	35591.92	0.5261	
WP5304	WP5306	12049.71	7155.059	-10316.6	34416.00	0.6306	
WP5303	WP5304	11408.48	7155.059	-10957.8	33774.77	0.6865	
WP5302	WP5304	9878.77	7155.059	-12487.5	32245.06	0.8081	
WP5307	WP5305	9352.12	7155.059	-13014.2	31718.41	0.8441	
WP5304	WP5308	9065.67	7155.059	-13300.6	31431.96	0.8620	
WP5307	WP5306	8176.19	7155.059	-14190.1	30542.48	0.9101	
WP5307	WP5308	5192.16	7155.059	-17174.1	27558.45	0.9900	
WP5308	WP5305	4159.96	7155.059	-18206.3	26526.25	0.9970	
WP5304	WP5307	3873.51	7155.059	-18492.8	26239.81	0.9980	
WP5308	WP5306	2984.03	7155.059	-19382.3	25350.32	0.9995	
WP5303	WP5302	1529.71	7155.059	-20836.6	23896.00	1.0000	/ /
WP5306	WP5305	1175.92	7155.059	-21190.4	23542.22	1.0000	

Oneway Anova of Bi-extensional viscosity, ηb [Pa·s] By Replicate

Analysis of Variance

Source	<mark>DF</mark>	Sum of Squares	Mean Square	F Ratio	<mark>Prob > F</mark>
Replicate	<mark>1</mark>	<mark>49919124.6</mark>	<mark>49919125</mark>	<mark>0.2231</mark>	<mark>6.393e-1</mark>
Error	<mark>40</mark>	<mark>8950222037</mark>	<mark>223755551</mark>		
C. Total	<mark>41</mark>	<mark>9000141162</mark>			

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%	
R1	21	148475.73	12555.969	2739.937	142760.32	154191.13	
R2	21	146295.31	17025.239	3715.2117	138545.52	154045.11	

Means Comparisons

Connecting Letters Report

Level		Mean
R1	А	148475.73
R2	А	146295.31

Levels not connected by same letter are significantly different.





Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
R1	R2	2180.413	4616.281	-7149.55	11510.38	0.6393	3

The repeatability of the measurements of the bi-extensional viscosity (at test speed of 12mm/min and biaxial strain of 1.0) was indicated by the insignificant differences between cooking replicate means by one-way and two-way ANOVA. On the other hand, the mean BEV of the genotypes were significantly different between the genotypes. The genotype has the most significant effect on BEV of Eba. The genotype*replicate effect was not significant.

Discriminance between genotypes based on BEV of Eba

Discriminant

Discriminant analysis showed good discrimination between the genotypes. The most discriminately distant genotypes are WP5305 and WP5303. The genotypes WP5305, WP5306 & WP5308, and WP5307 & WP5304, and WP5302 & WP5303 are not very discriminant from another.



Hierarchical classes

The genotypes were clustered in 3 classes of hierarchies representing good, poor and intermediate textural quality for making Eba. The genotypes WP5302 and WP5303 are closely associate in texture, as WP5304 and WP5307 are also closely associated, while WP5305, WP5306 and WP5308 are also closely associated in texture. The WP5302 is the farthest genotype from WP5308.



Summary

The preferred test speed and biaxial strain for the measurement of bi-extensional viscosity are 12mm/min and 1.0, respectively. The LSF protocol was repeatable between the replicates and discriminant between the genotypes. The discriminant and hierarchical analyses show the relationship between the genotypes in terms of the bi-extensional viscosity.





References

Santiago Arufe Vilas, Oluwatoyin Ayetigbo, Romain Domingo and Christian Mestres (2022). Standard Operating Protocol for Determination of Bi-extensional viscosity of Pounded yam by Lubricated Squeezing Flow (LSF) Method. Montpellier, France: RTBfoods Laboratory Standard Operating Procedure, 17 p. https://doi.org/10.18167/agritrop/00686

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4.2 Annex 2: Review of data on instrumental uniaxial extensibility of pounded yam on mission2 in IITA (+FSA-UAC, Benin partners), Ibadan, Nigeria

Genotype

	Genotype	Quality of pounded yam			
1	Variety 1	White (Dioscorea) yam			
2	Variety 2	Yellow yam			

Procedure

Texture measurements of uniaxial extensibility of pounded yam made from 2 varieties of yam obtained from the local market, using the texture analyser was carried out by preparing pounded yam based on the validated RTBfoods SOP for pounded yam textural analysis in BOWEN, Nigeria (Otegbayo et al. 2022), and adapting the uniaxial extensibility protocol for pounded yam (Ayetigbo et al. 2022). Two preparations or cooking replicates per genotype were considered. About 3-4 measurements per cooking replicate was collected. Measurements were made at 30 oC.



Yam Variety 2

Sample preparation room

Temperature measurement

Statistical accuracy of uniaxial extensibility textural parameters

	Genotype	Ν	Mean	Std Dev	Std Err	CV (%)	CV, mean (%)
Hardness (N)	variety 1	7	0.486	0.12	0.04	24	9
	variety 2	8	0.337	0.07	0.03	22	8
Extensibility (mm)	variety 1	7	13.5	1.69	0.64	12	5
	variety 2	8	10.5	2.47	0.87	24	8
Area between to and Fmax	variety 1	7	3.6	1.02	0.39	28	11
	variety 2	8	2.1	0.82	0.29	40	14





ANOVA and repeatability of uniaxial extensibility textural parameters of pounded yam

Oneway Anova of Hardness (N) By Genotype

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	<mark>Prob > F</mark>
<mark>Genotype</mark>	1	<mark>0.08244724</mark>	<mark>0.082447</mark>	<mark>8.7908</mark>	1.095e-2
Error	<mark>13</mark>	<mark>0.12192436</mark>	<mark>0.009379</mark>		
C. Total	<mark>14</mark>	<mark>0.20437160</mark>			

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Genotype	1	1	0.09110833	11.9776	5.325e-3
Replicate	1	1	0.00110933	<mark>0.1458</mark>	7.098e-1
Genotype*Replicate	1	1	0.03791426	<mark>4.9844</mark>	4.731e-2

Means and Std Deviations

Level	Number Mean		Std Dev	Std Err Mean	Lower 95%	Upper 95%	
variety 1	7	0.4858571	0.1182236	0.0446843	0.3765186	0.5951957	
variety 2	8	0.33725	0.0737404	0.0260712	0.2756015	0.3988985	

Means Comparisons

Connecting Letters Report

Level			Mean
variety 1	А		0.48585714
variety 2		В	0.33725000

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
variety 1	variety 2	0.1486071	0.0501216	0.0403235	0.2568908	1.1e-2	

Oneway Anova of Hardness (N) By Replicate

<mark>Source</mark>	<mark>DF</mark>	Sum of Squares	<mark>Mean Square</mark>	F Ratio	Prob > F	
Replicate	1	<mark>0.00000472</mark>	<mark>4.725e-6</mark>	<mark>0.0003</mark>	<mark>9.864e-1</mark>	
<mark>Error</mark>	<mark>13</mark>	<mark>0.20436688</mark>	<mark>0.015721</mark>			
C. Total	<mark>14</mark>	<mark>0.20437160</mark>				





Means and Std Deviations

Level	Number Mean		Std Dev	Std Err Mean	Lower 95%	Upper 95%	
R1	8	0.407125	0.090042	0.0318346	0.331848	0.482402	
R2	7	0.406	0.1568513	0.0592842	0.2609367	0.5510633	

Means Comparisons

Connecting Letters Report

Level		Mean
R1	А	0.40712500
R2	А	0.40600000

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value					
R1	R2	0.0011250	0.0648911	-0.139067	0.1413169	9.9e-1			Т		

Oneway Anova of Extensibility (mm) By Genotype

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
<mark>Genotype</mark>	<mark>1</mark>	<mark>35.485330</mark>	<mark>35.4853</mark>	<mark>7.7060</mark>	1.574e-2
Error	<mark>13</mark>	<mark>59.863812</mark>	<mark>4.6049</mark>		
C. Total	<mark>14</mark>	<mark>95.349142</mark>			

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Genotype	1	1	36.778567	7.7621	1.771e-2
Replicate	1	1	0.454680	0.0960	7.625e-1
Genotype*Replicate	1	1	6.968928	<mark>1.4708</mark>	2.506e-1

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%	
variety 1	7	13.543143	1.685711	0.6371389	11.98412	15.102165	
variety 2	8	10.460125	2.4731149	0.8743782	8.3925492	12.527701	





Means Comparisons

Connecting Letters Report

Level			Mean
variety 1	А		13.543143
variety 2		В	10.460125

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value		
variety 1	variety 2	3.083018	1.110611	0.6836331	5.482403	1.6e-2		

Oneway Anova of Extensibility (mm) By Replicate

Analysis of Variance

Source	<mark>DF</mark>	Sum of Squares	Mean Square	F Ratio	<mark>Prob > F</mark>
Replicate	<mark>1</mark>	<mark>1.698391</mark>	<mark>1.69839</mark>	<mark>0.2358</mark>	<mark>6.354e-1</mark>
Error	<mark>13</mark>	<mark>93.650751</mark>	<mark>7.20390</mark>		
C. Total	<mark>14</mark>	<mark>95.349142</mark>			

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	8	12.213625	2.4076591	0.8512361	10.200772	14.226478
R2	7	11.539143	2.9741383	1.1241186	8.7885237	14.289762

Means Comparisons

Connecting Letters Report

Level		Mean
R1	А	12.213625
R2	А	11.539143

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value					
R1	R2	0.6744821	1.389107	-2.32657	3.675533	6.4e-1					:





Oneway Anova of Area between t_{o} and $F_{\text{max}}\,\text{By}$ Genotype

Analysis of Variance

<mark>Source</mark>	DF	Sum of Squares	<mark>Mean Square</mark>	<mark>F Ratio</mark>	<mark>Prob > F</mark>
<mark>Genotype</mark>	<mark>1</mark>	<mark>9.136889</mark>	<mark>9.13689</mark>	<mark>10.7594</mark>	<mark>5.971e-3</mark>
Error	<mark>13</mark>	<mark>11.039618</mark>	<mark>0.84920</mark>		
C. Total	<mark>14</mark>	20.176507			

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Genotype	1	1	9.8321724	12.8438	4.29e-3
Replicate	1	1	0.0388554	0.0508	8.259e-1
Genotype*Replicate	1	1	<mark>2.6135696</mark>	<mark>3.4141</mark>	9.169e-2

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
variety 1	7	3.6162857	1.0249518	0.3873954	2.6683634	4.564208
variety 2	8	2.051875	0.8225796	0.2908258	1.3641812	2.7395688

Means Comparisons

Connecting Letters Report

Level			Mean
variety 1	А		3.6162857
variety 2		В	2.0518750

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value		
variety 1	variety 2	1.564411	0.4769326	0.5340371	2.594784	6e-3		

Oneway Anova of Area between to and Fmax By Replicate

Source	<mark>DF</mark>	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	<mark>1</mark>	<mark>0.020483</mark>	<mark>0.02048</mark>	<mark>0.0132</mark>	<mark>9.102e-1</mark>
<mark>Error</mark>	<mark>13</mark>	<mark>20.156024</mark>	<mark>1.55046</mark>		
C. Total	<mark>14</mark>	<mark>20.176507</mark>			





Means and Std Deviations

Level	Number	Mean	lean Std Dev Std Err Mean		Lower 95%	Upper 95%		
R1	8	2.8165	1.05675	0.3736175	1.9330349	3.6999651		
R2	7	2.7424286	1.434049	0.5420196	1.4161544	4.0687027		

Means Comparisons

Connecting Letters Report

Level		Mean
R1	А	2.8165000
R2	А	2.7424286

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value					
R1	R2	0.0740714	0.6444398	-1.31819	1.466331	9.1e-1	-				

The repeatability of the measurements of the uniaxial extensibility parameters was indicated by the insignificant differences between cooking replicate means by one-way and two-way ANOVA. On the other hand, the genotypes were significantly different between one another in all the uniaxial extensibility parameters, especially in the order area between to and Fmax> extensibility> hardness. Therefore, the most discriminant parameter was area between to and Fmax.

Discriminance between genotypes based on uniaxial extensibility parameters of pounded yam

PCA

A PCA showed the first two components accounted for 99.7% of variation in uniaxial extensibility parameters. The two varieties are in different components of the PCA, and are significantly different in uniaxial extensibility textural parameters. Variety 1 is closely associated with the uniaxial extensibility textural parameters, and may be considered the preferred variety between the two varieties. The extensibility textural parameters are all closely associated within the component spaces.







PCA- uniaxial extensibility for cooked pounded yam.

Discriminant

Discriminant analysis showed good discriminant between the genotypes. The most discriminant textural parameter was the area between to and Fmax, and the least discriminant is the hardness.



Hierarchical classes

There are not sufficient varieties analysed for hierarchical classification.





Correlations between instrumental uniaxial extensibility textural parameters Multivariate

Correlations

	Hardness (N)	Extensibility (mm)	Area between t_o and F_{max}				
	Coefficient						
Hardness (N)	1.0000						
Extensibility (mm)	0.7921	1.0000					
Area between t_o and F_{max}	0.9527	0.9244	1.0000				
		Probabi	ility				
Hardness (N)	0e+0						
Extensibility (mm)	4.3e-4	0e+0					
Area between to and Fmax	4.3e-8	8.4e-7	0e+0				

Significant correlation was found between all the uniaxial extensibility parameters, although no sensory textural data is available yet for correlation.

Summary

The uniaxial extensibility protocol was repeatable between the replicates and discriminant between the two varieties. The most discriminant uniaxial textural parameter was the area between to and Fmax. The PCA and discriminant analyses show the relationship between the genotypes. A recommendation for the next yam harvest season is made for more genotypes to be analysed to arrive at more conclusive outcomes.

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