Training Report

Training on Instrumental Textural Characterization of Extensibility of Pounded Yam

Biophysical Characterization of Quality Traits, WP2

4/10/2022 – 7/10/2022, Iwo, Nigeria

Oluwatoyin AYETIGBO, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France

Christian MESTRES, CIRAD, Montpellier, France (Validator)

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Ethics: 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 panellists and from consumers participating in activities.

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This document has been reviewed by:	
Christian MESTRES (CIRAD) Santiago ARUFE VILAS (CIRAD)	09/01/2023 10/01/2023
Final validation by:	
Christian MESTRES (CIRAD)	10/01/2023

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ABSTRACT

The stretchability of pounded yam is considered a key textural attribute that is preferred by consumers of pounded yam who regard the attribute as important for accepting the pounded yam as high quality food product. In order to describe the sensory perception of stretchability of pounded yam quantitatively, it is necessary to develop instrumental protocols that can measure the sensory texture reliably. Instrumental extensibility can represent the sensory stretchability of pounded yam. The new SOPs for uniaxial extensibility and bi-extensional viscosity developed for pounded yam at CIRAD was used to determine the extensibility of pounded yam using the texture analyser instrument.

The mission was conducted between 4-7 October 2022 to train the BOWEN University, Iwo team to set up the texture analyser and measure bi-extensional viscosity (BEV) of pounded yam using the lubricated squeezing flow (LSF) protocol; and to measure the extensibility of pounded yam using the uniaxial extensibility protocol.

After demonstration of the protocols using available genotypes (Kamilu & Ewura), statistical evaluations were presented to inform of the accuracy, repeatability, and discriminability of the protocols. A few challenges were however encountered in the use of the Perten Instruments texture analyser for the measurements. These challenges were not encountered by the use of a Stable microsystems texture analyser at International Institute of Tropical Agriculture (IITA), where the training was eventually concluded. A new version of the software may be a solution. Also accessories adapted to this PERTEN analyser is required.

Key Words: Pounded yam, Bi-extensional viscosity, Uniaxial extensibility, ANOVA, Texture,Stretchability

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

1.2 Specific objectives

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

1.3 Organizing committee

- Bolanle OTEGBAYO, Professor, Food Science &Technology, Bowen University

1.4 Support team

NAME First name	Gender (F/M)	External OR Position / Responsibilities within RTBfoods	Background –Expertise	Institute / Company + COUNTRY	Email Contact	Consent to Picture use (YES/NO)
AYETIGBO Oluwatoyin	M	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	Institute + COUNTRY	WP	Email Contact	Consent to Picture use (YES/NO)
1	OTEGBAYO Bolanle	F	Professor, Head	Food Science & Technology	BOWEN, NIGERIA	2	bolanle.otegbayo@bowen.edu.ng	YES
2	ORONIRAN Oluyinka	F	Lecturer, Nutritionist	Food, Nutrition & dietetics	BOWEN, NIGERIA	2	oluyinkaoroniran@gmail.com	YES
3	TANIMOLA Abiola	F	Lecturer, Food Scientist	Food Science & Technology	BOWEN, NIGERIA	2	oladeleabiola12@gmail.com	YES
5	ALAMU Ayomide	F	Research assistant	Food Science & Technology	BOWEN, NIGERIA	2	ayomidedorcas811@gmail.com	YES

1.6 Experience level of staff supported / trained

Bolanle Otegbayo is the lead Food Scientist at the Department of Food Science. She manages the department and laboratory, and is an expert on the texture measurement procedures.

Tanimola Abiola and Oroniran Oluyinka are staffs of the department, and are skilled in the use of the texture analyser

Alamu Ayomide is the primary technologist focussed on handling the texture analyser.

2 SUPPORT IMPLEMENTATION

2.1 Support mission agenda

4 October	5 October	6 October	7 October
• Arrival and familiarisation with staff, lab protocol and materials • Collection of 2 contrasting varieties of yam (kamilu, ewura) • Review of new SOP on determination of bi-extensional viscosity (BEV) by lubricated squeezing flow (LSF) with the trainees • Discussion with team and work plan breakdown	• Setting up, calibration, and test run of texture analyser (PERTEN Instrument) • Preparation of trial samples and measurement of BEV of 1 genotype at test speed of 120 mm/min, and two replications • Data collection could be viewed on screen but was not possible to download due to software not being able to download raw data for calculation of BEV	• Setting up, calibration, and test run of texture analyser (STABLE Microsystems) at IITA • Preparation of pounded yam from the 2 varieties and measurement of BEV at test speed of 120 mm/min, and two replications • Review of new SOP on determination of uniaxial extensibility • measurement of uniaxial extensibility of the 2 varieties • Data collection • Statistics tutorial with JMP 16 • Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of BEV and uniaxial extensibility	• Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of BEV and uniaxial extensibility

2.2 Daily progress of the support mission

DAY 1

Who: Abiola, Ayomide, Oroniran

Where: Texture lab

What:
- Arrival and familiarisation with staff, lab protocol and materials
- Collection of 2 contrasting varieties of yam (kamilu, ewura)
- Review of new SOP on determination of bi-extensional viscosity (BEV) by lubricated squeezing flow (LSF)
- Discussion with team and work plan breakdown

Specific Methods & Tools Used:

- Discussions
- Reviewing of validated SOP on BEV by LSF to explain the merits and demerits of the protocol
- The PERTEN instrument texture analyser was checked for compatibility with the probes and other accessories brought from CIRAD.

Challenges Faced:

- The training started late in the day due to transportation challenges, and trainees were in faculty meetings.
- Due to the late yam harvest which is planned for December 2022, there was no available elite clones. Therefore, the only 2 varieties available were obtained from a farm associated with BOWEN, with established well known landrace varieties.
- The probes and other accessories brought from CIRAD for the LSF & uniaxial extensibility measurements were not compatible with the PERTEN instrument texture analyser.

Output(s) – Result(s):

- The validated SOP on BEV by LSF was discussed and presented

DAY 2

Who: Abiola, Ayomide, Oroniran

Where: Texture lab

What:
- Setting up, calibration, and test run of texture analyser (PERTEN Instrument). The available probe (35mm diameter), instrument and software of the PERTEN texture analyser in BOWEN were adapted to the ones used in the SOP.
- Preparation of trial samples and measurement of BEV of 1 genotype at test speed of 120 mm/min and two replications
- Data collection was not possible due to software not being able to download raw data for calculation of BEV.

Specific Methods & Tools Used:

- Discussions & demonstration to trainees

- The PERTEN instrument texture analyser was checked for compatibility with the probes and other accessories brought from CIRAD

Challenges Faced:

- Data collection was not possible due to software not being able to download raw data for calculation of BEV. Data could only be captured as a picture which could not display the full range of data points (force, distance, time). Without the raw data, it was not possible to calculate BEV. We recommend an updated version of software to solve this problem.
- The probes and other accessories brought from CIRAD for the LSF & uniaxial extensibility measurements were not compatible with the PERTEN instrument texture analyser, so adaptations had to be made (such as changing mould size to 25mm height and 35mm diameter to align with PERTEN instrument probe of 35mm diameter).

Output(s) – Result(s):

- Using the available probe, instrument and software of the PERTEN texture analyser in BOWEN, the measurement parameters were set for the texture analyser as below:

  Sample height: 25 mm

  Starting distance from sample: 30 mm

  Compression: (88 %, equivalent to 22 mm of compression on 25 mm sample height)

  Initial speed: 1 mm/s

  Test speed: 2 mm/s

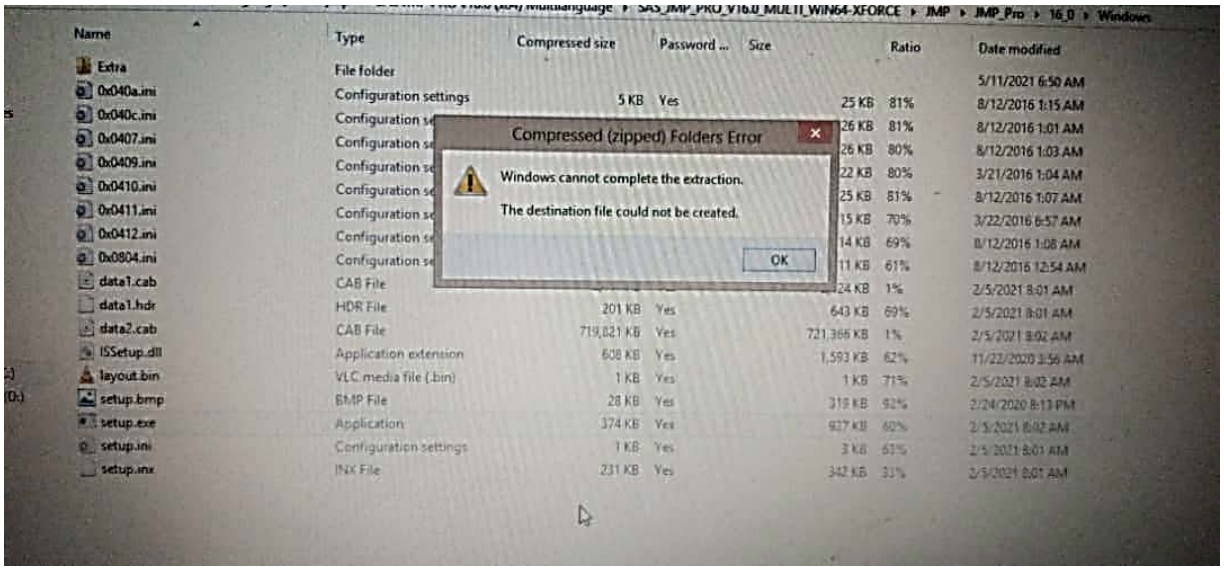
  Retract speed: 10 mm/s

  Trigger force: 0.049N (5g force)

  Data rate: 200

  Distance above trigger: 27 mm

  Nonetheless, at the end of first set of measurements, data download was impossible due to software limitations as shown in snapshot below:



DAY 3

Who: Abiola, Ayomide, Oroniran

Where: Texture lab

What:

- Setting up, calibration, and test run of texture analyser (STABLE Microsystems) at IITA.
- Preparation of pounded yam from the 2 varieties and measurement of BEV at test speed of 120 mm/min and two replications
- Review of new SOP on determination of uniaxial extensibility
- measurement of uniaxial extensibility of the 2 varieties
- Data collection
- Statistics tutorial with JMP 16
- Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of BEV and uniaxial extensibility

Specific Methods & Tools Used:

- Discussions
- Reviewing of validated SOP on uniaxial extensibility to explain the merits and demerits of the protocol

Challenges Faced:

- Due to the limitations of the PERTEN texture analyser and its software, the trainees were relocated to the International institute of Tropical Agriculture (IITA), Ibadan to conduct the rest of the trainings.
- The uniaxial extensibility protocol was not able to measure the extensibility of the pounded yam made from the variety *Ewura* due to its high fracturability.

Output(s) – Result(s):

- Data on uniaxial extensibility
- Trainees learned to use the statistical software JMP 16 for basic statistical analyses associated with the data collected.
- The protocols were accurate, repeatable and discriminant, although, it will require analyses of higher number of genotypes to arrive at more conclusive results.
- Detailed results are shown in Appendices 1 & 2.

DAY 4

Who: Abiola, Ayomide, Oroniran

Where: Texture lab

What:

- Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of BEV and uniaxial extensibility

Specific Methods & Tools Used:

- Reviewing of the BEV and uniaxial extensibility data

Challenges Faced:

- Due to the late yam harvest slated for December 2022, elite clones were not available. Therefore, the only 2 varieties available were obtained from a farm associated with BOWEN, with established well known landrace varieties.
- The training sessions were moved to IITA, Ibadan. This was due to the limitations of the PERTEN texture analyser and its software.

Output(s) – Result(s):

- Data on BEV and uniaxial extensibility of pounded yam. Detailed results are shown in Appendices 1 & 2
- The protocols were accurate, repeatable and discriminant, although, it will require analyses of higher number of genotypes to arrive at more conclusive results.
- Trainees had hands-on experimentation, and can conduct the tests individually.

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.
- 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 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 pounded yam, and the protocols may be useful for screening large populations of yam genotypes for consumer-preferred quality.
- 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

- Since yam harvest is slated for December2022/January 2023, elite clones were not available for analyses during the training. Therefore, the only 2 varieties available were obtained from a farm associated with BOWEN, with established well known landrace varieties.
- The training sessions in BOWEN was moved to IITA, Ibadan on the third day of training. This was due to the limitations of the PERTEN texture analyser and its software.

- The uniaxial extensibility protocol could not be applied to the variety *Ewura*, due to its high fracturability/mealiness.

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

- With the upcoming yam harvest in December 2022/January 2023, trainees will be expected to utilise the protocols in analysing the stretchability of pounded yam from a wider range of yam genotypes in order to obtain more conclusive data and results.
- The team lead in BOWEN suggested that the lab will request for an update of the PERTEN texture analyser software and purchase probes and accessories relevant to the protocols to overcome the challenges encountered during the training.

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	**No**

4 APPENDICES

4.1 Annex 1: Review of data on instrumental uniaxial extensibility of Pounded yam on mission2 in BOWEN, Iwo, Nigeria

Genotype

	Genotype	Quality of *pounded yam*
1	Kamilu	good
2	Ewura	bad

Procedure

Texture measurements of uniaxial extensibility of pounded yam made from 2 varieties of yam (Kamilu & Ewura) obtained from a research farm in Iwo, was carried out using the texture analyser 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 measurements per cooking replicate was collected. Measurements were made at 30 ^{o}C.

Statistical accuracy of uniaxial extensibility of pounded yam

	Genotype	Replicate	N	Mean	Std Dev	Std Err	CV (%)	CV, mean (%)
Hardness (N)	Kamilu	R1	3	0.39	0.05	0.03	12	7
		R2	3	0.44	0.17	0.10	40	23
Extensibility (mm)	Kamilu	R1	3	14.34	0.75	0.43	5	3
		R2	3	15.97	7.67	4.43	48	28
Area between to and Fmax (N.mm)	Kamilu	R1	3	3.17	0.57	0.33	18	10
		R2	3	2.80	0.94	0.54	34	19

**The 2nd variety *Ewura* did not form a sheet, was too fracturable/mealy

ANOVA and repeatability of uniaxial extensibility of pounded yam

Oneway Anova of Hardness (N) By Replicate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	0.00294817	0.002948	0.1829	0.6909
Error	4	0.06447467	0.016119		
C. Total	5	0.06742283			

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	3	0.394	0.0461194	0.0266271	0.279433	0.508567
R2	3	0.4383333	0.1735233	0.1001837	0.0072776	0.8693891

Means Comparisons

Connecting Letters Report

Level		Mean
R2	A	0.43833333
R1	A	0.39400000

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
R2	R1	0.0443333	0.1036618	-0.243478	0.3321449	0.6909	

Oneway Anova of Extensibility (mm) By Replicate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	3.99514	3.9951	0.1347	0.7322
Error	4	118.65844	29.6646		
C. Total	5	122.65357			

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	3	14.342333	0.7529305	0.4347046	12.47195	16.212716
R2	3	15.974333	7.6656581	4.4257698	-3.068217	35.016884

Means Comparisons

Connecting Letters Report

Level		Mean
R2	A	15.974333
R1	A	14.342333

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
R2	R1	1.632000	4.447067	-10.7150	13.97904	0.7322	

Oneway Anova of Area between to and Fmax (N.mm) By Replicate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	0.1976535	0.197654	0.3255	0.5989
Error	4	2.4291800	0.607295		
C. Total	5	2.6268335			

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	3	3.167	0.5725531	0.3305637	1.7446994	4.5893006
R2	3	2.804	0.9416863	0.5436828	0.4647217	5.1432783

Means Comparisons

Connecting Letters Report

Level		Mean
R1	A	3.1670000
R2	A	2.8040000

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	0.3630000	0.6362887	-1.40362	2.129622	0.5989	

The repeatability of the measurements of the uniaxial extensibility was indicated by the insignificant differences between cooking replicate means by one-way ANOVA. This result is inconclusive since only one variety was eventually measured. A test involving several varieties or genotypes will provide more reliable results.

Summary

As far as the data available is concerned, the uniaxial extensibility protocol was repeatable between the cooking replicates. No discrimination between the genotypes can be ascertained due to insufficient data on genotypes, although, it is clear the varieties are different in uniaxial extensibility as *Kamilu* can form dough sheet for measurement, but *Ewura* cannot. It is recommended that proper tests and measurements should be conducted when harvests are conducted in late December and many genotypes of yams are available for analyses.

References

Ayetigbo Oluwatoyin, Domingo Romain, Arufe Vilas Santiago, Mestres Christian, Akissoé Noël, Otegbayo Bolanle. 2022. Standard operating protocol for the instrumental determination of extensibility of pounded yam. Biophysical characterization of quality traits, WP2. Montpellier: RTBfoods Project-CIRAD, 18 p.https://doi.org/10.18167/agritrop/00684

Otegbayo Bolanle, Oroniran Oluyinka, Tanimola Abiola, Bolaji Oluwatomilola, Alamu Ayomide, Mestres Christian, Ayetigbo Oluwatoyin (2022). Standard operating protocol for textural characterization of pounded yam. Biophysical characterization of quality traits, WP2. Iwo: RTBfoods Project-CIRAD, 22 p. https://doi.org/10.18167/agritrop/00613

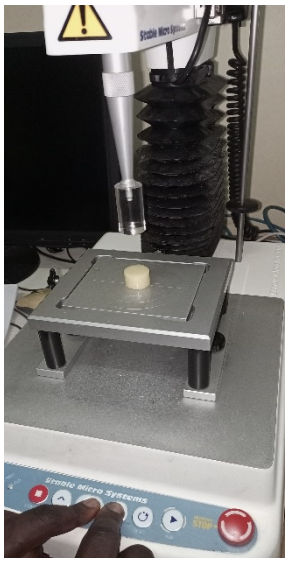
4.1.1 Annex 2: Review of data on instrumental biaxial extensional viscosity (BEV) of Pounded yam on mission2 in BOWEN, Iwo, Nigeria

Genotype

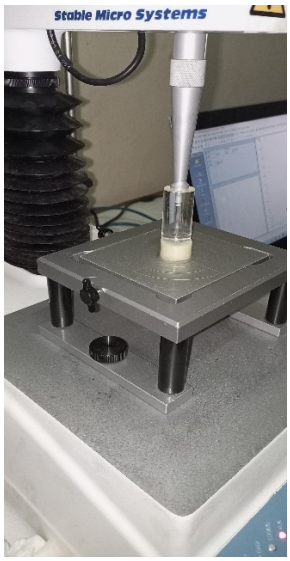
	Genotype	Quality of pounded yam
1	Kamilu (rotundata)	good
2	Ewura (alata)	bad

Procedure

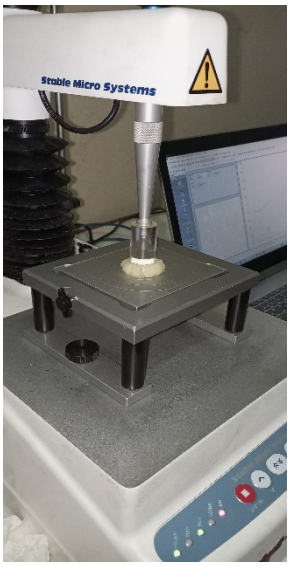
Texture measurements of biaxial extensional viscosity (BEV) of pounded yam made from two genotypes of yam (Kamilu, Ewura) using the texture analyser was carried out by preparing pounded yam based on the validated RTBfoods SOP for pounded yam textural analysis in BOWEN, Nigeria (Otegbayo et al. 2022), and adapting the SOP for biaxial extensional viscosity by lubricated squeezing flow (LSF) protocol for pounded yam (Santiago Arufe Vilas 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 test speed of 120 mm/min and biaxial strain of 1.0, and at temperature of 30 ^{o}C.



LSF compression prior to measurement



LSF compression during measurement



LSF compression at end of measurement

Statistical accuracy of bi-extensional viscosity of pounded yam

	Genotype	N	Mean	Std Dev	Std Err	CV (%)	CV, mean (%)
Extensional viscosity [Pa·s]	ewura	8	36659	5312	1878	14	5
	kamilu	7	29396	2151	813	7	3

ANOVA and repeatability of bi-extensional viscosity (test speed = 120mm/min, biaxial strain = 1.0) of pounded yam

Oneway Anova of Extensional viscosity [Pa·s] By Genotype

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	1	196936061	196936061	11.3637	5.014e-3
Error	13	225293373	17330259		
C. Total	14	422229434			

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Genotype	1	1	183213682	9.9241	9.238e-3
Replicate	1	1	1589464	0.0861	7.747e-1
Genotype*Replicate	1	1	21392772	1.1588	3.048e-1

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
ewura	8	36658.781	5312.0597	1878.0967	32217.788	41099.774
kamilu	7	29395.811	2151.2604	813.1	27406.227	31385.395

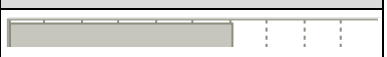
Means Comparisons

Connecting Letters Report

Level			Mean
ewura	A		36658.781
kamilu		B	29395.811

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
ewura	kamilu	7262.970	2154.538	2608.267	11917.67	5e-3	

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	3639351	3639351	0.1130	7.421e-1
Error	13	418590083	32199237		
C. Total	14	422229434			

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	8	32808.64	6995.6462	2473.3344	26960.133	38657.147
R2	7	33795.973	3559.4152	1345.3325	30504.063	37087.883

Means Comparisons

Connecting Letters Report

Level		Mean
R2	A	33795.973
R1	A	32808.640

Levels not connected by same letter are significantly different.

Ordered Differences Report

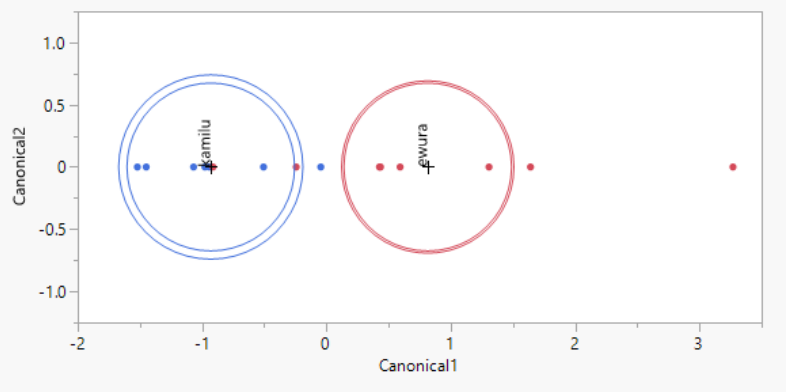
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
R2	R1	987.3329	2936.800	-5357.38	7332.049	0.7421	

The repeatability of the measurements of the bi-extensional viscosity (at test speed of 120mm/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 two genotypes. The genotype has the most significant effect on BEV of pounded yam. The genotype*replicate effect was not significant.

Discriminance between genotypes based on BEV of pounded yam

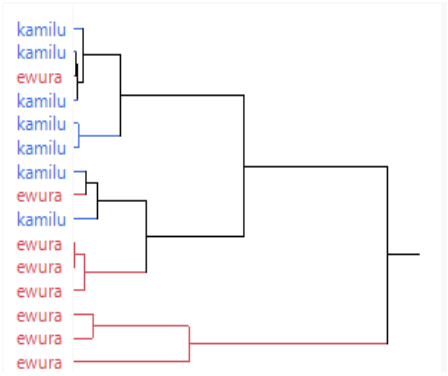
Discriminant

Discriminant analysis showed good discrimination between the 2 genotypes.



Hierarchical classes

The genotypes were clustered in about 2 classes of hierarchies with minimal incursions.



Summary

The LSF protocol was repeatable between the replications, and discriminant between the genotypes. The discriminant and hierarchical analyses show the relationship between the genotypes in terms of the bi-extensional viscosity.

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Institute: Cirad – UMR QualiSud

Address: C/O Cathy Méjean, TA-B95/15 - 73 rue Jean-François Breton - 34398 Montpellier Cedex 5 - France

Tel: +33 4 67 61 44 31

Email: rtbfoodspmu@cirad.fr

Website: https://rtbfoods.cirad.fr/