

## Training on Instrumental Textural Characterization of Extensibility of Fufu

Biophysical Characterization of Quality Traits, WP2

19/09/2022 - 23/09/2022, Umudike, 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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The stretchability of fufu is considered as a key textural attribute preferred by consumers of fufu as an acceptable quality parameter. The NRCRI team were trained in Nigeria between 19-23 September, 2022. The new SOP for uniaxial extensibility already developed for pounded yam was considered to determine the extensibility of fufu, while the new SOP for bi-extensional viscosity already developed for pounded yam was adapted to measure the bi-extensional viscosity of fufu using a test speed of 120mm/min and biaxial strain of 1.0. The training was done to determine instrumental extensibility texture of fufu using a texture analyser, which may represents the sensory stretchability of fufu.

The mission was conducted to train the National Roots Crop Research Institute (NRCRI) team to set up and measure extensibility of fufu using three protocols; uniaxial extensibility, lubricated squeezing flow (LSF) and Kieffer dough gluten extensibility (KDGE) rig.

Some modifications were introduced in the protocols, specific to the food product profile of fufu, and statistical evaluations were presented to show the accuracy, repeatability, and discriminance of the protocols.

Key Words: Extensibility, PCA, Discriminant analysis, Fufu, Texture analysis, LSF





## **1 GENERAL OVERVIEW**

## 1.1 Interest of this support mission in RTBfoods framework

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

## **1.2 Specific objectives**

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

## **1.3 Organizing committee**

• Ugo CHIJIOKE, Food Technologist, National Roots Crop Research Institute (NRCRI)





## **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	М	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	CHIJIOKE Ugo	F	Food Scientist / Lead	Food Science	NRCRI NIGERIA	2	ugochijioke4@gmail.com	YES
2	OKORONKWO Justice	М	Lab technologist	Biochemistry	NRCRI NIGERIA	2	justice_okoronkwo@yahoo.com	YES
3	ACHONWA Oluchi	F	Lab technologist	Biochemistry	NRCRI NIGERIA	2	olyachonwa@gmail.com	YES
4	IRO Ugochi Jane	F	Research Assistant	Food Science	NRCRI NIGERIA	2	ugochijaneiro@gmail.com	YES
5	UDOKA Precious	F	Lab technologist	Science lab technology	NRCRI NIGERIA	2	preudoka@yahoo.com	YES
6	CHIKERE Juliet	F	Lab technologist	Food Science	NRCRI NIGERIA	2	Julieddy4real@gmail.com	YES
7	OGUNKA Amaka	F	Lab technologist	Food Science	NRCRI NIGERIA	2	Pinozichora268@gmail.com	YES

## **1.6 Experience level of staff supported / trained**

Ugo Chijioke is the lead Food Scientist at the Institute. She manages the lab and coordinates the team, and has good knowledge on texture measurement procedures.

Okoronkwo Justice is the primary laboratory technologist focussed on the use of the texture analyser.

Oluchi Achonwa is an assistant laboratory technologist focussed on assisting on the use of the texture analyser

The other assisting staff have varying skills such as in sample preparation prior to textural measurement, and sensory texture evaluation.





## **2 SUPPORT IMPLEMENTATION**

## 2.1 Support mission agenda

19 September	20 September	21 September	22 September	23 September
<ul> <li>Arrival and familiarisation with staff, lab protocol and materials</li> <li>Collection of samples from fufu mash processor and conditioning of test materials (fermented fufu mash from 5 contrasting varieties of cassava)</li> <li>Review of new SOP on determination of uniaxial extensibility with trainees</li> <li>Discussion with team and work plan breakdown</li> <li>Setting up, calibration, and test run of texture analyser</li> <li>First set of measurements of uniaxial extensibility of 1 genotype (TME 419) with and without dough relaxation in order to determine if dough relaxation was necessary or not</li> </ul>	<ul> <li>Sample preparation of fufu from cooked mash following established SOP for fufu preparation</li> <li>Calibration and setting up of texture analyser</li> <li>Measurements of uniaxial extensibility of 4 genotypes (TME 419, F9P002, F1053P00010, F68P007) without dough relaxation</li> <li>Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of uniaxial extensibility protocol</li> </ul>	<ul> <li>Review of new SOP on determination of biaxial extensional viscosity (BEV) by lubricated squeezing flow (LSF) protocol with trainees</li> <li>Discussion with team and work plan breakdown</li> <li>Setting up, calibration, and test run of texture analyser</li> <li>Sample preparation of fufu from cooked mash following established SOP for fufu preparation</li> <li>First set of measurements of biaxial extensional viscosity of 2 genotypes (F68P007, F1053P00010) without dough relaxation, and at three test speeds (6, 12, 120 mm/min) and two replications</li> <li>Data repository</li> </ul>	<ul> <li>Setting up, calibration, and test run of texture analyser</li> <li>Sample preparation of fufu from cooked mash following established SOP for fufu preparation</li> <li>Measurements of biaxial extensional viscosity of 2 genotypes (F9P002, TME 419) without dough relaxation, and at three test speeds (6, 12, 120 mm/min) and two replications</li> <li>Data repository</li> </ul>	<ul> <li>Setting up, calibration, and test run of texture analyser</li> <li>Sample preparation of fufu from cooked mash following established SOP for fufu preparation</li> <li>Measurements of uniaxial extensibility and biaxial extensibility and biaxial extensional viscosity of 1 genotype (Nwaocha) without dough relaxation</li> <li>Statistical evaluation of data with trainees to determine the best operating parameters (test speed, biaxial strain), and to determine accuracy, repeatability and discriminant of biaxial extensional viscosity by LSF protocol</li> <li>Demonstration of KDGE protocol to trainees on sample preparation, texture analyser set up, calibration, and determination of fufu extensibility using a genotype (Nwaocha)</li> <li>Statistical evaluation of data with trainees to determine accuracy, and repeatability of extensibility by KDGE protocol</li> </ul>





## 2.2 Daily progress of the support mission

#### DAY 1

Who: Ugo, Justice, Oluchi, Jane, Precious, Amaka, Juliet

- Where: Analytical and Texture lab, NRCRI.
- What: Arrival and acquaintance with staff, lab management and outlay
  - Collection of samples (5 genotypes: F68P007, F1053P00010, F9P002, TME 419, Nwaocha) from fufu mash processor, and preparation of test materials (fermented fufu mash from the 5 contrasting varieties of cassava)
  - Review of new SOP on determination of uniaxial extensibility with trainees. The trainees were shown the technical aspects of the SOP, precautions, advantages and limitations of the SOP
  - Discussion with team and work plan breakdown for trainees
  - Setting up, calibration, and test run of texture analyser
  - First set of measurements of uniaxial extensibility of 1 genotype (TME 419) was collected with and without the dough relaxation procedure in order to determine if dough relaxation was necessary or not. It was determined that the dough relaxation process was not necessary since the fufu doughs formed very homogeneous and smooth sheet after rolling using a pasta roller. Also there was low variability in uniaxial extensibility parameter for dough without relaxation.

#### Specific Methods & Tools Used:

- Discussions
- Demonstrations
- Validated SOP on uniaxial extensibility
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface

#### Challenges Faced:

- The cassava fufu mash of a genotype (Nwaocha) was not ready on arrival at NRCRI. It was being hurriedly processed within the week
- Institute was on strike and access was delayed into building.
- Partner does not have some of the instrument's accessories (HDP, P/1SP ball probe, roller) to conduct uniaxial extensibility tests. The accessories used were brought in from CIRAD, and returned after the training.

#### Output(s) – Result(s):

- It was determined that the fufu doughs did not require the relaxation step in the SOP since they formed smooth homogeneous sheets after rolling without relaxation
- The repeatability of the measurements of the uniaxial extensibility parameters was indicated by the insignificant differences between cooking replicate means by one-way ANOVA (except hardness).
- Uniaxial extensibility measurements raw data for genotype TME 419
- Detailed results are shown in the Appendix 1





#### DAY 2

- <u>Who</u>: Justice, Jane, Precious, Amaka
- Where: Analytical and Texture lab, NRCRI
- <u>What:</u> Sample preparation of fufu from cooked mash following established SOP for fufu preparation
  - Calibration and setting up of texture analyser
  - Measurements of uniaxial extensibility of 4 genotypes (TME 419, F9P002, F1053P00010, F68P007) without dough relaxation
  - Statistical evaluation of data with trainees to determine accuracy, repeatability and discriminant of uniaxial extensibility protocol

#### Specific Methods & Tools Used:

- Demonstrations
- Validated SOP on uniaxial extensibility
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface

#### Challenges Faced:

- The cassava fufu mash of a genotype (Nwaocha) was not ready on arrival at NRCRI. It was being hurriedly processed within the week
- Institute was on strike and access was delayed into building.
- 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):

- Uniaxial extensibility measurements raw data for genotypes TME 419, F9P002, F1053P00010, F68P007
- The repeatability of the measurements of the uniaxial extensibility parameters was indicated by the insignificant differences between cooking replicate means by one-way ANOVA (except hardness).
- Detailed results are shown in the Appendix 1

#### DAY 3

- Who: Justice, Jane, Precious, Amaka
- Where: Analytical and Texture lab, NRCRI
- What:
   Review of new SOP on determination of biaxial extensional viscosity (BEV) by lubricated squeezing flow protocol with the trainees in order for them to understand the principles, challenges and advantages of the protocol over the uniaxial extensibility protocol
  - Discussion with team and work plan breakdown for the day
  - Setting up, calibration, and test run of texture analyser
  - Sample preparation of fufu from cooked mash following established SOP for





fufu preparation

- First set of measurements of biaxial extensional viscosity of 2 genotypes (F68P007, F1053P00010) without dough relaxation, and at three test speeds (6, 12, 120 mm/min) and two replications
- Data repository

#### Specific Methods & Tools Used:

- Demonstrations
- Validated SOP on bi-extensional viscosity
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface
- Refined vegetable oil

#### Challenges Faced:

• The cassava fufu mash of a genotype (Nwaocha) was not ready on arrival at NRCRI. It was being hurriedly processed within the week

#### Output(s) – Result(s):

- Biaxial extensibility measurements raw data for genotypes F68P007 and F1053P00010
- The repeatability of the measurements of the biaxial extensibility was indicated by the insignificant differences between cooking replicate means by one-way ANOVA
- Detailed results are shown in the Appendix 2

#### DAY 4

What:

- Who: Justice, Jane, Precious, Amaka
- Where: Analytical and Texture lab, NRCRI
  - Discussion with team and work plan breakdown for the day
    - Setting up, calibration, and test run of texture analyser
    - Sample preparation of fufu from cooked mash following established SOP for fufu preparation
    - Measurements of biaxial extensional viscosity of 2 genotypes (F9P002, TME 419) without dough relaxation, and at three test speeds (6, 12, 120 mm/min) and two replications
    - Data repository

#### Specific Methods & Tools Used:

- Demonstrations
- Validated SOP on bi-extensional viscosity
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface





#### **Challenges Faced:**

• The cassava fufu mash of a genotype (Nwaocha) was not ready on arrival at NRCRI. It was being hurriedly processed within the week

#### Output(s) – Result(s):

- Biaxial extensibility measurements raw data for genotypes F9P002 and TME 419
- The repeatability of the measurements of the biaxial extensibility was indicated by the insignificant differences between cooking replicate means by one-way ANOVA
- Detailed results are shown in the Appendix 2

#### DAY 5

- <u>Who:</u> Justice, Jane, Precious, Amaka
- Where: Analytical and Texture lab, NRCRI
- What: Setting up, calibration, and test run of texture analyser
  - Sample preparation of fufu from cooked mash following established SOP for fufu preparation
  - Measurements of uniaxial extensibility and biaxial extensional viscosity of 1 genotype (Nwaocha) without dough relaxation
  - Statistical evaluation of data with trainees to determine the overall best operating parameters (test speed, biaxial strain), and to determine accuracy, repeatability and discriminant of biaxial extensional viscosity by LSF protocol
  - Demonstration of Kieffer dough gluten extensibility (KDGE) protocol to trainees on sample preparation, texture analyser set up, calibration, and determination of fufu extensibility using a genotype (Nwaocha)
  - Statistical evaluation of data with trainees to determine accuracy, and repeatability of extensibility by KDGE protocol

#### Specific Methods & Tools Used:

- Demonstrations
- Validated SOP on uniaxial extensibility and bi-extensional viscosity
- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface

#### Challenges Faced:

- The cassava fufu mash of a genotype (Nwaocha) was now ready on the last day of the mission at NRCRI.
- The uniaxial extensibility protocol was not applicable for the genotype Nwaocha because the fufu dough was too sticky and difficult to handle or roll into a sheet using a pasta roller
- We could not obtain full KDGE extensibility data for all the 5 genotypes due to insufficient time, therefore, only a demonstration was conducted using one genotype (Nwaocha).

#### Output(s) – Result(s):

- Uniaxial extensibility data for Nwaocha was not possible to collect due to poor sheet





formation. Biaxial extensibility measurements raw data for genotype Nwaocha was collected.

- The repeatability of the measurements of the uniaxial extensibility and biaxial extensibility was indicated by the insignificant differences between cooking replicate means by one-way ANOVA
- Genotype Nwaocha was particularly significantly discriminant from the other genotypes
- Detailed results are shown in the Appendices 1, 2 & 3

## 2.3 List of material / documents distributed

• Validated SOP for the Instrumental Determination of Extensibility of Pounded yam.

This SOP was adapted to the fufu food product profile.

 Standard Operating Protocol for Determination of Bi-extensional viscosity of Pounded yam by Lubricated Squeezing Flow (LSF) Method.

## 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 fufu 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 fufu, and the protocols may be useful for screening large populations of cassava genotypes for fufu of consumer-preferred quality.
- The best operating conditions for measurements were obtained for bi-extensional viscosity.
- Demonstration of the KDGE protocol for determination of extensibility of fufu to the trainees

### **3.2 Challenges faced – paths for improvement**

- The partner institute was on labour union strike at the time the mission was conducted, thereby affecting staff availability for sensory evaluation.
- Among the two protocols trained on the mission, the uniaxial extensibility protocol is limited in use for analysing genotypes that produce fufu of sticky or adhesive texture. Such genotypes are not cohesive enough to be rolled into a homogeneous dough sheet. This disadvantage is not applicable to the LSF protocol.
- Partner does not have some of the instrument's accessories to conduct uniaxial extensibility tests. Accessories used were brought in from CIRAD
- There was insufficient time to develop a SOP for determination of extensibility of fufu on Day 5 by the KDGE protocol. Trainees will develop the new SOP after the mission following the demonstration to the protocol.





## 3.3 Feedbacks from staff trained - general remarks from support team

 Request for further statistical training in cleaning textural data and statistical analyses and further support in preparation of the SOP on determination of extensibility of fufu by KDGE system.

## 3.4 Next steps

- Sensory analyses of the stretchability of fufu 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 a wider range of fufu from populations of cassava genotypes.
- A new SOP is expected to be developed on determination of extensibility of fufu by KDGE protocol.
- Plan to purchase some of the instrument's accessories to conduct uniaxial extensibility tests.

#### List of documents attached to the report

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





## **4 APPENDICES**

## 4.1 Annex 1: Review of data on instrumental uniaxial extensibility of *Fufu* in NRCRI, Umudike, Nigeria

#### Genotype

	Genotype	Quality of fufu
1	TME419	Good check
2	F9P002	?
3	F1053P00010	?
4	F68P007	?
5	Nwaocha	Landrace

#### Procedure

Texture measurements of uniaxial extensibility of fufu made from four genotypes of cassava using the texture analyser was carried out by preparing fufu based on the validated RTBfoods SOP for Fufu textural analysis in NRCRI, Nigeria (Chijioke et al. 2022), and adapting the uniaxial extensibility protocol for pounded yam (Ayetigbo et al. 2022) to fufu. A modification of the protocol, however, did not include the relaxation in the incubator for 12 min step, rather, the cooked fufu was wrapped in aluminium foil immediately and kept in a Styrofoam box for about 5 min prior to rolling into sheet and measurements. Two preparations or cooking replicates per genotype were considered. About 3-9 measurements per cooking replicate was collected. Measurements were made at 30 °C.



Preparation of cooked fufu



Genotype (TME 419) with good sheet formation



Genotype (F68P007) with poor sheet formation

#### Statistical accuracy of uniaxial extensibility textural parameters

	Genotype	Ν	Mean	Std Dev	Std Err	CV (%)	CV, mean (%)
Hardness (N)	F9P002	16	0.45	0.08	0.02	17	4
	F68P007	7	0.26	0.11	0.04	44	17
	F1053P00010	16	0.34	0.06	0.01	17	4
	TME419	17	0.41	0.06	0.01	15	4
Extensibility (mm)	F9P002	16	12.62	1.78	0.45	14	4





	Genotype	Ν	Mean	Std Dev	Std Err	CV (%)	CV, mean (%)
	F68P007	7	7.17	2.00	0.76	28	11
	F1053P00010	16	11.81	1.33	0.33	11	3
	TME419	17	14.41	1.15	0.28	8	2
Area between to and Fmax (N.mm)	F9P002	16	3.34	0.60	0.15	18	5
	F68P007	7	1.20	0.66	0.25	55	21
	F1053P00010	16	2.55	0.52	0.13	20	5
	TME419	17	3.69	0.69	0.17	19	5

\*\*Variety Nwaocha was not analysed due to not being able to form a dough sheet

#### ANOVA and repeatability of uniaxial extensibility textural parameters

#### Hardness (N) By Genotype

#### One way Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	3	0.22561450	0.075205	14.1663	7.197e-7
Error	52	0.27605406	0.005309		
C. Total	55	0.50166855			

#### **Effect Tests**

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Genotype	3	3	0.23785438	27.0770	2.14e-10
Cooking Replicate	1	1	0.01362004	4.6515	3.607e-2
Genotype*Cooking Replicate	3	3	0.13225519	15.0557	4.881e-7

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
F9P002	16	0.451625	0.0778356	0.0194589	0.4101493	0.4931007
F68P007	7	0.2572857	0.1131897	0.0427817	0.1526027	0.3619687
F1053P00010	16	0.3404375	0.0577789	0.0144447	0.3096493	0.3712257
TME419	17	0.4080588	0.0603277	0.0146316	0.3770412	0.4390765





#### **Connecting Letters Report**

Level			Mean
F9P002	A		0.45162500
TME419	A		0.40805882
F1053P0001 0		В	0.34043750
F68P007		В	0.25728571

Levels not connected by same letter are significantly different.

#### **Ordered Differences Report**

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
F9P002	F68P007	0.1943393	0.0330180	0.106706	0.2819722	<.0001*	
TME419	F68P007	0.1507731	0.0327211	0.063928	0.2376181	0.0002*	
F9P002	F1053P00010	0.1111875	0.0257603	0.042817	0.1795578	0.0004*	
F1053P00010	F68P007	0.0831518	0.0330180	-0.004481	0.1707847	0.0687	
TME419	F1053P00010	0.0676213	0.0253786	0.000264	0.1349787	0.0488*	
F9P002	TME419	0.0435662	0.0253786	-0.023791	0.1109235	0.3256	

#### Hardness (N) By Cooking Replicate

#### One-way Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cooking Replicate	1	0.00135045	0.001350	0.1458	7.041e-1
Error	54	0.50031811	0.009265		
C. Total	55	0.50166855			

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
1	28	0.3774286	0.0958654	0.0181169	0.3402558	0.4146013
2	28	0.38725	0.0966443	0.0182641	0.3497753	0.4247247





#### **Connecting Letters Report**

Level		Mean
2	A	0.38725000
1	A	0.37742857

Levels not connected by same letter are significantly different.

#### **Ordered Differences Report**

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
2	1	0.0098214	0.0257254	-0.041756	0.0613990	0.7041	

#### Extensibility (mm) By Genotype

#### One way Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	3	264.50959	88.1699	38.3260	3.28e-13
Error	52	119.62713	2.3005		
C. Total	55	384.13672			

#### **Effect Tests**

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Genotype	3	3	259.31238	47.3512	2.22e-14
Cooking Replicate	1	1	3.43997	1.8844	1.762e-1
Genotype*Cooking Replicate	3	3	25.39533	4.6373	6.305e-3

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
F9P002	16	12.617063	1.7807388	0.4451847	11.668174	13.565951
F68P007	7	7.174	2.001183	0.7563761	5.3232144	9.0247856
F1053P00010	16	11.814063	1.3347152	0.3336788	11.102843	12.525282
TME419	17	14.405059	1.1541042	0.2799114	13.811673	14.998444





#### **Connecting Letters Report**

Level				Mean
TME419	A			14.405059
F9P002		В		12.617063
F1053P00010		В		11.814063
F68P007			С	7.174000

Levels not connected by same letter are significantly different.

#### **Ordered Differences Report**

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
TME419	F68P007	7.231059	0.6811540	5.42321	9.038909	<.0001*	
F9P002	F68P007	5.443063	0.6873344	3.61881	7.267316	<.0001*	
F1053P00010	F68P007	4.640063	0.6873344	2.81581	6.464316	<.0001*	
TME419	F1053P00010	2.590996	0.5283062	1.18882	3.993173	<.0001*	
TME419	F9P002	1.787996	0.5283062	0.38582	3.190173	0.0072*	
F9P002	F1053P00010	0.803000	0.5362511	-0.62026	2.226263	0.4464	

#### Extensibility (mm) By Cooking Replicate

#### **Oneway Analysis of Variance**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cooking Replicate	1	12.47968	12.4797	1.8132	1.837e-1
Error	54	371.65704	6.8825		
C. Total	55	384.13672			

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
1	28	12.722107	2.9542114	0.5582935	11.576584	13.867631
2	28	11.777964	2.2444844	0.4241677	10.907644	12.648284





#### **Connecting Letters Report**

Level		Mean
1	A	12.722107
2	A	11.777964

Levels not connected by same letter are significantly different.

#### **Ordered Differences Report**

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
1	2	0.9441429	0.7011489	-0.461610	2.349896	0.1837	

#### Area between to and Fmax (N.mm) By Genotype

#### **Oneway Analysis of Variance**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	3	35.835478	11.9452	31.3249	1.05e-11
Error	52	19.829240	0.3813		
C. Total	55	55.664719			

#### **Effect Tests**

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Genotype	3	3	35.716956	41.4922	2.24e-13
Cooking Replicate	1	1	0.101193	0.3527	5.554e-1
Genotype*Cooking Replicate	3	3	5.300212	6.1572	1.255e-3

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
F9P002	16	3.342125	0.6047497	0.1511874	3.0198766	3.6643734
F68P007	7	1.1951429	0.6611879	0.2499055	0.583646	1.8066397
F1053P00010	16	2.554125	0.5175312	0.1293828	2.2783521	2.8298979
TME419	17	3.6901176	0.6938485	0.168283	3.3333737	4.0468616





#### **Connecting Letters Report**

Level				Mean
TME419	A			3.6901176
F9P002	A			3.3421250
F1053P00010		в		2.5541250
F68P007			С	1.1951429

Levels not connected by same letter are significantly different.

#### **Ordered Differences Report**

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
TME419	F68P007	2.494975	0.2773215	1.75894	3.231013	<.0001*	
F9P002	F68P007	2.146982	0.2798377	1.40427	2.889699	<.0001*	
F1053P00010	F68P007	1.358982	0.2798377	0.61627	2.101699	<.0001*	
TME419	F1053P0001 0	1.135993	0.2150918	0.56512	1.706868	<.0001*	
F9P002	F1053P0001 0	0.788000	0.2183265	0.20854	1.367460	0.0037*	
TME419	F9P002	0.347993	0.2150918	-0.22288	0.918868	0.3777	

#### Area between to and Fmax (N.mm) By Cooking Replicate

#### **Oneway Analysis of Variance**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cooking Replicate	1	1.443216	1.44322	1.4373	2.358e-1
Error	54	54.221502	1.00410		
C. Total	55	55.664719			

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
1	28	3.1147857	1.2059449	0.2279022	2.6471691	3.5824023
2	28	2.7937143	0.744245	0.1406491	2.5051262	3.0823024





#### **Connecting Letters Report**

Level		Mean
1	A	3.1147857
2	A	2.7937143

Levels not connected by same letter are significantly different.

#### **Ordered Differences Report**

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
1	2	0.3210714	0.2678088	-0.215866	0.8580089	0.2358	

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 (except hardness). On the other hand, the genotypes were significantly different between one another in all the uniaxial extensibility parameters, especially in the order extensibility>area between  $t_o$  and  $F_{max}$ >hardness.

#### Discriminance between genotypes based on uniaxial extensibility parameters of fufu

#### PCA

A PCA showed the first two components accounted for 99.8% of variation in uniaxial extensibility parameters. The extensibility and area between  $t_o$  and  $F_{max}$  are closely related. Genotype TME 419 is associated with extensibility and area between  $t_o$  and  $F_{max}$ , and is regarded as a good check variety. Genotype F9P002 is associated with hardness, and is close to genotype TME 419. Genotype F1053P00010 is also close to genotype TME 419, but genotype F68P007 is very different in texture from all the other genotypes, as it is clustered in a different component space.



PCA- uniaxial extensibility for cooked fufu.

\*\*Variety Nwaocha was not analysed due to not being able to form a dough sheet





#### Discriminant

Discriminant analysis showed good discriminant between the genotypes. The genotype F68P007 is very discriminant from all the other genotypes. The other genotypes are more closely related though. The most discriminant textural parameter was the extensibility, and the least discriminant is the hardness.



\*\*Variety Nwaocha was not analysed due to not being able to form a dough sheet

#### **Hierarchical classes**

The genotypes were clustered in 3 classes of hierarchies representing good, poor and intermediate textural quality for making fufu. Since TME419 is a good check genotype, the genotype F9P002 is also considered good, while genotypes F1053P00010 and F68P007 are considered intermediate and poor quality.



\*\*Variety Nwaocha was not analysed due to not being able to form a dough sheet

	Hardness (N)	Extensibility (mm)	Area between $t_o$ and $F_{max}$ (N.mm)
	coefficients		
Hardness (N)	1.0000		
Extensibility (mm)	0.8676	1.0000	
Area between $t_o$ and $F_{max}$ (N.mm)	0.9371	0.9823	1.0000
	probability		
	Hardness (N)	Extensibility (mm)	Area between $t_o$ and $F_{max}$ (N.mm)
Hardness (N)	<.0001		

#### Correlations between instrumental uniaxial extensibility textural parameters





	Hardness (N)	Extensibility (mm)	Area between $t_o$ and $F_{max}$ (N.mm)
Extensibility (mm)	0.1324	<.0001	
Area between $t_o$ and $F_{max}$ (N.mm)	0.0629	0.0177**	<.0001

\*\* Significant at 5% level. Genotype Nwaocha was not analysed due to not being able to form a dough sheet

The only significant correlation found between the uniaxial extensibility parameters are between extensibility and area between  $t_o$  and  $F_{max}$ .

#### Summary

The uniaxial extensibility protocol was repeatable between the replicates and discriminant between the genotypes. The most discriminant uniaxial textural parameter was the extensibility. The PCA, discriminant and hierarchical analyses show the relationship between the genotypes.

#### 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

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# 4.2 Annex 2: Review of data on instrumental biaxial extensional viscosity (BEV) of Fufu on mission2 in NRCRI, Umudike, Nigeria

#### Genotype

	Genotype	Quality of <i>fufu</i>
1	TME419	Good check
2	F9P002	?
3	F1053P00010	?
4	F68P007	?
5	Nwaocha	Landrace

#### Procedure

Texture measurements of biaxial extensional viscosity of fufu made from five genotypes of cassava using the texture analyser was carried out by preparing fufu based on the validated RTBfoods SOP for Fufu textural analysis in NRCRI, Nigeria (Chijioke et al. 2022), and adapting the biaxial extensional viscosity protocol for pounded yam (Santiago Arufe Vilas et al. 2022) to fufu. A modification of the protocol, however, did not include the relaxation in the incubator for 12 min step, rather, the cooked fufu was wrapped in aluminium foil immediately and kept in a Styrofoam box as soon as sample is ready prior to measurements. Two preparations or cooking replicates per genotype were considered. About 4 measurements per cooking replicate was collected. Measurements were made at 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) at temperature of 30 °C.

#### Statistical accuracy of bi-extensional viscosity of fufu

	Genotype	Ν	Mean	Std Dev	Std Err	CV (%)	CV, mean (%)
extensional visc, η₀ [Pa⋅s]	F9P002	8	42748	6434	2275	15	5
	F68P007	8	45546	4016	1419	9	3
	F1053P00010	8	44970	13336	4715	30	10
	Nwaocha	8	25396	3735	1321	15	5
	TME419	8	40285	4393	1553	11	4



## Statistical evaluation to select best test speed and biaxial strain to measure bi-extensional viscosity

#### For 6mm/min

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Biaxial strain (eb)	5	4.5072e+12	9.014e+11	54.1689	6.518e-5
Error	6	9.9848e+10	1.664e+10		
C. Total	11	4.607e+12			

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

#### **Means and Std Deviations**

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.10	2	2636659	52862.596	37379.5	2161707.9	3111611.1
0.25	2	2043374	176433.04	124757	458186.02	3628562
0.40	2	1682233	160749.41	113667	237956.83	3126509.2
0.50	2	1495344	150100.38	106137	146745.55	2843942.5
0.75	2	1078927	106377.85	75220.5	123160.43	2034694.6
1.00	2	766401	78979.585	55847	56797.584	1476004.4

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

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	8.7726e+10	8.773e+10	0.1941	6.689e-1
Error	10	4.5193e+12	4.519e+11		
C. Total	11	4.607e+12			

#### For 12mm/min

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

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Biaxial strain (eb)	5	1.0696e+12	2.139e+11	10.7030	5.977e-3
Error	6	1.1992e+11	1.999e+10		
C. Total	11	1.1895e+12			





#### Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.10	2	1305388	156513.84	110672	-100833.1	2711609.1
0.25	2	1081796.5	173766.54	122871.5	-479433.9	2643026.9
0.40	2	875292.5	155008.41	109607.5	-517402.8	2267987.8
0.50	2	776577	142259.98	100593	-501578.3	2054732.3
0.75	2	567168.5	112709.29	79697.5	-445484.3	1579821.3
1.00	2	415647	90860.393	64248	-400701.2	1231995.2

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

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	1.1513e+11	1.151e+11	1.0716	3.25e-1
Error	10	1.0743e+12	1.074e+11		
C. Total	11	1.1895e+12			

#### For 120mm/min

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

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Biaxial strain (eb)	5	1.3156e+10	2.6312e+9	42.1928	1.345e-4
Error	6	374163699	62360617		
C. Total	11	1.353e+10			

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0.10	2	135470	4088.4914	2891	98736.362	172203.64
0.25	2	133858.5	12902.577	9123.5	17933.441	249783.56
0.40	2	105837	9275.8268	6559	22497.003	189177
0.50	2	92559.5	7962.7295	5630.5	21017.214	164101.79
0.75	2	65442	5624.3273	3977	14909.424	115974.58
1.00	2	45546	3145.211	2224	17287.401	73804.599





<b>Oneway Anova Extensional</b>	viscosity (Pa.s)	By Replicate
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Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	202097376	202097376	0.1516	7.051e-1
Error	10	1.3328e+10	1.3328e+9		
C. Total	11	1.353e+10			

#### Oneway Anova of Extensional viscosity (Pa.s) By Speed (mm/min) Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Speed (mm/min)	2	1.3878e+13	6.939e+12	39.4134	1.797e-9
Error	33	5.81e+12	1.761e+11		
C. Total	35	1.9688e+13			

#### Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
6	12	1617156	647164.46	186820.29	1205967.8	2028345.2
12	12	836978	328836.86	94927.024	628045.28	1045911.2
120	12	96452	35071.364	10124.231	74168.885	118735.45

#### Compilation of mean bi-extensional viscosity (BEV) at various bi-axial strains and test speeds

Speed	Test speed	Number	Mean BEV	Std Dev	Std Err Mean	Lower 95%	Upper 95%	CV (%	CV, mean (%)
6	0.10	2	2636659.5	52862.596	37379.5	2161707.9	3111611.1	2.00	1.42
6	0.25	2	2043374	176433.04	124757	458186.02	3628562	8.63	6.11
6	0.40	2	1682233	160749.41	113667	237956.83	3126509.2	9.56	6.76
6	0.50	2	1495344	150100.38	106137	146745.55	2843942.5	10.04	7.10
6	0.75	2	1078927.5	106377.85	75220.5	123160.43	2034694.6	9.86	6.97
6	1.00	2	766401	78979.585	55847	56797.584	1476004.4	10.31	7.29
12	0.10	2	1305388	156513.84	110672	-100833.1	2711609.1	11.99	8.48
12	0.25	2	1081796.5	173766.54	122871.5	-479433.9	2643026.9	16.06	11.36
12	0.40	2	875292.5	155008.41	109607.5	-517402.8	2267987.8	17.71	12.52
000	1								



Speed	Test speed	Number	Mean BEV	Std Dev	Std Err Mean	Lower 95%	Upper 95%	CV (%	CV, (%)	mean
12	0.50	2	776577	142259.98	100593	-501578.3	2054732.3	18.32	12.95	
12	0.75	2	567168.5	112709.29	79697.5	-445484.3	1579821.3	19.87	14.05	
12	1.00	2	415647	90860.393	64248	-400701.2	1231995.2	21.86	15.46	
120	0.10	2	135470	4088.4914	2891	98736.362	172203.64	3.02	2.13	
120	0.25	2	133858.5	12902.577	9123.5	17933.441	249783.56	9.64	6.82	
120	0.40	2	105837	9275.8268	6559	22497.003	189177	8.76	6.20	
120	0.50	2	92559.5	7962.7295	5630.5	21017.214	164101.79	8.60	6.08	
120	0.75	2	65442	5624.3273	3977	14909.424	115974.58	8.59	6.08	
120	1.00	2	45546	3145.211	2224	17287.401	73804.599	6.91	4.88	

To select the best biaxial strain (i.e. the strain with the most repeatable and accurate bi-extensional viscosity), we selected one genotype F68P007 and then, we determined the ANOVA of the bi-extensional viscosity at each test speed (6, 12, 120 mm/min) and selected the biaxial strain with the least standard error of mean and CV (%), which is at biaxial strain of 1.0 (except for strain of 0.1 which can't be used because the material has not been significantly deformed enough to measure extensibility at that strain). In addition, the ANOVA showed no significant differences between the replicates at each speed and biaxial strains. 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 120mm/min. The selected biaxial strain and test speed were used for the other genotypes (same statistics was found for genotype F1053P00010).

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

#### Oneway Anova extensional visc, ηb [Pa·s] By Genotype

#### Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Genotype	4	2209277664	552319416	10.2805	1.3e-5
Error	35	1880375055	53725002		
C. Total	39	4089652719			





#### **Two-way ANOVA / Effect Tests**

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Genotype	4	4	2209277664	13.2503	2.483e-6
Replicate	1	1	233189851	5.5943	2.468e-2
Genotype*Replicate	4	4	396683567	2.3791	7.397e-2

#### Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
F9P002	8	42747.734	6433.8669	2274.7154	37368.886	48126.581
F68P007	8	45545.963	4015.8469	1419.8163	42188.63	48903.295
F1053P00010	8	44970.435	13336.263	4715.0812	33821.04	56119.83
Nwaocha	8	25395.6	3735.1693	1320.5818	22272.92	28518.28
TME419	8	40284.735	4392.7128	1553.0585	36612.335	43957.135

#### **Connecting Letters Report**

Level			Mean
F68P007	А		45545.963
F1053P00010	A		44970.435
F9P002	A		42747.734
TME419	A		40284.735
Nwaocha		в	25395.600

#### **Ordered Differences Report**

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
F68P007	Nwaocha	20150.36	3664.867	9613.64	30687.09	3.3e-5	
F1053P00010	Nwaocha	19574.84	3664.867	9038.11	30111.56	5.4e-5	
F9P002	Nwaocha	17352.13	3664.867	6815.41	27888.86	3.3e-4	
TME419	Nwaocha	14889.14	3664.867	4352.41	25425.86	2.3e-3	
F68P007	TME419	5261.23	3664.867	-5275.50	15797.95	6.1e-1	
F1053P00010	TME419	4685.70	3664.867	-5851.02	15222.42	7.1e-1	





Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
F68P007	F9P002	2798.23	3664.867	-7738.49	13334.95	9.4e-1	
F9P002	TME419	2463.00	3664.867	-8073.72	12999.72	9.6e-1	
F1053P00010	F9P002	2222.70	3664.867	-8314.02	12759.42	9.7e-1	]
F68P007	F1053P00010	575.53	3664.867	-9961.20	11112.25	1e+0	<pre>/ : : h: : :/ : : : :</pre>

#### Oneway Anova extensional visc, ηb [Pa·s] By Replicate

#### Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	233189851	233189851	2.2978	1.378e-1
Error	38	3856462868	101485865		
C. Total	39	4089652719			

#### Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	20	37374.407	10446.387	2335.883	32485.347	42263.466
R2	20	42203.38	9687.3494	2166.1572	37669.561	46737.199

#### **Connecting Letters Report**

Level		Mean
R2	A	42203.380
R1	A	37374.407

#### **Ordered Differences Report**

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value	
R2	R1	4828.974	3185.685	-1620.18	11278.12	1.4e-1	

The repeatability of the measurements of the bi-extensional viscosity was indicated by the insignificant differences between cooking replicate means by one-way ANOVA. On the other hand, the bi-extensional viscosity means of the genotypes were significantly different between one another. The genotype Nwaocha was significantly different from the other genotypes.





#### Discriminance between genotypes based on BEV of fufu

#### Discriminant

Discriminant analysis showed good discriminant between the genotypes. The most discriminately distant genotypes are Nwaocha and F68P007, and other genotypes. The closest genotypes to the genotype TME 419 a check, is genotype F9P002.



#### **Hierarchical classes**

The genotypes were clustered in 3 classes of hierarchies representing good, poor and intermediate textural quality for making fufu. The hierarchical classification of uniaxial extensibility parameters is similar to that of bi-extensional viscosity. Since TME419 is a good check genotype, the genotype F9P002 is also considered good, while genotype F1053P00010 is considered of intermediate quality.



Uniaxial extensibility



\*\*Variety Nwaocha was not analysed for uniaxial extensibility due to not being able to form a dough sheet

#### Summary

The preferred test speed and biaxial strain for the measurement of bi-extensional viscosity are 120mm/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. Under Review.

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### 4.3 Annex 3: Demonstration of extensibility texture of Fufu using KDGE rig on mission2 in NRCRI, Umudike, Nigeria

#### Genotype

	Genotype	Quality of <i>fufu</i>
1	Nwaocha	?



KDGE rig

KDGE Teflon dough form unit

Active test of fufu by KDGE

#### Procedure

#### Sample Preparation:

Texture measurements of extensibility of fufu made from a genotypes of cassava (Nwaocha) using the KDGE rig of the texture analyser was carried out first, by preparing fufu based on the validated RTBfoods SOP for Fufu textural analysis in NRCRI, Nigeria (Chijioke et al. 2022).

Next, we apply a small amount of paraffin oil to both sides of the teflon dough form, to avoid sample adhesion. Place about 10g of the fufu dough sample onto the grooved base of the form. Position the upper block of the form on top of the sample and push down firmly until the two blocks come together. Remove excess dough cleanly from sides, using a knife/spatula and clamp the dough form in the form press for 12 minutes (this cuts the sample into strips, allows the dough to relax and prevents loss of moisture). Scrape off any excess dough sample that is forced out from the sides of the form. Loosen the dough press and carefully slide the upper form block backwards over the grooved base to uncover the first dough strip. Tighten the press in this position, and using the upper form block as a cutting edge, score along the ridge of a groove to separate the strip of dough. To remove the strip of dough from the grooved base, dip the spatula in oil, and carefully slide it under the sample. Take care not to stretch or deform the dough strip sample.

#### Test Set-Up:

Position the Kieffer rig on the machine base. Ensure that the hook probe is covered with the plastic sleeve to prevent it from shearing through the sample. Lower the hook probe to just above the upper surface of the spring loaded clamp. Place the strip of dough onto the grooved region of the sample plate and, holding down the spring loaded clamp lever, insert the plate into the rig. Release the handle slowly. Commence the tensile test under the following parameter settings:





Load cell: 5kg Trigger force 5g (0.049N) Test mode: Tension Pre-test speed: 2 mm/s Test speed: 3.3 mm/s Post-test speed: 10 mm/s Distance: 75mm

#### Results

#### Statistical accuracy of extensibility textural parameters measured by KDGE

Extensibility parameter	N	Mean	Std Dev	Std Err	CV (%)	CV, mean (%)
Extensogram peak force (N)	14	0.09	0.01	0.00	10	3
Stretch area (N.s)	14	0.67	0.18	0.05	27	7
Stretch length (mm)	14	5.47	1.68	0.45	31	8
Rupture area (N.s)	14	0.60	0.06	0.02	11	3
Rupture length (mm)	14	7.09	0.97	0.26	14	4
Total area (N.s)	14	1.27	0.18	0.05	14	4
Total length (mm)	14	12.55	1.64	0.44	13	3

\*\*Only one genotype was analysed in two replicates of seven measurements per replicate.

#### Oneway Anova Extensogram peak force (N) By Replicate

#### Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	0.00039114	0.000391	7.6910	1.686e-2
Error	12	0.00061029	0.000051		
C. Total	13	0.00100143			

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	7	0.0937143	0.007868	0.0029738	0.0864376	0.1009909
R2	7	0.0831429	0.0063095	0.0023848	0.0773076	0.0889782





#### **Connecting Letters Report**

Level			Mean
R1	A		0.09371429
R2		В	0.08314286

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	0.0105714	0.0038119	0.0022660	0.0188769	1.7e-2	

#### Oneway Anova Stretch area (N.s) By Replicate

#### Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	0.04885207	0.048852	1.5051	2.434e-1
Error	12	0.38949743	0.032458		
C. Total	13	0.43834950			

#### Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	7	0.6114286	0.1976325	0.0746981	0.428649	0.7942082
R2	7	0.7295714	0.160803	0.0607778	0.5808534	0.8782894

#### **Means Comparisons**

**Connecting Letters Report** 

Level		Mean
R2	A	0.72957143
R1	A	0.61142857

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	0.1181429	0.0963003	-0.091678	0.3279639	2.4e-1	

#### Oneway Analysis of Stretch length (mm) By Replicate

#### **Oneway Anova**

#### Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	11.842241	11.8422	5.7692	3.34e-2
Error	12	24.631934	2.0527		
C. Total	13	36.474175			

#### Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	7	4.5468571	1.299297	0.4910881	3.3452078	5.7485065
R2	7	6.3862857	1.5547185	0.5876284	4.9484109	7.8241605

#### **Means Comparisons**

#### **Connecting Letters Report**

Level			Mean
R2	A		6.3862857
R1		В	4.5468571

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	1.839429	0.7658163	0.1708524	3.508005	3.3e-2	





#### Oneway Analysis of Rupture area (N.s) By Replicate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	0.00000350	3.5e-6	0.0008	9.782e-1
Error	12	0.05401971	0.004502		
C. Total	13	0.05402321			

#### Oneway Analysis of Variance

#### Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	7	0.6031429	0.0499614	0.0188836	0.5569363	0.6493495
R2	7	0.6041429	0.0806669	0.0304892	0.5295385	0.6787473

#### **Means Comparisons**

#### **Connecting Letters Report**

Level		Mean
R2	A	0.60414286
R1	A	0.60314286

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	0.0010000	0.0358634	-0.077140	0.0791399	9.8e-1	

#### Oneway Analysis of Rupture length (mm) By Replicate

#### Oneway Anova

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	0.447858	0.447858	0.4599	5.105e-1
Error	12	11.684833	0.973736		
C. Total	13	12.132691			





#### **Means and Std Deviations**

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	7	6.9074286	0.6652804	0.2514523	6.2921469	7.5227103
R2	7	7.2651429	1.2267331	0.4636615	6.1306039	8.3996818

#### **Means Comparisons**

#### **Connecting Letters Report**

Level		Mean		
R2	A	7.2651429		
R1	A	6.9074286		

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	0.3577143	0.5274564	-0.791519	1.506947	5.1e-1	

#### Oneway Analysis of Total area (N.s) By Replicate

#### **Oneway Analysis of Variance**

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	0.04968257	0.049683	1.7052	2.161e-1
Error	12	0.34962514	0.029135		
C. Total	13	0.39930771			

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	7	1.2145714	0.2117663	0.0800401	1.0187203	1.4104226
R2	7	1.3337143	0.1158702	0.0437948	1.2265522	1.4408764





#### **Connecting Letters Report**

Level		Mean
R2	A	1.3337143
R1	A	1.2145714

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	0.1191429	0.0912382	-0.079649	0.3179345	2.2e-1	

#### Oneway Analysis of Total length (mm) By Replicate

#### Oneway Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Replicate	1	16.896029	16.8960	11.3070	5.647e-3
Error	12	17.931591	1.4943		
C. Total	13	34.827620			

#### Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
R1	7	11.454286	1.4274151	0.5395122	10.134147	12.774425
R2	7	13.651429	0.9752357	0.3686044	12.749486	14.553371

#### **Means Comparisons**

**Connecting Letters Report** 

Level			Mean
R2	A		13.651429
R1		В	11.454286

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	2.197143	0.6534085	0.7734831	3.620803	5.6e-3	

The protocol was repeatable for most of the KDGE extensibility parameters measured. The repeatability of the protocol cannot be conclusive since only one genotype was analysed. Recommendations were made for the trainees to test at least 5-6 genotypes for more reliable results.

#### References

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