Standard Operating Procedure for Determination of Extensibility of Pounded Yam using Kieffer Dough Extensibility Method

Cotonou, Benin, 12/02/2024

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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.

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RTBfoods / RTB Breeding

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ABSTRACT

This SOP describes the procedure for determining the extensibility of pounded yam using the Kieffer dough extensibility method. The SOP also reviews the procedure for preparation of pounded yam by adjusting the dry matter of the pounded yam to 30% during pounding. The SOP reviewed the existing method of preparing pounded yam from *Dioscorea rotundata* and *Dioscorea alata* genotypes mechanically by modifying the method through adjusting the dry matter of the pounded yam to 30% in order to determine the amount of water to be added during pounding. Results showed that the extensibility and area under extension curve were the most discriminant textural parameters while extensogram peak force was the least discriminant textural parameter. The method was able to differentiate between the extensibility of *D. alata* genotypes and *D. rotundata* genotypes. The precautions required to conduct the procedure correctly and the statistical recommendations of minimum number of observations to be conducted are given.

Key Words: Pounded yam, Dry matter, Extensibility, Discriminant, Texture

1 Scope and Application

This SOP presents the procedure of preparation of pounded yam based on adjustment of dry mater of pounded yam, by reviewing the previous procedures hitherto developed *(Ayetigbo et al.,* 2023*; Otegbayo et al*., 2022). The SOP also describes the procedure for determining the extensibility of pounded yam produced from different varieties of yams from *D.alata* and *D.rotundata* using a texture analyser with Kieffer dough probe under fixed conditions. In order to develop this SOP, different preliminary experiments were conducted in various locations (Cote d'Ivoire, Benin, Nigeria and Montpellier, France) using various genotypes of yam and traditional or laboratory procedures. As a result, process parameters were harmonized as much as possible in order to be able to analyse pounded yam under similar conditions. This SOP makes it possible to integrate consumer preferences through the final dry matter content that end-products should have according to regions and consumption habits.

2 References

Ayetigbo, O., Domingo, R., Arufe-Vilas, S., Mestres, C., Akissoe, N., & Otegbayo, B. (2023). *Standard Operating Protocol for the Instrumental Determination of Extensibility of Pounded Yam* (p. 18). RTBfoods- CIRAD & UAC-FSA. https://doi.org/https://doi.org/10.18167/agritrop/00684

Otegbayo, B., Oroniran, O., Tanimola, A., Bolaji, O., Alamu, A., Mestres, C., & Ayetigbo, O. (2022). *Standard Operating Protocol for Textural Characterization of Pounded Yam* (pp. 1–22). RTBfoods - Bowen University, Iwo. https://doi.org/10.18167/agritrop/00613

Stable Micro Systems. (2022). *Extensibility of dough and measure of gluten quality*. Stable Micro Systems Ltd.

3 Principle

The new procedure is based on a more consumer-focused representative textural quality of pounded yam with the consideration that, traditionally, water is sometimes added to pounded yam during pounding process due to the high dry matter of some genotypes (most landraces and *rotundata* yam varieties have dry matter above 30%, while most *alata* varieties have comparably lower dry matter). The Kieffer dough extensibility method is based on the application of tensile force on strand configuration of pounded yam formed by the Kieffer mould, and extending the strands until it breaks. The three important textural parameters derived from the texture analyser were extensogram peak force representing (hardness, unit in Newton), extensibility (representing extension length at failure, unit in mm) and extension area (representing the work done to extend the strand until failure). Generally, the extensibility represents the instrumental equivalence of stretchability, which is scored by sensory panel evaluation.

4 Reagents

Paraffin oil (chemical grade)

5 APPARATUS

1. Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface.
2. Kieffer dough standard probe and rig (A/KIE, Stable microsystems, Surrey, England).
3. Yam Pounding machine (QZP/6000 model, Cheerfengly Ind. Co. Ltd, Taipei, Taiwan)
4. Infra-red thermometer
5. Chronometer
6. Bowls
7. Incubator
8. Air oven (105 °C)
9. Moisture cans
10. Weighing balance ±0.01 g
11. Plastic spoon
12. Aluminium foil
13. Stainless steel knives
14. Cutting boards

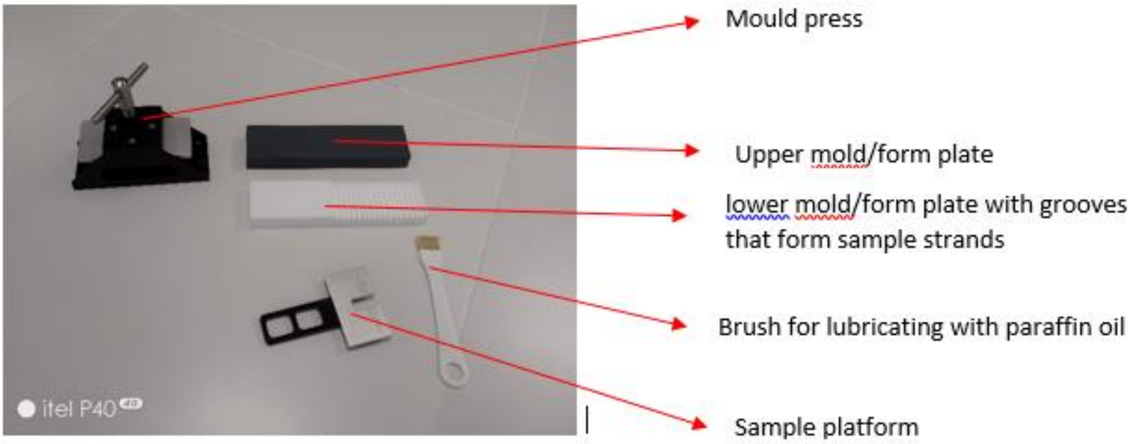


Figure 1 : Sample preparation kit

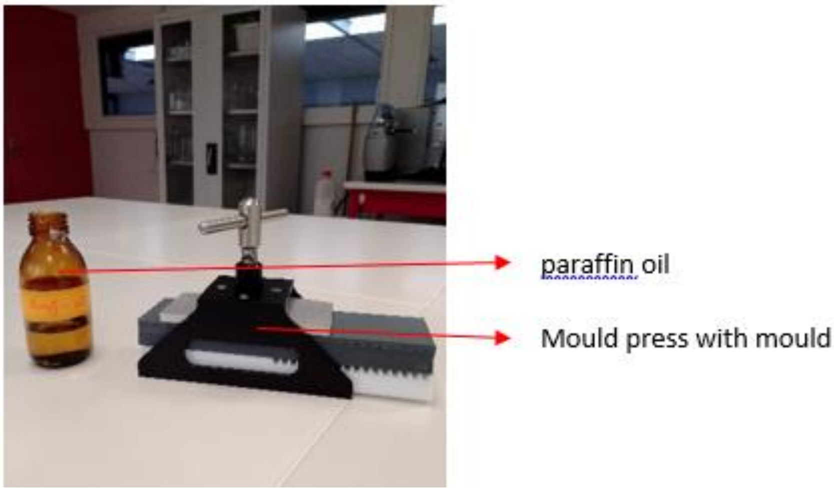


Figure 2 : Paraffin oil and Kieffer mould

6 PROCEDURE

6.1 Calibration of texture analyser

The texture analyser must be switched on at least 15 min before calibration and measurements. The probe height and force are calibrated following the calibration instructions provided in the operation manual. The force calibration is done using a 2 kg standard mass and a 5 kg load cell on the texture analyser. The calibration of probe distance (40 mm) is done afterwards. The test probe used is a Kieffer hook (A/KIH) tension probe (Figure 3) with the Kieffer dough extensibility rig (A/KIE) (Figure 4).

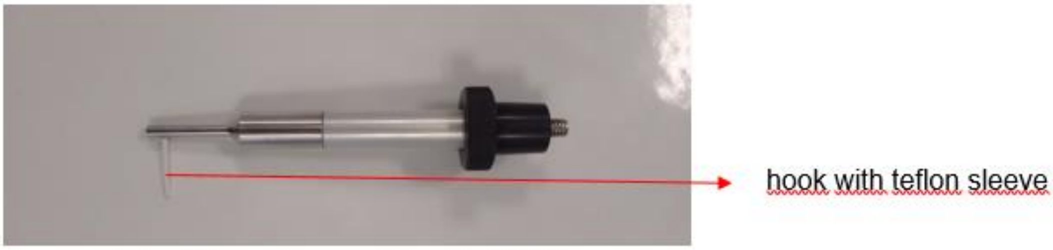


Figure 3 : **Kieffer probe with teflon sleeve**

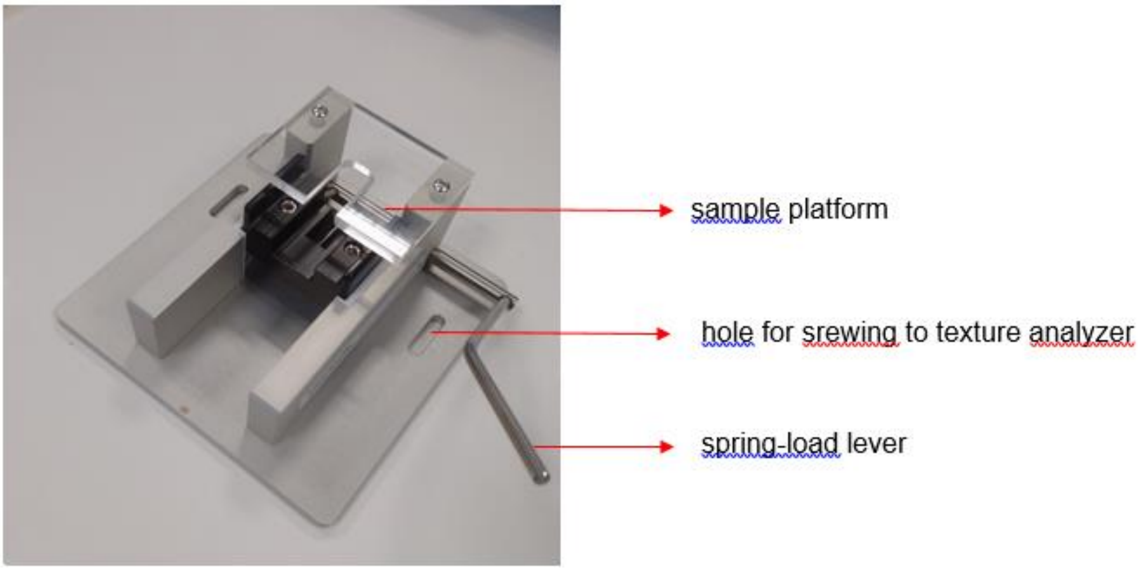


Figure 4 : **Kieffer rig**

6.2 Test conditions for measurement of extensibility

The test settings considered are:

Test Mode: Tension

Pre-Test Speed: 3.00 mm/s

Test Speed: 2.00 mm/s

Post-Test Speed: 10.00 mm/s

Target Mode: Distance

Distance: 40 mm

Trigger Force: 0.049 N (or 5g)

6.3 Preparation of pounded yam dough and measurement of extensibility

For preparation of the pounded yam, similar procedure published (Otegbayo *et al.*, 2022) was used with some modifications as briefly described below:

1. Longitudinally cut two yam tubers into two halves, remove the proximal and distal sections from both ends, about 1/10 of the length of each tuber (Figure 5).

Figure 5 : Longitudinal halves of yam tubers after cutting proximal and distal sections

2. From the two central sections, pieces of about 2 cm cubes are cut from both representative central sections (Figure 6) after peeling.

Figure 6 : **Fresh yam cubes (about 2 cm dimension) from central section of the tuber after peeling**

3. A known mass of the fresh yam cubes (typically 450g for big tubers, or less for smaller tubers, however, the cubes should be representative of the tubers used) is placed in the cooking chamber of the yam pounding machine (Figure 7). The dry matter of the fresh yam must be determined beforehand (air oven at 105°C for 16-24h). The pounding machine's receptacle is filled with 250 mL of tap water. The yam cubes were steam cooked for about an average of 23 min (usually the alarm sounds around 23 min when cooking is finished, but cooking time may vary between 19-26 min depending on variety and quantity of samples). Alternatively, the cubes may be steamed in a separate steamer for 20 min and pounded later in the yam pounding machine.

Figure 7. Yam Pounding machine

4. After steaming in the pounded yam machine, the excess water was drained from the steamed tubers, then the mass of the steamed yam was noted. The dry matter of steamed yam was determined earlier, and used in calculation of amount of water to be added during pounding. The steamed yam was pounded for between 1.5 to 4 min, with occasional scraping and pulling the dough together in the pounding receptacle until a smooth dough was formed. This mimics the kneading of the pounded yam in the traditional method.
5. A calculated volume of warm water (at about 65°C) may be added from time to time (not all at once) during pounding to adjust the dry matter of the pounded yam to 30 %. With regard to this pounding cycle, it is advisable to apply a first pounding stage (about 0.5 min) before starting to add water. This will allow the cooked yam pieces to be destructured/crushed, which will facilitate the subsequent absorption of water and reduce the quantity of lumps. It should be noted that water addition is only required when the dry matter of steamed yam exceeds 30%. Whenever the dry matter of the steamed yam is below 30%, there is no need for water addition or calculation of water to be added. The actual mass of pounded yam produced is recorded and the actual dry matter of the pounded yam is also determined. These values can be compared with the expected/calculated mass of pounded yam and the expected dry matter (*i.e.,* 30%) of pounded yam.
6. After preparation of the pounded yam, a sufficient amount of the dough (about 50-60g) at 40°C has to be rolled into a ball mass and placed on the lubricated lower form plate/mould and pressed with the lubricated upper form plate/mould until fully compressed by the turning the screw, and getting rid of the excess dough on the edges of the strip grooves. Note that no relaxation time was considered for the dough strands because a 30 min relaxation led to reduction in extension area and extensibility by 50% in a preliminary test, probably due to retrogradation. After retracting the upper form plate to reveal the dough strands (neglecting the first two strands which may not be full-strands), the strand is removed with a thin lubricated spatula and placed on the Kieffer sample platform for measurement on the texture analyser (Figure 8).

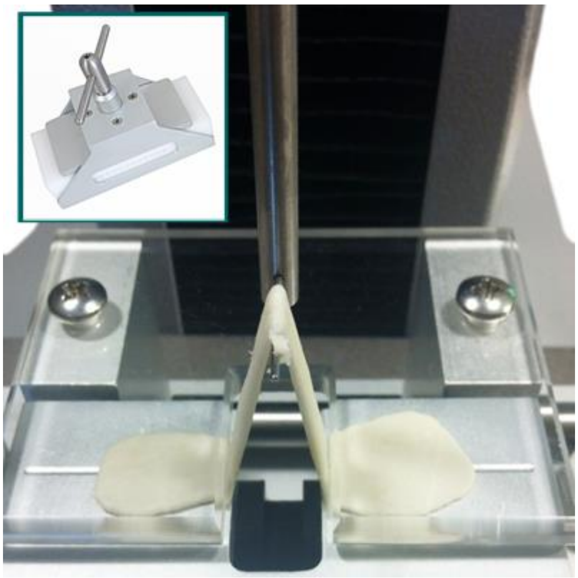


Figure 8 : **Sample placement showing the extension of a sample strand**

6.4 Calculation of the amount of water addition to adjust dry matter of pounded yam during pounding

The water to be added to pounded yam during pounding can be calculated based on dry matter of steamed yam determined experimentally beforehand, or based on dry matter of fresh yams and water absorbed during steaming.

6.4.1 Calculation of water addition based on pre-determined dry matter of steamed yam

The mass of water to be added (*W*) to adjust the final dry matter of pounded yam to 30 g of dry matter/100 g of wet matter is determined (Figure 9) by solving the Equation (1) :

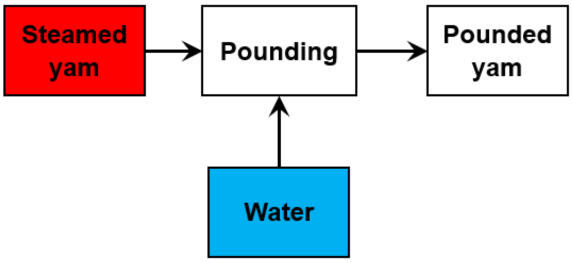


Figure 9 : **Mass balance diagram for calculation of water addition to pounded yam**

$$W = \frac{DM_{SY} \cdot M_{SY}}{DM_{PY}} - M_{SY} \quad (1)$$

where M_{SY} is mass of the steamed yam (g), DM_{SY} is the dry matter of the steamed yam (g of dry matter/100 g of wet matter) determined beforehand, M_{PY} is the expected or calculated mass of pounded yam (g), and DM_{PY} is the expected dry matter of pounded yam (g of dry matter/100 g of wet matter).

6.4.2 Calculation of water addition based on dry matter of fresh yams and water absorbed during steaming

In order to avoid time lapse between pre-determining dry matter of steamed yam in method 1 above, the dry matter of the steamed yam may be estimated from the dry matter of fresh yam and water absorbed during steaming or boiling, assuming no solids loss or gain. To do this the mass and dry matter of fresh yam and mass of steamed yam must be known.

When the dry matter of raw tuber (fresh yam) is lower than to 30%, no water is added to the pounded yam during pounding, but when the dry matter exceed 30%, the quantity of water to be added in order to obtain a final pounded yam with a specific dry matter content of 30% is calculated following Equation (2) :

$$W = \frac{DM_{FY} \cdot M_{FY}}{DM_{PY}} - M_{SY} \quad (2)$$

where DM_{SY} and DM_{PY} are the dry matter of fresh yam (g of dry matter/100 g of wet matter) determined beforehand and pounded yam (to be fixed at 30 g of dry matter/100 g of wet matter), respectively and M_{FY} and M_{SY} are the measured mass of fresh and steamed yam (g).

7 EXPRESSION OF RESULTS

The result of the test is obtained from the textural parameters as shown in Figure 10.

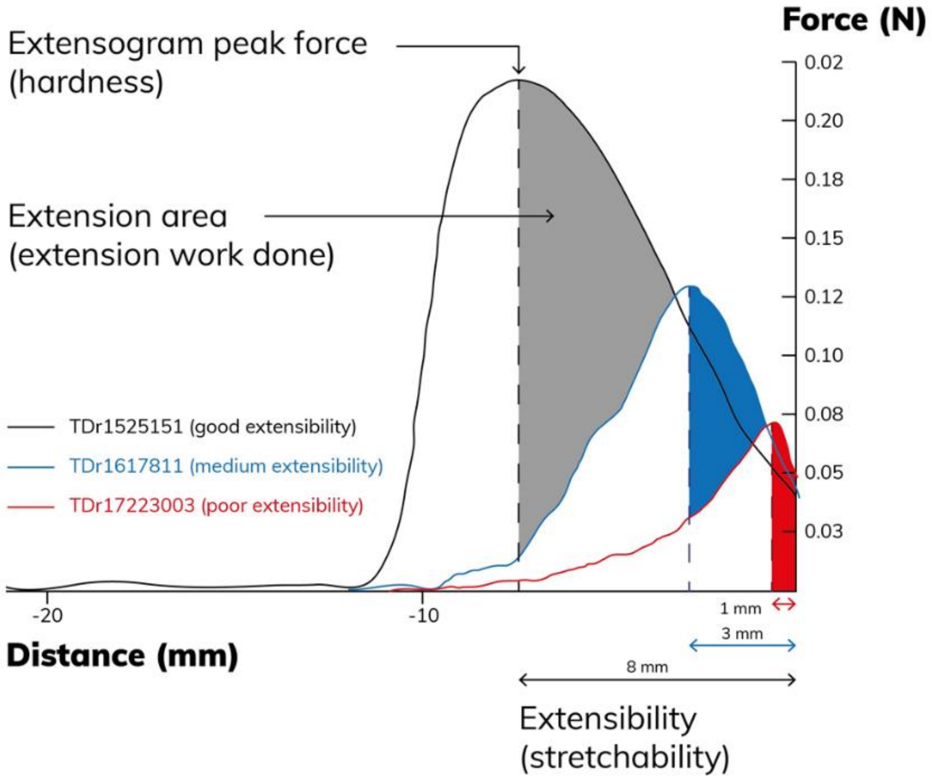


Figure 10: Example of extensogram showing the extensibility parameters of pounded yam

7.1 Statistics and number of replications and measurements

Theorical results of power test for ANOVA or Kruskal Wallis tests in the case of a strong effect size (Cohen's f = 0.4), significant level of 0.05 (α) and different number of groups (k from 5-20) can be observed in Figure 11. The minimum number of observations per group in order to achieve a power of 0.95 were 25, 15, 12 and 10 for 5, 10, 15 and 20 groups respectively. However, a power test analysis was performed on a preliminary dataset of 5 groups (Table 1) and it was observed that the effect size is much larger than 0.4 (1.24 in this case) which implied that for α= 0.05 and k = 5 a power value of 0.95 was achieved with only 5 observations.

Table 1 : Extension (mm) values for 5 different genotypes

TDa1510119	TDa1511008	TDa1520050	Oweigbo	Oju iyawo
10.15	2.36	2.15	0.57	1.22
17.45	5.78	0.79	0.80	2.86
6.67	4.12	0.37	1.13	1.44
6.33	2.64	0.96	1.61	0.93
4.19	2.16	0.49	1.00	0.84
7.03	2.20	0.63	1.27	1.19
5.64	2.47	0.68	0.36	1.14
5.87	7.78	0.27	0.44	0.88
5.36	2.40	0.60	0.72	0.44
11.71	2.20	1.09	0.84	1.18
4.27	2.47	0.67	0.96	0.36
4.97	2.45	1.41	0.64	1.21
10.78	1.16	0.96	0.63	0.64
3.57	0.28	1.64	0.45	0.65
3.08	0.71	1.00	0.69	0.92
6.26	0.73	0.45	0.43	0.72
6.15	0.57	1.48	0.66	0.80
6.79	0.49	1.01	1.12	1.24
4.30	0.58	1.16	0.68	0.64
1.98	2.07	1.15	1.00	0.50

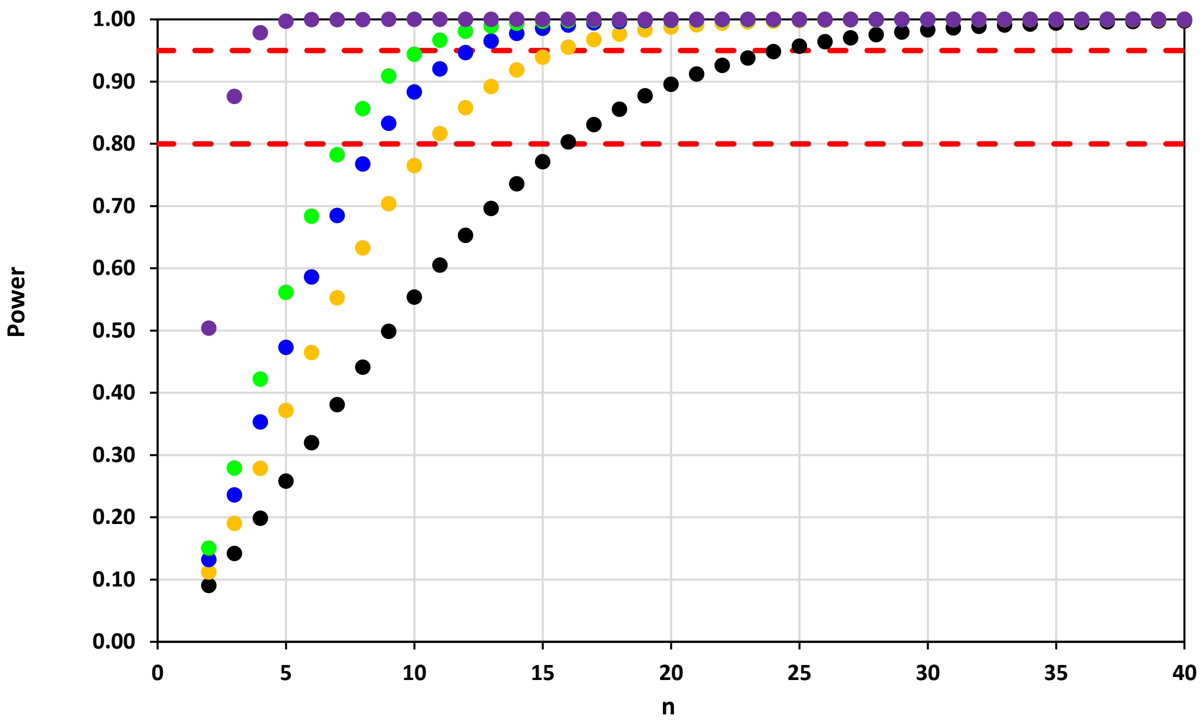


Figure 11. Power values as function of the number of observations for α = 0.95, f = 0.4 and k = 5 (●), 10 (●), 15 (●) and 20 (●). Violet pots (●) correspond to preliminary essay α = 0.95, f = 1.24 and k = 5.

Taking the aforementioned information into account, a minimum of two replicates should be considered per genotype, and not less than 5 measurements should be made per replicate. If ANOVA analysis is desired the total number of measurements should be 20 at least.

8 CRITICAL POINTS OR NOTE ON THE PROCEDURE

- It must be ensured that the texture analyser is switched on at least 15 minutes prior to measurements and the texture analyser must be calibrated for force and distance on each experiment day prior to measurements.
- It should be noted that when the dry matter of steamed yam does not exceed 30 %, water addition will not be necessary during pounding. This is usually the case for many *alata* genotypes.
- The pounded yam produced should be as smooth and homogeneous as possible. Sometimes, some yam genotypes are difficult to pound and may have lumps, therefore, it may be necessary to remove the lumps before pressing to form sample strands.
- Ensure to place the Kieffer mould in an incubator at 40°C prior to pressing the sample. The pounded yam dough should be at about 40°C when pressing in the Kieffer mould to form sample strands.
- The Kieffer mould must be well lubricated with paraffin oil before pressing to form pounded yam sample strands. After pressing to form sample strands, carefully slide the top mould to reveal the sample strands. Disregard the first 2-3 strands, which are not well formed, and ensure to pick only well-formed full strands that have no defects. Do not expose the remaining strands in the mould to avoid rapid temperature decline and retrogradation.
- The pounded yam sample strands must be carefully removed with a well lubricated spatula.
- The sample strand must be placed in the middle of the sample platform at equal distances.
- It should be noted that there are some limitations with this procedure. For example, addition of considerable amount of water calculated may result in some doughs of pounded yam (e.g. the rotundata check landrace, *Oju iyawo*) becoming more sticky and soft, which affects the ease of handling. However, the lubrication applied on the Kieffer mold ameliorates this effect. Also, water addition may influence the native stretchability of pounded yam compared to when water is not added. Nevertheless, water addition is understandably necessary to mimic traditional practices of pounded yam preparation and consumer appreciation.

APPENDICES

Annex 1: Example of extensibility data on pounded yam (IITA, Nigeria) by KDGE method

Genotypes

Oweigbo (landrace, alata, check)
TDa1510119 (hybrid, alata)
TDa1511008 (hybrid, alata)
TDa1520050 (hybrid, alata)
TDr1437005 (hybrid, rotundata)
TDr1439027 (hybrid, rotundata)
TDr1500042 (hybrid, rotundata)
TDr1500100 (hybrid, rotundata)
Oju iyawo (landrace, rotundata, check)

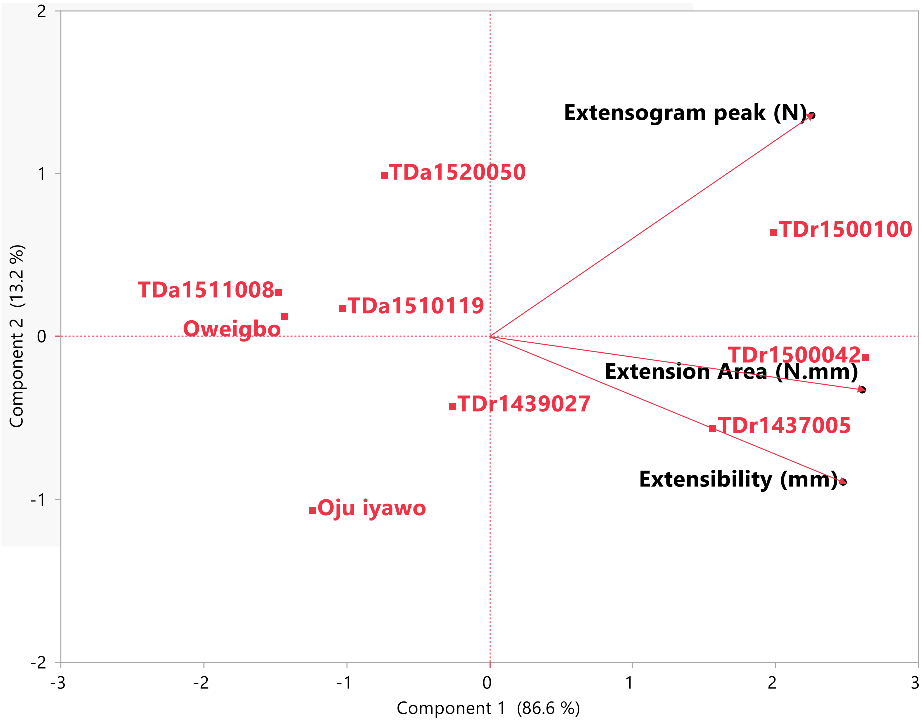
Parameter	Genotype	N	Mean	Std Dev	Std Err	CV (%)	CV, mean (%)
Extensogram peak (N)	Oju iyawo	17	0.062	0.019	0.005	30.1	7.3
	Oweigbo	21	0.085	0.017	0.004	20.4	4.4
	TDa1510119	20	0.092	0.028	0.006	30.2	6.7
	TDa1511008	21	0.087	0.029	0.006	33.5	7.3
	TDa1520050	21	0.114	0.021	0.005	18.7	4.1
	TDr1437005	21	0.113	0.023	0.005	20.0	4.4
	TDr1439027	20	0.090	0.027	0.006	30.4	6.8
	TDr1500042	21	0.137	0.021	0.005	15.5	3.4
	TDr1500100	21	0.144	0.046	0.010	31.8	6.9
Extensibility (mm)	Oju iyawo	17	2.306	1.082	0.262	46.9	11.4
	Oweigbo	21	1.229	0.360	0.079	29.3	6.4
	TDa1510119	19	1.586	0.513	0.118	32.3	7.4
	TDa1511008	19	1.076	0.189	0.043	17.6	4.0
	TDa1520050	21	1.278	0.286	0.062	22.4	4.9
	TDr1437005	21	4.311	1.390	0.303	32.3	7.0
	TDr1439027	18	2.770	0.797	0.188	28.8	6.8
	TDr1500042	21	4.792	0.779	0.170	16.3	3.5
	TDr1500100	21	3.640	1.190	0.260	32.7	7.1
Extension Area (N.mm)	Oju iyawo	17	0.134	0.086	0.021	64.7	15.7
	Oweigbo	21	0.080	0.020	0.004	24.8	5.4
	TDa1510119	19	0.110	0.027	0.006	24.3	5.6
	TDa1511008	19	0.074	0.023	0.005	30.5	7.0
	TDa1520050	21	0.102	0.019	0.004	18.2	4.0
	TDr1437005	21	0.369	0.167	0.036	45.2	9.9
	TDr1439027	18	0.187	0.034	0.008	18.3	4.3
	TDr1500042	21	0.465	0.109	0.024	23.5	5.1
	TDr1500100	21	0.387	0.214	0.047	55.2	12.1

Analysis of Variance

Parameter	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Extensogram peak	8	0.11040818	0.013801	18.7868	3.64e-20
Extensibility	8	326.88817	40.8610	58.4065	9.68e-45
Extension Area	8	3.7907955	0.473849	42.5416	1.07e-36

All the KDGE parameters are discriminant, especially the textural parameters extensibility and extension area. The extensibility parameter discriminates the *alata* genotypes uniquely in a cluster separate from the *rotundata* genotypes.

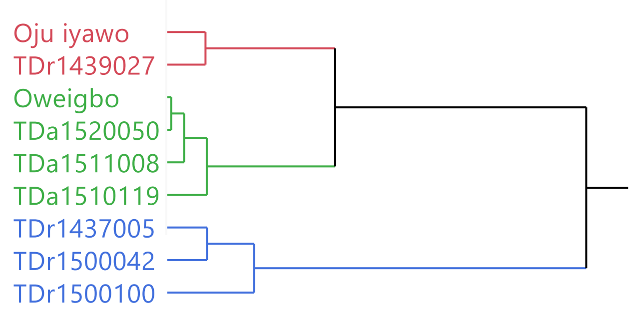
PCA



PCA components 1 and 2 account for 99.8% of data variation. The extensibility and extension area are correlated significantly, and associated particularly with genotypes TDr1500042 and TDr1437005, while TDr1500100 is associated with harder texture for pounded yam. *Alata* genotypes are clustered together away from the *rotundata* genotypes.

Hierarchical clustering

The 3-class hierarchical clustering of the genotypes reveal that the *alata* genotypes are clustered in one group separately from the *rotundata* genotypes.



3 class hierarchical clustering of genotypes based on pounded yam extensibility parameter

Correlations between Extensibility parameters and DM of fresh, steamed and pounded yam

	Avg DM fresh yam (%)	Avg DM steamed yam (%)	Avg Actual DM of PY (%)
Extensogram peak (N)	-0.2023	-0.3228	0.7098
Extensibility (mm)	0.4458	0.3267	0.8175
Extension Area (N.mm)	0.2597	0.1465	0.8592
	Avg DM fresh yam (%)	**Avg DM steamed yam (%)**	**Avg Actual DM of PY (%)**
Extensogram peak (N)	0.6017	0.3969	0.0322
Extensibility (mm)	0.2291	0.3908	0.0071
Extension Area (N.mm)	0.4998	0.7068	0.0030

There is significant relationship between dry matter of pounded yam and the extensibility texture parameters of pounded yam.

The actual dry matter of pounded yam produced in this study was between 25-34 % after adjustment of dry matter by addition of water.