Laboratory Standard Operating Procedure

SOP for Determination of Bi-extensional Viscosity of Pounded Yam by Lubricated Squeezing Flow (LSF) Method

Biophysical Characterization of Quality Traits, WP2

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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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SOP: Determination of Bi-extensional Viscosity of Pounded Yam by Lubricated Squeezing Flow (LSF) Method

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ABSTRACT

This SOP describes the method for determining the bi-extensional viscosity (BEV) by application of lubricated squeezing flow (LSF) on pounded yam produced from different yam varieties using a texture analyser under fixed conditions. A full description of the sample preparation (pounded yam), conducting the experimental measurements, calculation using the obtained raw data and results expression are proposed. This SOP could be adapted for other pasty products such as Eba, Fufu or Matooke in order to obtain the corresponding bi-extensional viscosity that may be related to other sensory quality traits.

Key Words: bi-extensional viscosity, rheology, texture, compression, Hencky strain, strain rate.

1 Scope and Application

This SOP describes the method for determining the bi-extensional viscosity (BEV) by application of lubricated squeezing flow (LSF) of pounded yam produced from varieties of yams using a texture analyser under fixed conditions.

2 References

Otegbayo, B., Oroniran, O., Tanimola, A., Bolaji, O., Alamu, A., Mestres, C & Ayetigbo, O. (2022). Standard Operating Protocol for Textural Characterization of Pounded Yam. Biophysical Characterisation of Quality Traits, WP2. Iwo, Nigeria: RTBfoods Laboratory Standard Operating Procedure, 22 p. https://doi.org/10.18167/agritrop/00613

Ayetigbo, O., Domingo, R., Arufe, S., Mestres, C., & Akissoé, N., Otegbayo, B. (2022). Standard Operating Protocol for the Instrumental Determination of Extensibility of Pounded yam. Biophysical Characterization of Quality Traits, WP2. Montpellier, France: RTBfoods Laboratory Standard Operating Procedure, 18 p. https://doi.org/10.18167/agritrop/00684

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Van Vliet, T. (2008). Strain hardening as an indicator of bread-making performance: A review with discussion. Journal of Cereal Science 48, 1-9. DOI: http://dx.doi.org/10.1016/j.jcs.2007.08.010.

Launay G., Michon C. (2008) Biaxial extension of wheat flour dough: lubricated squeezing flow and stress relaxation properties. J Texture Stud 39:496–529. DOI: https://doi.org/10.1111/j.1745-4603.2008.00156.x

3 Principle

The standard operating protocol (SOP) for determination of bi-extensional viscosity of pounded yam using lubricated squeezing flow measures the normal force applied to make the sample flow, the time and the distance of the texture analyse probe in order to calculate the Hencky strain and the strain rate during compression of pounded yam between two plates.

Squeezing flow between parallel plates can be achieved in many food rheology laboratories. When this deformation is executed between lubricated plates, biaxial extensional flow is achieved and bi-extensional viscosity can be calculated (Steffe,1996). In practice, three squeezing geometries may be used: (a) the upper and lower plates have the same diameter as the un-squeezed sample, (b) the upper compression plate and the sample have the same diameter, but the lower plate is much larger and (c) both the upper and lower compression plates are much larger than the sample. In the case of this SOP (a) geometry, that lead to a constant surface of contact between the sample and the plates was selected, Figure 1.

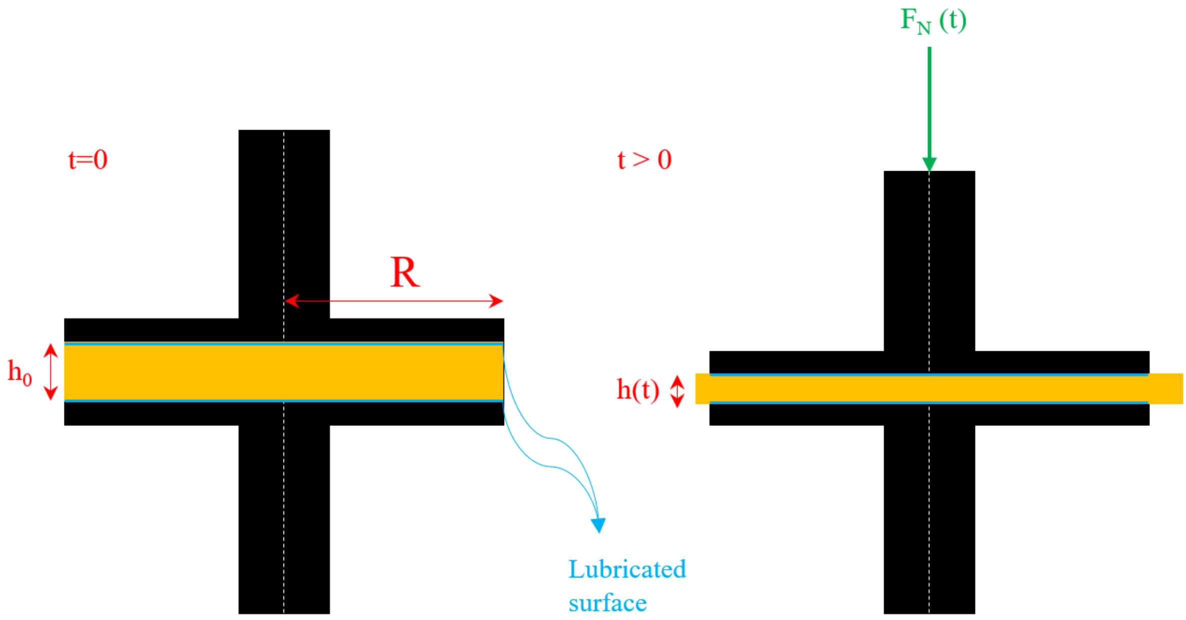

Figure 1. Lubricated squeezing flow with sample completely filling the gap between two parallel plates. R: radius of the plate, F_N: Normal force applied to the sample.

4 REAGENTS

Paraffin oil (CAS Number 8012-95-1)

5 APPARATUS

- Texture analyser (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with Exponent Software Interface.
- P/20P standard cylinder probe (20mm of diameter, Perspex,10kg compression / 5kg tension)
- Yam Pounding machine (QZP/6000 model, Cheerfengly Ind. Co. Ltd, Taipei, Taiwan)
- Infra-red thermometer
- Chronometer
- Bowls
- Plastic moulds (14 mm height, 20 mm diameter, Teflon)
- Incubator (WTC binder, Tuttlingen, Germany)
- Sharp stainless-steel knives
- Cutting boards
- Mass balance ± 0.01 g (Precisa, Precisa Gravimetrics AG, Switzerland)
- Plastic spoon
- Plastic film roll

6 PROCEDURE

6.1 Calibration of texture analyser

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 weight. The test probe used is shown in Figure 2:

Figure 2. P/20P standard cylinder probe of 20mm diameter, Perspex (Stable microsystems).

6.2 Test conditions for measurement of BEV

Preliminary essays based on bibliography (Launay and Michon 2008, Van Vliet 2008) were carried out in order to find the best conditions for test measurement (please, see Annex 1 on the Appendix). After the evaluation of the preliminary data the selected test conditions were as follows:

- Test mode: compression
- Test probe: P/20P
- Load module: 5 kg
- Pre-test speed: 60 mm/min
- Test speed: 120 mm/min
- Post-test speed: 600 mm/min
- Trigger force: 0.25 N (25g)
- Target mode: distance
- Distance: 13 mm
- Sample dimensions: 20 mm of diameter and 14 mm of height

6.3 Preparation of pounded yam dough and measurement

The pounded yam dough is prepared following the RTBfoods SOP for pounded yam sample preparation recommended for texture analysis (Otegbayo et al., 2022) with some modifications as described below.

- Step 1: Two yam tubers are cut longitudinally into two halves and the proximal and distal sections are cut off from both ends (Figure 3).

Figure 3. Longitudinal halves of yam tubers after cutting proximal and distal sections.

- Step 2: From the two central sections, cubes of about 2 cm dimension are cut from both representative central sections (Figure 4).

Figure 4. Obtained yam cubes (≈ 2 cm side) from central section of the tuber after peeling and cutting.

- Step 3: 450 g of the yam cubes is weighed and placed in the cooking chamber of the yam pounding machine (Figure 5) above its receptacle containing 250 mL of water. The yam cubes are cooked for about 21 - 24 minutes (alarm sounds when cooking is finished), and pounded for 1.5 minutes in the pounding machine.

Figure 5. Yam Pounding machine (QZP/6000 model, Cheerfengly Ind. Co. Ltd, Taipei, Taiwan)

- Step 4: The yam dough is spooned out, 12 pre-lubricated moulds (20 mm of internal diameter, 14 mm of height) are filled; dough is cut out to fit their shape. The mould filled with dough sample is wrapped with plastic film (Figure 6).

Figure 6. Prepared samples.

- Step 5: These are then placed in an incubator at 55°C for 10 - 15 minutes to allow for stress relaxation and temperature equilibration.
- Step 6: The samples are demoulded, covered with paraffin oil and the compression test is applied with the aforementioned probe also covered with paraffin oil, for all replicates at constant temperature ≈30°C (Figure 7).

Figure 7. Moulded samples lubricated with paraffin oil before and during measurement.

7 EXPRESSION OF RESULTS

7.1 Obtained raw data

After experimentation, raw data of normal force, F_N defined as the force applied perpendicularly to the sample, time (min) and distance (mm) are obtained during compression (total time is fixed to achieve compression until 1 mm).

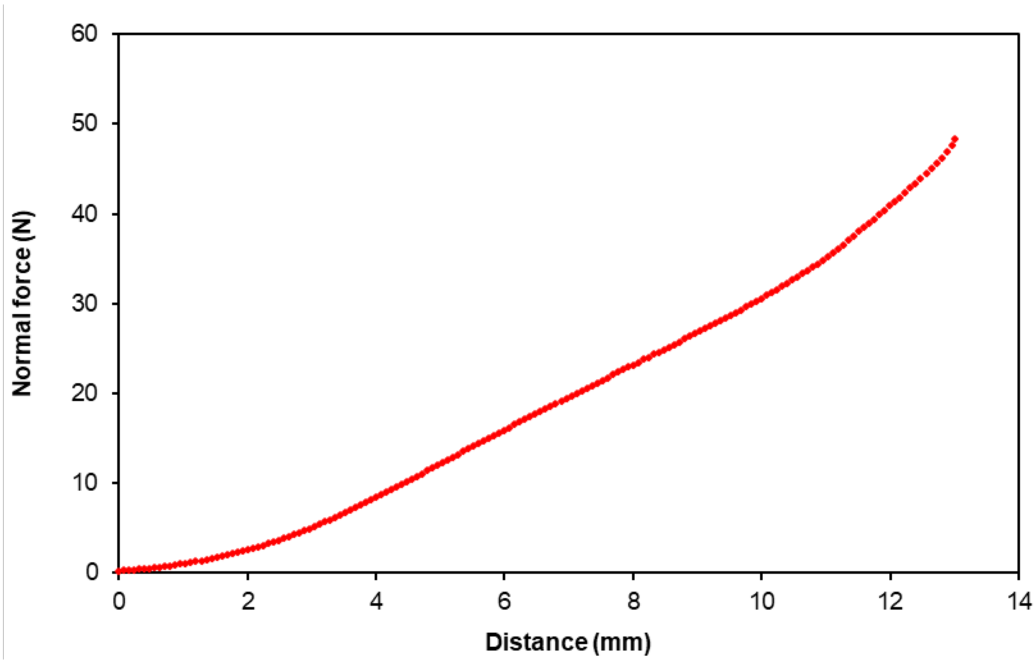

Figure 8. Obtained raw data after measurement. Normal force (N) *vs* Distance (mm).

Once raw data are obtained, extensional viscosity can be calculated as described in Section 7.2.

7.2 Method of calculation

7.2.1 Calculation

Considering the configuration of the experiment, Figure 1, the **logarithmic strain** ε, also called *Hencky strain* of the sample is given by Eq. (1):

$$\varepsilon_H = -ln\frac{h(t)}{h_0} \tag{1}$$

where h_0 is the initial sample height (14 mm) and h(t) the height at time t. The biaxial (Hencky) strain $\square_b$ of sample can be then calculated, Eq. (2):

$$\varepsilon_b = -\frac{1}{2}ln\frac{h(t)}{h_0} \tag{2}$$

Once the biaxial (Hencky) strain $\square_b$ is calculated, it can be related to the time in order to calculate the strain rate, $\dot{\varepsilon_b}$ (s^{-1}), Eq. (3).

$$\dot{\varepsilon_b} = \frac{d\varepsilon_b}{dt} \tag{3}$$

The stress applied by the plates to compress the sample, □ (Pa) is calculated as the ratio between the normal force applied and the surface area of the plate:

$$\sigma = \frac{F_N}{S} \qquad (4)$$

where F_N (N) corresponds to the normal force applied for dough deformation recorder by the software and S (m^2) is the surface of contact between the plates and the sample which is constant through the whole experiment (S=□·R^2=□□·$(10\cdot10^{-3})^2$= 3.14·10^{-4} m^2). Finally, $\dot{\varepsilon_b}$ can be related to □ in order to obtain the bi-extensional viscosity, □$_e$ (Pa·s), Eq. (5).

$$\eta_e = \frac{\sigma}{\dot{\varepsilon_b}} \qquad (5)$$

Once raw data are obtained, calculations are performed to obtain bi-extensional viscosity and can be facilitated in an Excel sheet (Microsoft Office Package) as follows:

Force [N]	Distance [mm]	Time [min]	h (t) [mm]	ε_H [-]	ε_B [-]	$\dot{\varepsilon_b}$ [s^{-1}]	□ [N·m^{-2}]
				Eq. (1)	Eq. (2)	Eq. (3)	Eq. (4)

where blue cells correspond to raw data and grey cells to calculated data. Finally, a common □$_b$ must be selected for all the studied samples. Once □$_b$ value has been selected (*i.e.* □$_b$ = 1), bi-extensional viscosity can be calculated using Eq. (5) and the corresponding values of □ and $\dot{\varepsilon_b}$ associated to □$_b$.

7.2.2 Expression of the results

Results should be expressed using the International System of Units. The values of bi-extensional viscosity should be then expressed in Pa·s (or KPa·s). Moreover, the selected value of □$_b$ (-) and the correspondent $\dot{\varepsilon_b}$ must be clearly indicated (please, see Section 9).

7.3 Statistics

7.3.1 Analysis of Variance (ANOVA)

Experimental data were statistically analysed. The significant differences between means were identified by one-factor analysis of variance (ANOVA), followed by the Tukey test and considering significant P-values ≤ 0.05 using RStudio package (please, See Section 9).

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.
- The dough must be relaxed for a period of 10-15 minutes at 50-55°C.
- Paraffin oil must be applied to each sample and to the probes between each measurement.
- Temperature of the dough must be monitored and measurement must be done at ≈30°C. Large variations in temperature may result in inaccurate results as the dough retrograde rapidly.

9 TEST REPORT

Table 1. Bi-extensional viscosity (KPa·s) of pounded yam cooking replicates obtained from different varieties at biaxial Hencky strain of 1, strain rate of 0.523 s^{-1}. (N: number of replicates, SD: standard deviation, SEM: standard error of the mean, CV: coefficient of variation of the mean).

Parameter	Cultivar	Cooking replicate	N	Mean	SD	SEM	CV
Bi-extensional viscosity (Pa.s)	Aga	1	2	106.6	52.5	37.1	49
		2	2	74.1	0.9	6.1	1
	Crachi	1	4	185.7	4.6	2.3	2
		2	4	166.7	10.0	4.9	6
	Dodo	1	8	50.7	6.9	2.4	14
		2	8	55.1	5.5	1.9	10
	Irindou	1	4	142.5	31.0	15.5	22
		2	4	116.9	52.7	26.4	45
	Laboko	1	4	286.0	17.2	8.6	6
		2	4	220.6	18.8	9.4	9
	Wete	1	4	139.9	13.8	6.9	10
		2	4	103.4	13.0	6.5	12

9.1 ANOVA and repeatability of instrumental textural attribute

9.1.1 Bi-extensional viscosity (Pa·s) by Cultivar

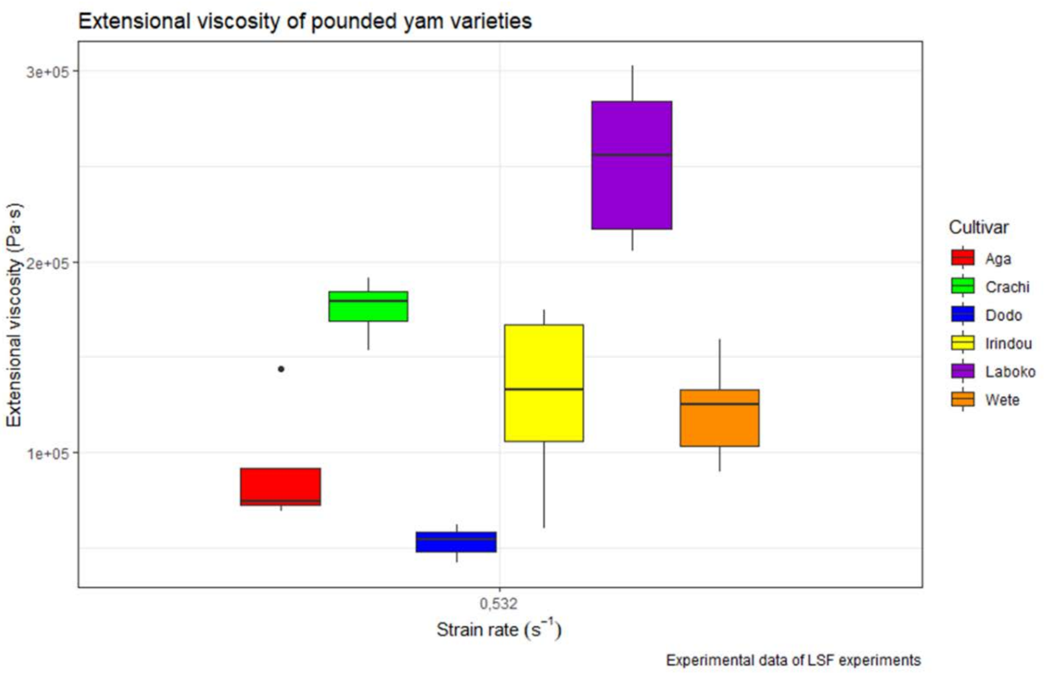

Figure 9. BEV of pounded yam varieties ($\dot{\varepsilon}_b$= 0.532 s^{-1}, ε_B = 1).

Table 2. One-way Analysis of Variance by cultivar.

	DF	Sum of Square	Mean Square	F value	Pr (>F)
Cultivar	5	$2.4 \cdot 10^{11}$	$4.8 \cdot 10^{10}$	68.54	$2.2 \cdot 10^{-16}$
Residuals	46	$3.2 \cdot 10^{10}$	$7.0 \cdot 10^{8}$		
C. Total	51	$2.7 \cdot 10^{11}$			

Table 3. Connecting Letters Report

	Aga	Crachi	Dodo	Irindou	Laboko	Wete
Level	AB	C	A	B	D	B

Table 4. Effect tests

	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cultivar	5	$2.4 \cdot 10^{11}$	$4.8 \cdot 10^{10}$	107.4	$<2 \cdot 10^{-16}$
Cooking replicate	1	$7.3 \cdot 10^{9}$	$7.3 \cdot 10^{9}$	16.3	$2.4 \cdot 10^{-4}$
Cultivar*Cooking replicate	5	$7.1 \cdot 10^{9}$	$1.4 \cdot 10^{9}$	3.2	$1.7 \cdot 10^{-2}$
Residuals	40	$1.8 \cdot 10^{10}$	$4.5 \cdot 10^{8}$		

9.1.2 Bi-extensional viscosity (Pa·s) by Cooking replicate

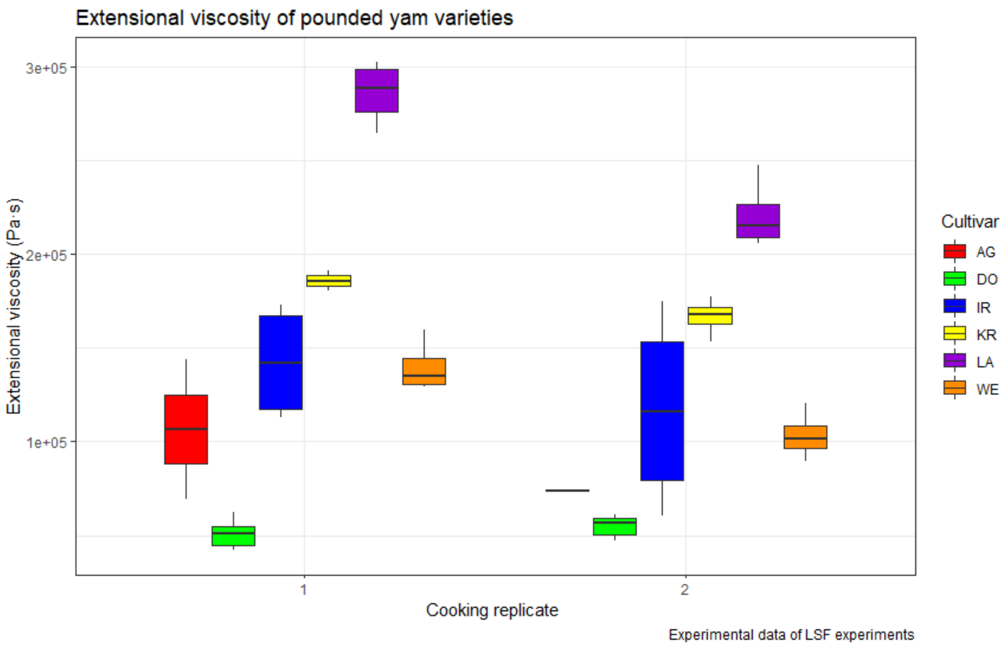

Figure 10. BEV of pounded yam varieties ($\dot{\varepsilon_b}$= 0.532 s^{-1}, ε_B = 1) by cooking replicate.

Table 5. One-way Analysis of Variance by cooking replicate.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Cooking replicate	1	$7.3 \cdot 10^{9}$	$7.3 \cdot 10^{9}$	1.373	0.247
Residuals	50	$2.7 \cdot 10^{11}$	$5.3 \cdot 10^{9}$		
C. Total	51	$2.7 \cdot 10^{11}$			

Table 6. Connecting Letters Report

Cooking replicate	1	2
Level	A	A

The studied cultivars were significantly different in bi-extensional viscosity between one another, as shown in Table 3. The two-way ANOVA explained that both the effects of cultivar and cooking replicate on bi-extensional viscosity are significant, Table 4, as well as the significant effect of the interaction between cultivar and cooking replicate. The cultivar effect was the most significant on bi-extensional viscosity and the interaction effect is the least. The measuring bi-extensional viscosity of pounded yam by the proposed protocol may be considered as a repeatable, accurate and discriminatory protocol. Moreover, it was found a significant ($p \leq 0.10$) relationship between overall liking of the studied genotypes and BEV, Pearson r=0.730 (data not shown). This fact indicates that LSF method results are related to overall liking which makes this method a potential useful tool for screening of pounded yam.

10 APPENDICES

10.1 Annex 1

Preliminary essays with different compression speeds were carried out in order to determine the most discriminant compression speed in the case of pounded yam.

Four different genotypes, Aga (*D. Alata*), and Irindou, Laboko and Wete (*D. Rotundata*), were tested at 6, 12 and 120 mm/min compression speeds (corresponding strain rates of 0.026, 0.053, 0.532 s^{-1}, respectively) in order to obtain the corresponding bi-extensional viscosity, Figure 11.

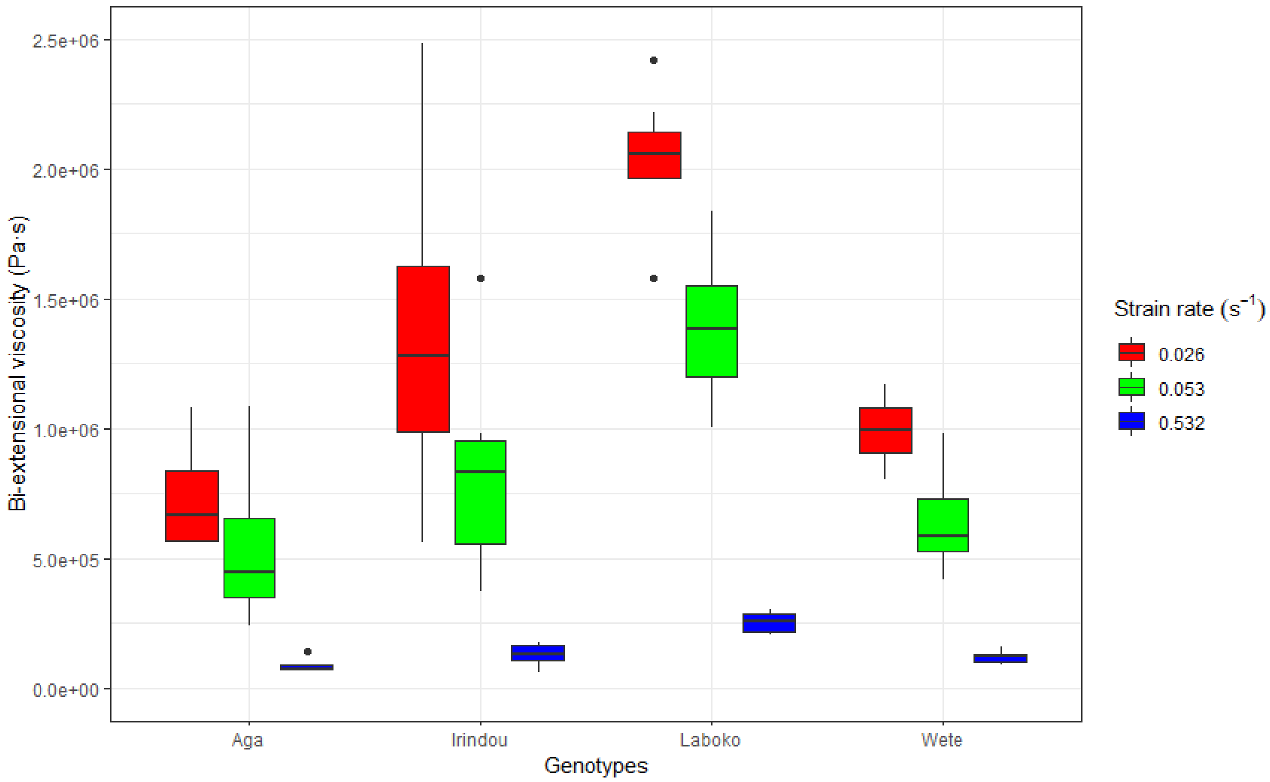

Figure 11. Bi-extensional viscosity of pounded yam obtained from different genotypes at 0.026, 0.053 and 0.532 s^{-1} strain rates.

Once bi-extensional viscosity of different genotypes at different strain rates was obtained, one-way ANOVA analysis was carried out in order to determine the best compression speed. Results, Table 7, shown that every tested compression speed lead to significant differences on the variance between the means of the populations, being the highest compression speed (associated to a strain rate of 0.532 s^{-1}) the one with the highest probability. Regarding the connecting letters reports after the application of Tukey test (Table 8), no difference of classification of groups for the studied population. Taking this into account, and in addition with the fact that the highest speed compression lead to the data obtaining in a shorter period of time, 120 mm/min was the compression speed selected for determination of bi-extensional viscosity of pounded yam.

Table 7. One-way Analysis of Variance by cultivar at a strain rate of 0.026 s^{-1}.

Strain rate (s^{-1})		DF	Sum of Square	Mean Square	F value	Pr (>F)
0.026	Cultivar	3	$6.4 \cdot 10^{12}$	$2.1 \cdot 10^{12}$	13.04	$2.96 \cdot 10^{-5}$
	Residuals	24	$3.9 \cdot 10^{12}$	$1.6 \cdot 10^{11}$		
0.053	Cultivar	3	$3.0 \cdot 10^{12}$	$9.9 \cdot 10^{11}$	10.75	$1.11 \cdot 10^{-4}$
	Residuals	24	$2.2 \cdot 10^{12}$	$9.3 \cdot 10^{10}$		
0.532	Cultivar	3	$1.1 \cdot 10^{11}$	$3.6 \cdot 10^{10}$	28.25	$4.81 \cdot 10^{-8}$
	Residuals	24	$3.1 \cdot 10^{10}$	$1.3 \cdot 10^{9}$		

Table 8. Connecting Letters Report as function of the used strain rate.

	Strain rate (s^{-1})	Aga	Irindou	Laboko	Wete
Level	0.026	A	A	B	A
	0.053	A	A	B	A
	0.532	A	A	B	A

Results shown that the use a strain rate of 0.532 s^{-1} (corresponding compression speed of 120mm/min) lead to the most discriminant results in terms of bi-extensional viscosity of the samples.

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