Laboratory Standard Operating Procedure

SOP for Protocol for Characterization of Cooking Time and Texture of Boiled Cassava: Texture-extrusion

Cali, Colombia, December 2019

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Ethics: The activities, which led to the production of this manual, 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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RTBfoods

WP2: Biophysical characterization of quality traits

SOP: Protocol for characterization of cooking time and texture of boiled cassava: Texture-extrusion

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1 SCOPE AND APPLICATION

This SOP describes the preparation of cassava root samples for boiling tests to measure optimum cooking time and texture of boiled cassava. The shape and size of the root pieces for boiling are selected to (1) be representative of the pieces of cassava roots typically used for boiling in Colombia; (2) be suitable for the texture-extrusion protocol developed at CIAT for RTBfoods in 2019 (Ottawa cell with 5-blade extrusion grid).

The SOP includes the handling of cassava roots after reception from the field on the day of harvest, preparation of samples for boiling and characterizations (cooking time, texture, closing angle), and allocation of identification codes for each sample. Further characterizations such as water absorption, conductivity, and NIRS of fresh roots and boiled roots are described in separate SOPs.

2 PRINCIPLES AND DEFINITIONS

When roots are delivered from the field by the breeders (WP4) for cooking tests and HTPP analyses (such as NIRS), they are transformed typically as follows: Washing and peeling; cutting into pieces of appropriate size for boiling; rasping the remaining parts of the root for dry matter and NIRS, and preservation by drying (45°C or lyophilisation) for further analyses (e.g. extraction and characterization of cell wall materials, CWM).

Optimum cooking time (OCT): The time the pieces of cassava roots need to stay immersed in boiling water for their texture to become soft and acceptable for consumption. For pieces of the same standard size, OCT depends on the genotype as well as environmental factors during the growth of cassava roots. As of writing the present SOP, OCT is determined by fork test, whereby a trained assessor probes the pieces during boiling and determines the time when the texture reaches optimum softness.

Pieces of cassava roots cut for the boiling experiments are placed in boiling water and subjected to two treatments: One set is boiled for 20 minutes (T20), the other set is boiled until optimum cooking time (OCT), as determined by probing the pieces with a fork. The 20 minutes boiling allows a comparison of texture across genotypes with discriminative power, as the roots are not fully cooked and present a wide range of textures depending on the genotype. Together, the two cooking treatments allow an assessment of changes in texture during cooking. The resulting pieces of boiled cassava roots are characterized by texture analysis.

Pieces of boiled roots are left to cool exactly 10 minutes after removal from boiling water, placed on the Ottawa texture-extrusion grid (figure 15), and pushed through the grid with a piston moving at constant speed (1 mm/s). The force necessary to maintain the speed at 1 mm/s is recorded as a function of the distance travelled. The resulting texture profile of the sample is used to analyse various parameters to characterize the texture of the sample, such as gradient at origin, maximum force, force at end of compression, area under the curve, linear length of the curve, etc.

Remark: This SOP focuses on boiling and texture analysis, however several biophysical characterizations described in other SOPs (boiling, dry matter, HCN, NIRS, CWM) form a package to be conducted on the same root in order to develop HTPP predictions from NIRS data. The high variability that is sometimes observed between roots of the same cassava genotype (and of the

same plant in some cases) makes necessary conducting all the biophysical characterizations and the HTPP analyses (NIRS) on the same root (or set of roots)**.**

3 PREREQUISITE

Using and managing a texture analyzer.

4 APPARATUS

a. Texture analyzer with load cell able to measure forces at least 50 kg (the model used for the development of this SOP is a TA-XTPlus by Stable Microsystem) equipped with an Ottawa texture-extrusion cell (A/OTC).

b. Gas cooker with several fires to boil several samples in parallel. Ensure the room/laboratory has good ventilation and the gas inlet valve is closed when not in use.

c. Large cooking pots (capacity at least 5 litres of water): (1) to enable boiling several root pieces in the same pot, ensuring boiling at the same temperature; (2) to minimize temperature variations upon introduction of the root pieces in the water.

d. Temperature probe or thermometer to check the temperature of boiling water.

e. Heat-resistant plastic nets (nylon, e.g. of the type used to hold fruits or vegetables) and labels to hold and identify the samples individually during boiling.

f. Fork to assess optimum cooking time.

g. Balance to weigh the pieces of cassava roots.

h. Chronometer.

5 PRODUCT PREPARATION

5.1 Sampling and preparation of cassava roots

1. **Select three roots per genotype as follows:**

Minimum length 25 cm (fig. 1) and minimum diameter 5.5 cm (fig. 2). These dimensions are important to be able to cut pieces of suitable size for boiling (fig. 3), and to prepare samples for NIRS analysis from the same root.

Remark: Three roots is the minimum to capture the variability of cooking behaviour within the same genotypes. If more roots of suitable size are available, it is recommended to use them and increase the number of samples for characterization of optimum cooking time and texture.

Figure 1. Minimum length of cassava root: 25 cm

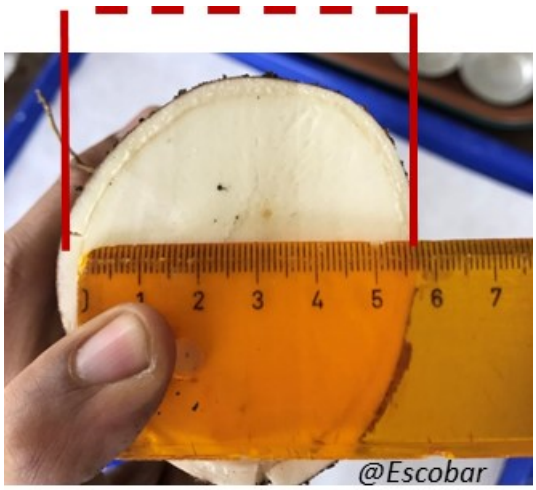

Figure 2: Minimum diameter 5.5 cm

Figure 3: Minimum radius 2.5 cm for texture test

2. After washing and peeling the roots, divide each root as follows:

Cut six pieces per root for boiling, in the shape of half cylinders (fig. 4): Three pieces for boiling 20 minutes (P01, P04, P05) and three pieces for optimal cooking time (P02, P03, P06). Each piece must be 6 cm long (fig. 5). Each piece is identified with a unique code attributed according to the guidelines of the SOP *Sampling and allocation of standardized codes* (Table 1): The three roots receive respectively codes P01 to P06, P11 to P16, and P21 to P26 (18 pieces per genotype in total).

Measure the diameter of the pieces and record it in the template for recording boiling experiments data (table 5 in Annex) for later (optional) normalization of the texture data (table 3).

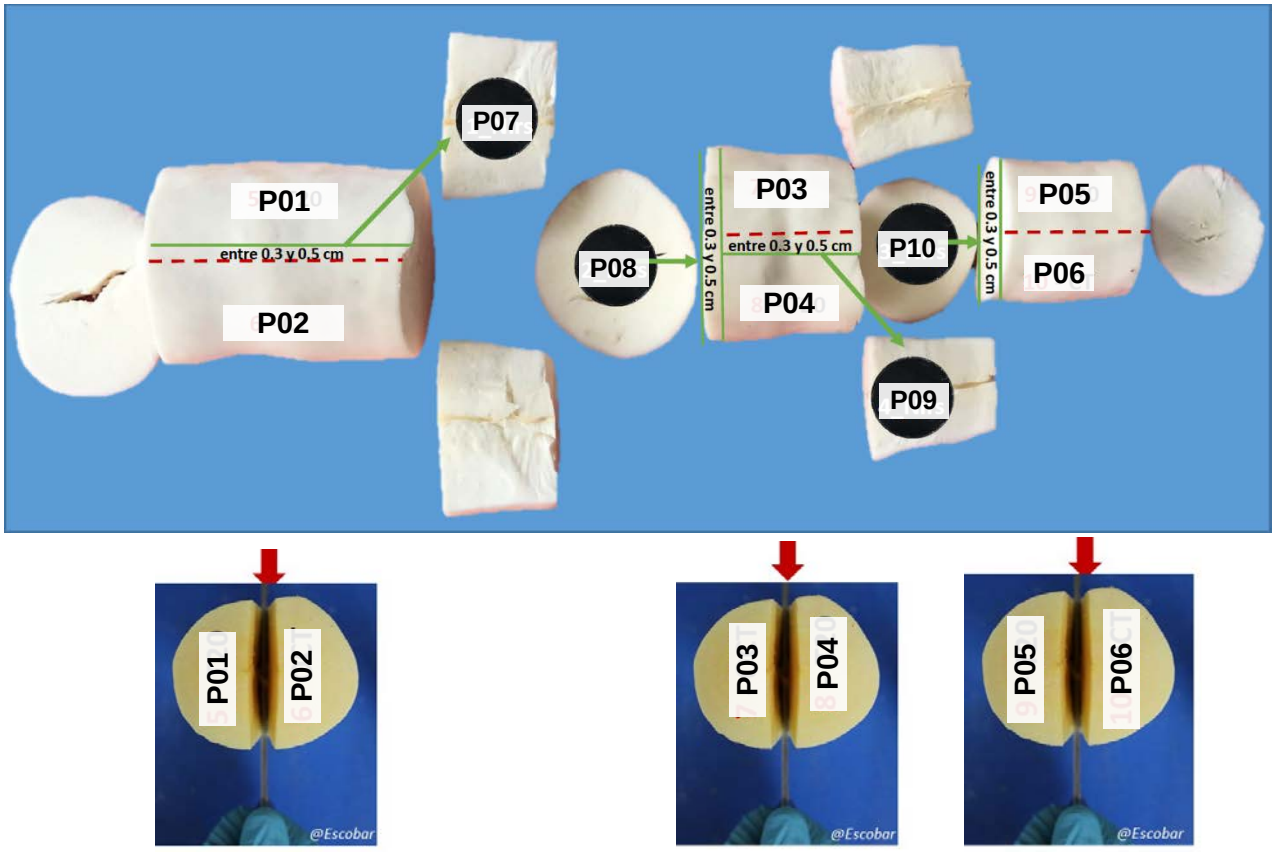

Figure 4: Cassava root cutting to prepare samples for texture and optimum cooking time (boiling). Pieces are numbered in advance from P01 to P06 for root 1, P11 to P16 for root 2 and P21 to P26 for root 3, according to the guidelines of the SOP *Sampling and allocation of standardized codes*.

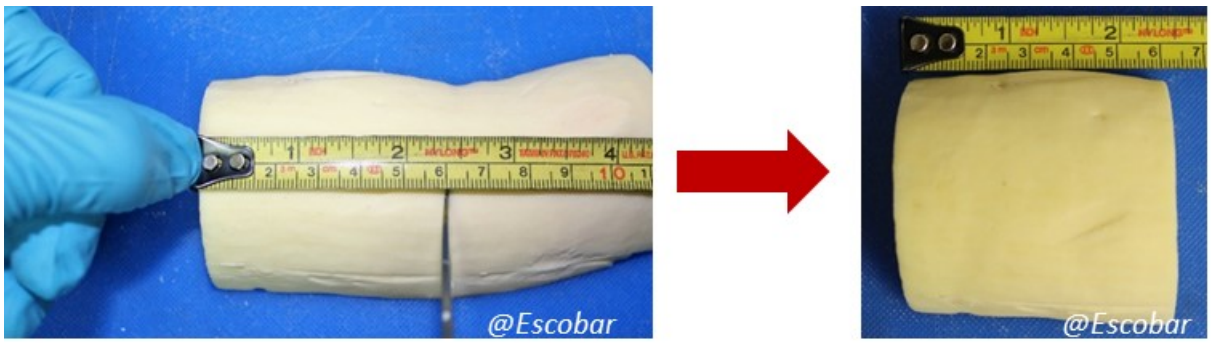

Figure 5: Length of the half cylinder for boiling: 6 cm

Table 1: Summary of the pieces to cut from three cassava roots for texture, optimum cooking time (boiling).

19G01P01	Proximal piece of Root 1 for boiling 20 minutes
19G01P02	Proximal piece of Root 1 for boiling to optimum cooking time
19G01P03	Central piece of Root 1 for boiling to optimum cooking time
19G01P04	Central piece of Root 1 for boiling 20 minutes
19G01P05	Distal piece of Root 1 for boiling 20 minutes
19G01P06	Distal piece of Root 1 for boiling to optimum cooking time
19G01P11	Proximal piece of Root 2 for boiling 20 minutes
19G01P12	Proximal piece of Root 2 for boiling to optimum cooking time
19G01P13	Central piece of Root 2 for boiling to optimum cooking time
19G01P14	Central piece of Root 2 for boiling 20 minutes
19G01P15	Distal piece of Root 2 for boiling 20 minutes
19G01P16	Distal piece of Root 2 for boiling to optimum cooking time
19G01P21	Proximal piece of Root 3 for boiling 20 minutes
19G01P22	Proximal piece of Root 3 for boiling to optimum cooking time
19G01P23	Central piece of Root 3 for boiling to optimum cooking time
19G01P24	Central piece of Root 3 for boiling 20 minutes
19G01P25	Distal piece of Root 3 for boiling 20 minutes
19G01P26	Distal piece of Root 3 for boiling to optimum cooking time

5.2 Cassava boiling 20 minutes for texture analysis (T20)

Pack and identify each piece of root separately in heat-resistant plastic nets (fig. 6). In a pot with boiling water, cook the pieces for 20 minutes exactly (fig. 7) of the different parts of each root: proximal (P01, P11, P21), central (P04, P14, P24) and distal (P05, P15, P25) (fig. 4). At 20 minutes of boiling, remove the pieces and allow them to cool to room temperature (fig. 8) for exactly 10 minutes before performing the texture-extrusion test. During cooling, place the pieces in a closed container to limit the loss of moisture by evaporation (fig. 9).

Figure 6: Packing

Figure 7: Cooking (boiling)

Figure 8: Cooling at room temperature

Figure 9: Cooling down of boiled cassava pieces for 10 minutes in a closed container to minimize moisture losses, before texture analysis

Pack and identify each piece of root separately in heat-resistant plastic nets. In a pot with boiling water, cook the root pieces until optimal cooking time (OCT), detected with the help of a fork (fig. 10). Record OCT (in mins). Remove the pieces and let them cool to room temperature (fig. 8) for exactly 10 minutes before performing the texture analysis. During cooling, place the pieces in a closed container to limit the loss of moisture by evaporation (fig. 9).

Figure 10: Evaluation of optimum cooking time by fork test

During the 10 minutes cooling period, take photographs of the pieces from the side and from above, for further analysis of the closing angle, i.e. the angle the half-cylinder closes during boiling (fig. 11).

To identify the samples, include in the photographs labels with the full sample code (prepare the labels in advance before the experiments).

Figure 11: Photographs of root pieces at optimum cooking time (OCT)

In the template for recording data of boiling experiments, record the optimum cooking time (OCT), closing angle, length of the whole root, diameter of each half-cylinder piece (table 5 in Annex).

Dimensions of the cassava root. The length of the whole root and diameter of half-cylinder pieces cut from it are reported in the template for recording data of cassava boiling experiment (table 5 in Annex). Unit to use: cm.

Optimum cooking time (OCT). The optimum cooking time is evaluated by a trained assessor using a fork, and is reported in the template for recording data of cassava boiling experiment (table 5 in Annex). Unit to use: minutes.

Closing angle of the half-cylinder after boiling to optimum cooking time (OCT) The angle of closing of the half-cylinder before boiling is defined as 0° (fig. 12). The closing angle □ after OCT boiling is measured using a protractor (fig. 13), and reported in the template for recording data of cassava boiling experiment (table 5 in Annex). Unit to use: degrees.

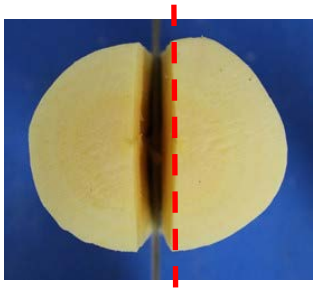

Figure 12: Closing angle before boiling is defined as 0°

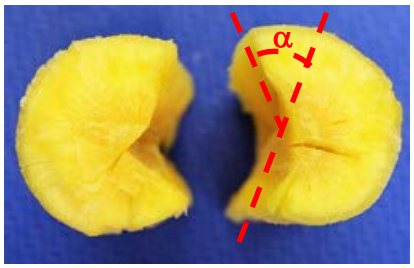

Figure 13: Closing angle □ after OCT boiling is measured as indicated, using a protractor

For the fork tests, the assessor needs to be trained to feel the changes in texture at various times during boiling (e.g. every five minutes: at 5, 10, 15, 20, etc. minutes), so as to detect accurately and in a repeatable way the optimum cooking time (OCT), i.e. the time when the root becomes soft enough to be acceptable for consumption. During training, the assessor should test several different genotypes, as changes in texture depend on the genotype. Before starting full-scale cassava boiling experiments, it is critical to demonstrate the accuracy and repeatability of the assessor, using blind testing of at least 20 pieces of cassava roots (half cylinders cut as described in this SOP: 6 cm long,

2.5 cm radius) prepared from at least 4 roots. When reporting cassava boiling experiments for RTBfoods, the results of the assessor's performance need to be included in the test report.

6 TEXTURE MEASUREMENT

Equipment: Texture analyser, in this case TA-XTPlus (Stable Microsystems)
Method: Extrusion using Ottawa cell with 5-blade grid

Pre-test speed	**2 mm/s**
Speed test	**1 mm/s**
Trigger force (when the probe touches the surface of the sample)	**1000 g**
Target distance	**20 mm**
Temperature of test	**X°C** in heart of product

Following the root cutting protocol above, one cassava root yields 6 pieces (half-cylinders 6 cm long x 2.5 cm diameter) for boiling. Use at least three cassava roots from each genotype for texture measurements, so as to have 18 pieces of boiled cassava per genotype.

- Among these 18 pieces, 9 are boiled for 20 minutes (T20) and 9 are boiled until optimum cooking time (OCT).
- Among the 9 pieces T20, 4 are analysed with the fibers positioned perpendicular to the blades of the extrusion grid (fig. 14 & fig. 16); and 4 are analysed with the fibers positioned parallel to the blades of the extrusion grid (fig. 15 & fig. 17). The last piece is used as spare in case of problem with one of the texture measurements.
- Among the 9 pieces OCT, 4 are analysed with the fibers positioned perpendicular to the blades of the extrusion grid; and 4 are analysed with the fibers positioned parallel to the blades of the extrusion grid. The last piece is used as spare in case of problem with one of the texture measurements.

The pieces allocated to each measurement are summarized in table 2.

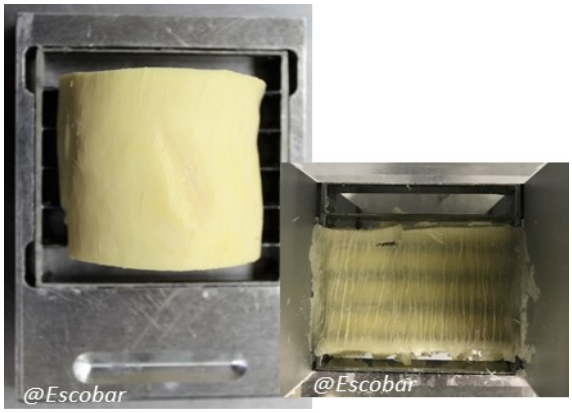

Figure 14: Fibers perpendicular to the blades

Figure 15: Fibers parallel to the blade

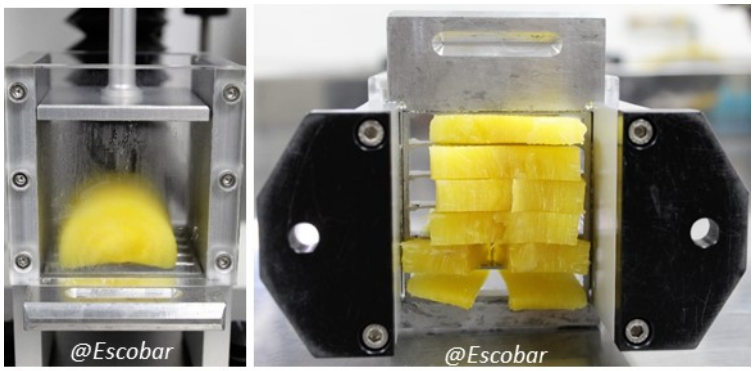

Figure 16: Texture analysis with fibers perpendicular to the blades

Figure 17: Texture analysis with fibers parallel to the blade

Table 2: Summary of the allocations of pieces of cassava roots for the texture-extrusion measurements

	Boiling time 20 mins (T20)	**Optimum cooking time (OCT)**
Fibers perpendicular to the blades	19G01P05: Distal part of Root 1 19G01P11: Proximal part of Root 2 19G01P15: Distal part of Root 2 19G01P24: Central part of Root 3	19G01P06: Distal part of Root 1 19G01P12: Proximal part of Root 2 19G01P16: Distal part of Root 2 19G01P23: Central part of Root 3
Fibers parallel to the blades	19G01P01: Proximal part of Root 1 19G01P04: Central part of Root 1 19G01P14: Central part of Root 2 19G01P25: Distal part of Root 3	19G01P02: Proximal part of Root 1 19G01P03: Central part of Root 1 19G01P13: Central part of Root 2 19G01P26: Distal part of Root 3
Spare pieces	19G01P21: Proximal part of Root 3	19G01P22: Proximal part of Root 3

Remark: Two cases for the OCT (Optimum Cooking Time):
- If the OCT is less than 20 minutes, measure the texture only at 20 minutes. Report the same data for texture T20 (cooking at 20 minutes) and texture OCT.
- If the OCT is higher than 20 minutes, measure and report the T20 texture and the OCT texture separately.

Grid used for the texture-extrusion test: The standard extrusion grid provided by Stable Microsystems for the TA-XTPlus texture analyser comes with 8 blades. However with 8 blades the extrusion of boiled cassava roots exceeds the maximum load (50 kg) of the instrument. Consequently an ad-hoc 5-blade grid was designed (fig. 18) to replace the standard 8-blade grid.

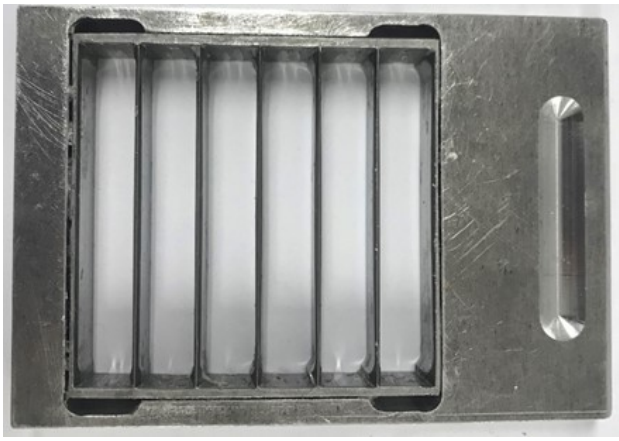

Figure 18: Ad-hoc 5-blade grid used for texture-extrusion analysis of boiled cassava roots

The settings of the texture-extrusion test are shown below (fig. 19).

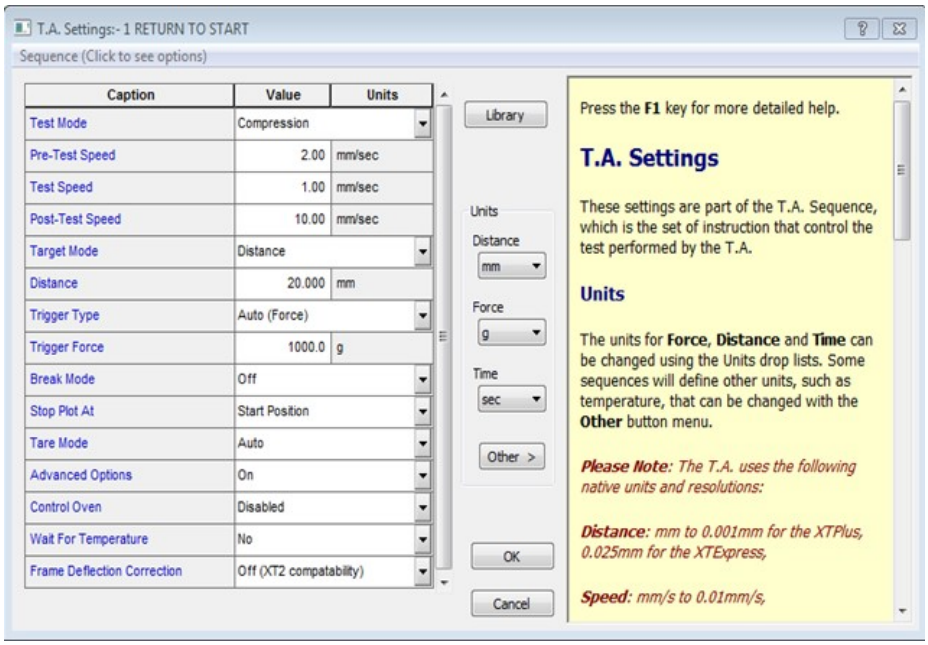

Figure 19: Parameters for the texture analysis by extrusion of boiled cassava roots

7 EXPRESSION OF RESULTS

7.1 Summary of parameters from texture-extrusion analysis

Parameters to analyze from the texture-extrusion curves (fig. 20) are summarized in table 3.

Table 3: Parameters to analyse from the texture-extrusion curves of boiled cassava

Parameter	Unit	Definition
Initial gradient	N/mm	Slope of the texture curve at the beginning of the extrusion, normalized by the initial surface of the sample in contact with the extrusion grid. The slope should be recorded between 0 and 1 mm travel distance (based on preliminary experiments)
Max. force	N	Maximum force recorded during the extrusion experiment, normalized by the initial surface of the sample in contact with the extrusion grid
Distance at max. force	mm	Distance at which the maximum force occurs during the extrusion experiment
End force at 20 mm	N	Force at the end of the extrusion, i.e. at the maximum travel distance of 20 mm, normalized by the initial surface of the sample in contact with the extrusion grid
Area under curve	N.mm	Full area under the curve, i.e. between 0 and 20 mm travel distance, normalized by the initial surface of the sample in contact with the extrusion grid. This area represents the work (energy) required to carry out the extrusion test.
Linear distance	N.mm	Linear length of the extrusion curve from 0 to the maximum distance (20 mm), normalized by the initial surface of the sample in contact with the extrusion grid. The linear distance can give an indication of the behaviour of the fibers within the sample; for example a sample with more fibers resisting breaking will show more peaks and throughs, and hence a longer linear distance.
End force / Maximum force ratio	/	Ratio calculated as follows: End force divided by Maximum force. This parameter gives an indication of the drop in hardness of the sample after the maximum force is reached. A large drop can indicate samples with a harder outer layer protecting a softer center; whereas a small drop can indicate a more homogeneous level of hardness between the center and the outer parts of the sample.

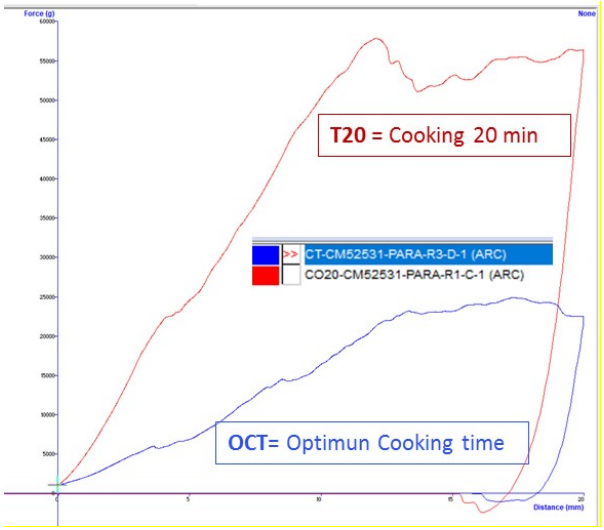

Figure 20: Example of two texture-extrusion curves of boiled cassava: (1) after 20 minutes (T20) and (2) at optimum cooking time (OCT). In this example, the same genotype was used, with fibers parallel to the blades of the extrusion grid.

7.2 Optional (under experimental development): Normalization of the texture-extrusion parameters

Due to natural variations between cassava roots, it is not always possible to prepare half-cylinders for boiling and texture, with exactly the same target diameter of 2.5 cm. Because the force recorded by the texture analyser during the extrusion test depends on the size of the half-cylinders, it may be necessary to normalize the texture parameters related to force measurements, by taking into account the dimensions of the root samples. This section describes an optional normalization procedure of the texture-extrusion parameters (still under development and testing).

A possible normalization factor is the initial surface S of the half-cylinder in contact with the extrusion grid, i.e.:

S = l x d

With: l = length of the half-cylinder = 60 mm (length fixed by the SOP)
d = diameter of the half-cylinder. d needs to be measured for each sample and reported in the template for recording data (table 5 in Annex).

Units: Length l and diameter d to be expressed in mm, and S in mm^2.

The normalized parameters are then calculated as follows (Table 4):

Normalized parameter = Parameter / S

Table 4: Optional: Normalized parameters to calculate from the texture-extrusion curves of boiled cassava

Parameter	Unit	Definition
Normalized Initial gradient	(N/mm)/ mm^2	Slope of the texture curve at the beginning of the extrusion, normalized by the initial surface of the sample in contact with the extrusion grid. The slope should be recorded between 0 and 1 mm travel distance (based on preliminary experiments)
Normalized Max. force	(N)/mm^2	Maximum force recorded during the extrusion experiment, normalized by the initial surface of the sample in contact with the extrusion grid
Distance at max. force	mm	Distance at which the maximum force occurs during the extrusion experiment
Normalized End force at 20 mm	(N)/mm^2	Force at the end of the extrusion, i.e. at the maximum travel distance of 20 mm, normalized by the initial surface of the sample in contact with the extrusion grid

Parameter	Unit	Definition
Normalized Area under curve	(N.mm)/ mm^2	Full area under the curve, i.e. between 0 and 20 mm travel distance, normalized by the initial surface of the sample in contact with the extrusion grid. This area represents the work (energy) required to carry out the extrusion test.
Normalized Linear distance	(N.mm)/ mm^2	Linear length of the extrusion curve from 0 to the maximum distance (20 mm), normalized by the initial surface of the sample in contact with the extrusion grid. The linear distance can give an indication of the behaviour of the fibers within the sample; for example a sample with more fibers resisting breaking will show more peaks and throughs, and hence a longer linear distance.
End force / Maximum force ratio	/	Ratio calculated as follows: End force divided by Maximum force. This parameter gives an indication of the drop in hardness of the sample after the maximum force is reached. A large drop can indicate samples with a harder outer layer protecting a softer center; whereas a small drop can indicate a more homogeneous level of hardness between the center and the outer parts of the sample.

8 CRITICAL POINTS AND NOTES ON THE PROCEDURE

The trigger force is high (1 kg) due to the half-cylinder shape of the sample: For most samples, the surfaces of the half-cylinder are not parallel to the surface of the moving probe. Consequently below 1 kg, the probe is still adjusting to get in full contact with the cylinder; during that initial phase of compression, the data recorded relates to the shape of the half-cylinder, not to the texture of the sample; hence it is not relevant and is not recorded (to avoid confusion and errors during data analysis).

We have tested various trigger forces and found that 1 kg ensures that the whole surface of the cylinder is in contact with the probe, before the beginning of the measurement.

Also, because the extrusion distance is fixed (2 cm), if we trigger too early (with a trigger force lower than 1kg), the piece of root goes through the grid only partially and we cannot capture all the information we need on the texture of the sample (and we get more variability due to the shape of the half-cylinders, not due to their texture).

Difference between two determinations carried out simultaneously by the same analyst on the same sample should not exceed the following limits:

- Dimensions: lengths and diameters: ± 0.1 cm (= 1 mm).
- Optimum cooking time using a fork test: ± 3 minutes.
- Closing angle: ± 2 degrees.

The standard deviations for all measurements need to be assessed and included in the reports for RTBfoods.

9 TEST REPORT

The test report shall indicate the method used and the results obtained (cf. template in table 6 in Annex). In addition, it shall mention operating conditions not specified in the present SOP or modified from the SOP, as well as any circumstances that may have influenced the results.

The test report shall include all details necessary for the complete identification of the sample, in particular the full identification code according to the guidelines of the SOP Sampling and allocation of standardized codes (WP2 and WP3).

10 REVISION RECORD

Date (DD/MM/YYYY)	Responsible person	Description of change

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