Laboratory Standard Operating Procedure

SOP for Characterization of Water Absorption, Cooking Time and Closing Angle of Boiled Cassava

Biophysical Characterization of Quality Traits, WP2

Cali, Colombia, February 2022

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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 water absorption, cooking time and closing angle of boiled cassava

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ABSTRACT

Cassava is the most widely grown tropical root and tuber crop and it is consumed in most of the tropical regions. Particularly, boiling cassava roots is one of the simplest modes of consumption. This Standard Operating Procedure (SOP) establish the main conditions of the boiling procedure of cassava root in order to obtain boiled cassava to be characterised by means of water absorption (WAB), optimum cooking time (CT) and closing angle of the half-cylinder of cassava root after boiling using the CT previously established. Apparatus (gas cooker, large cooking pots, temperature probe, heat-resistant trays, fork, balance and chronometer) and different steps of the procedure (sampling and preparation of cassava roots, preparation of root pieces for boiling and the experimental protocol for WAB, CT and closing angle determination) are clearly defined. Moreover, critical points of the procedure are pointed out and an example of template for reporting data is provided.

Key Words: water absorption, closing angle, cassava, root, cooking time, texture, sampling, fork

1 SCOPE AND APPLICATION

This SOP describes the preparation of cassava root samples for boiling tests to measure water absorption, optimum cooking time and closing angle 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 (water absorption, cooking time, closing angle), and allocation of identification codes for each sample. Further characterizations such as texture and NIRS of fresh roots and boiled roots are described in separate SOPs.

The protocol for water absorption and cooking time is also described in Tran et al. (2021): *Correlation of cooking time with water absorption and changes in relative density during boiling of cassava roots*. IJFST 56(3), 1193-1205. doi:10.1111/ijfs.14769

2 PRINCIPLES AND DEFINITIONS

When roots are delivered from the field by the breeders (RTBfoods 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” or “Cooking time” (CT): 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, CT depends on the genotype as well as environmental factors during the growth of cassava roots. As of writing the present SOP, CT 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 various boiling times depending on the type of analysis. For optimum cooking time (CT), the set of cassava roots pieces is boiled until optimum cooking time (CT), as determined by probing the pieces with a fork. For water absorption (WAB), the set of cassava roots pieces is boiled for 30 minutes (WAB30). The 30 minutes boiling allows a comparison of WAB across genotypes with discriminative power, as some genotypes are fully cooked while others are just right and others not cooked yet, thus presenting a wide range of WAB.

Regarding water absorption, in this SOP we describe a protocol to measure the kinetics of water absorption during boiling until 30 minutes and until optimum cooking time. If time is short or if there are many genotypes to characterize, it is possible to measure WAB after boiling 30 minutes only (WAB30) instead of measuring kinetics, in order to obtain an indication of the water absorption behaviour of various genotypes.

Remark: This SOP focuses on boiling and water absorption & cooking time, however several biophysical characterizations described in other SOPs (texture, NIRS, dry matter, HCN, 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

N/A.

4 APPARATUS

a. 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.

b. 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.

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

d. Heat-resistant trays or containers to hold pieces of roots above the bottom of the cooking pots. The trays should be pierced with holes to ensure unhindered flow of the water within the cooking pot and homogeneous temperature.

e. Fork to assess optimum cooking time.

f. Balance to weigh the pieces of cassava roots.

g. Chronometer.

5 PRODUCT PREPARATION: BOILED CASSAVA

5.1 Sampling and preparation of cassava roots

Select six 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: To carry out multiple analyses (texture, water absorption, NIRS, dry matter, etc.) of the same genotype, a minimum of nine roots is necessary to capture the variability of cooking behaviour. In particular water absorption and optimum cooking time measurements require six roots and texture analysis requires three roots. If more roots of suitable size are available, it is recommended to use them and increase the number of samples and representativeness of the results.

Figure 1. Minimum length of cassava root: 25 cm

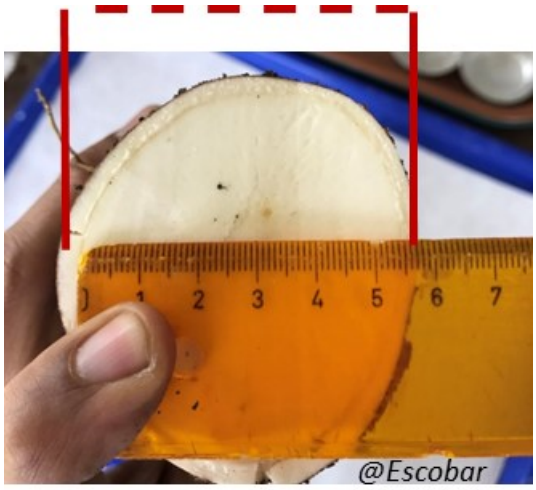

Figure 2: Minimum diameter 5.5 cm

Figure 3: Minimum radius 2.5 cm

5.2 Preparation of root pieces for boiling

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

For water absorption (WAB) and cooking time (CT), select six roots, and cut six pieces per root in the shape of half cylinders, about 6 cm long and 5.5 cm diameter (fig. 4 and 5). Identify the pieces individually by numbering the half-cylinders 01 to 06; 11 to 16; 21 to 26; 31 to 36; 41 to 46 and 51 to 56 for the roots 1, 2, 3, 4, 5 and 6, respectively. Of these 36 pieces, distribute 24 of them among three metal strainers (for instance, test sieves) for water absorption. Make sure that each strainer contains an even mix of pieces from the six roots as follows: Strainer 1: 01, 13, 25, 33, 21, 41, 53 and 56; Strainer 2: 03, 15, 23, 31, 05, 43, 52 and 46; Strainer 3: 06, 12, 24, 36, 16, 45, 51 and 54. Use the 12 remaining pieces for additional analyses, such as dry matter, HCN, NIRS of fresh roots, etc.

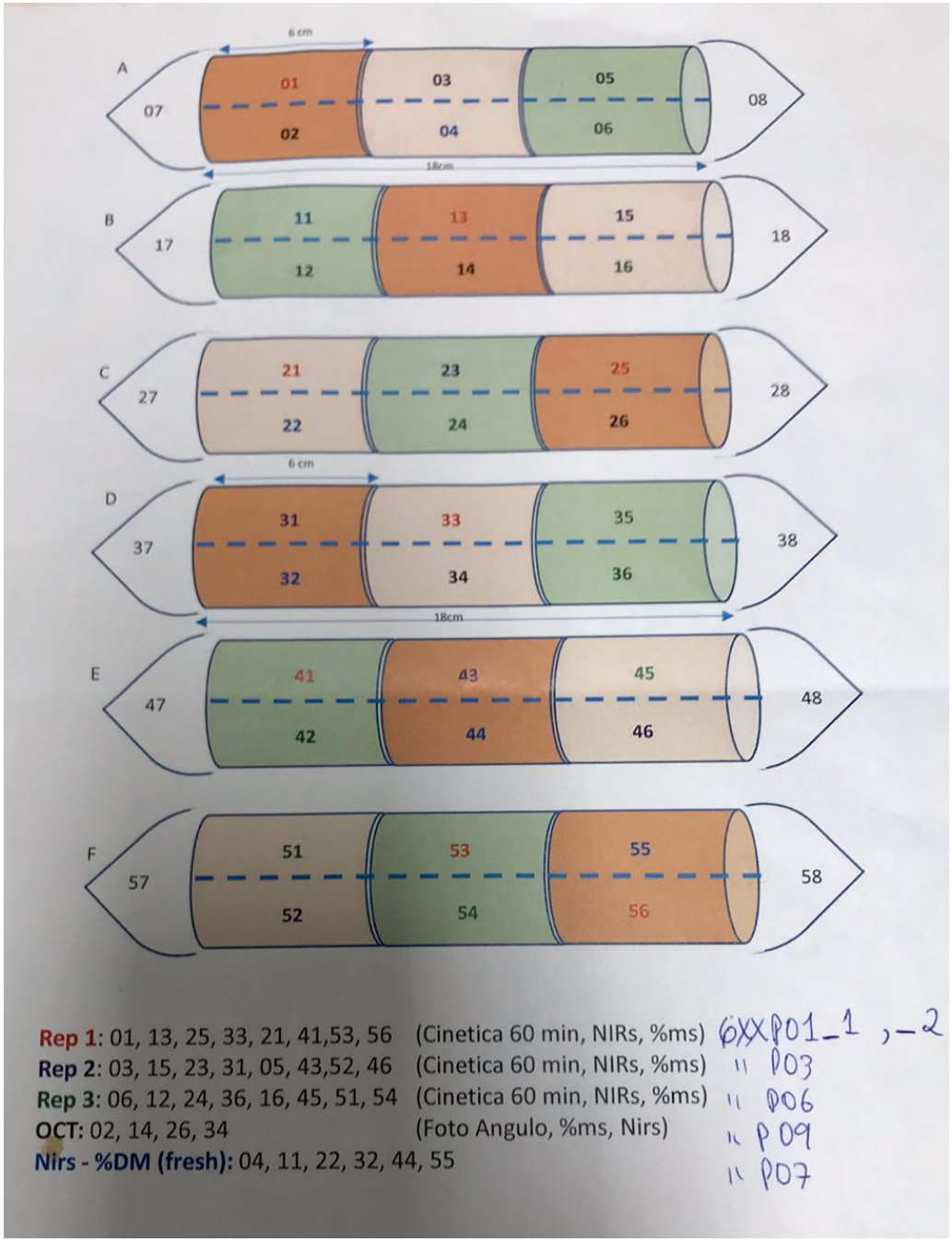

Figure 4: Cassava root cutting to prepare samples for determination of water absorption and optimum cooking time (boiling). Pieces are numbered in advance from 01 to 06 for root 1, 11 to 16 for root 2, etc. until 51 to 56 for root 6.

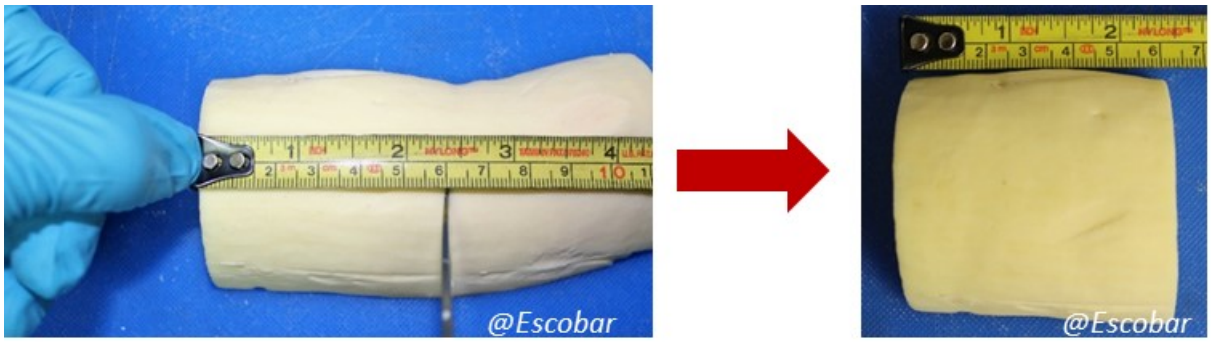

Figure 5: Length of the half cylinder for boiling: 6 cm

6 CASSAVA BOILING FOR DETERMINATION OF WATER ABSORPTION (WAB) AT 30 MINUTES AND OPTIMUM COOKING TIME (CT)

Distribute the 24 pieces of roots for WAB and CT measurements among three metal strainers as described in section 5.1 (fig. 6). Record the weights of the three strainers before boiling (time t = 0). Place the first strainer (Rep1) in a cooking pot with water already boiling. Place the second strainer (Rep2) in a second pot with boiling water two minutes later. Similarly, place the third strainer (Rep3) in a third pot with boiling water another two minutes later. The pots must contain a quantity of water significantly larger (4 L per 400-600 g roots) than the weight of the roots and their strainer, so as to minimize the drop in temperature upon introduction of the roots in the water.

Measurement of WAB during boiling: When Rep1 reaches ten minutes immersed in boiling water, remove the strainer from the cooking pot, dry the bottom with paper towel during 30s, record the weight with a 0.01g precision digital scale and immediately place the strainer back in the boiling water. Record the time the strainer and roots spend outside the boiling water with a timer (typically it takes 1 min ± 10s to do this weight measurement). Follow the same procedure for Rep2 and Rep3. Repeat the measurement of each strainer every ten minutes until 30 minutes or until reaching CT in roots requiring longer than 30 minutes to reach CT (the protocol to evaluate CT is described in the following paragraph). At that point, stop the experiment and remove the strainers from boiling water. Calculate water absorption (WAB) of each repetition (Rep1, Rep2, Rep3) as their change in weight expressed as percentage of their initial weight, for the various times recorded (t = 0, t = 10, t = 20, t = 30min, up to t = CT), according to the following equation:

WAB (%) = (Weight at t – Weight at t=0min) / Weight at t=0min x 100

With t = time of boiling, from t=10min up to t=Cooking time.

Remark: In practice, measuring WAB at t=30min only provides enough information on the water absorption behaviour. It is therefore possible to simplify the procedure if the objective is to increase the throughput of samples to analyse.

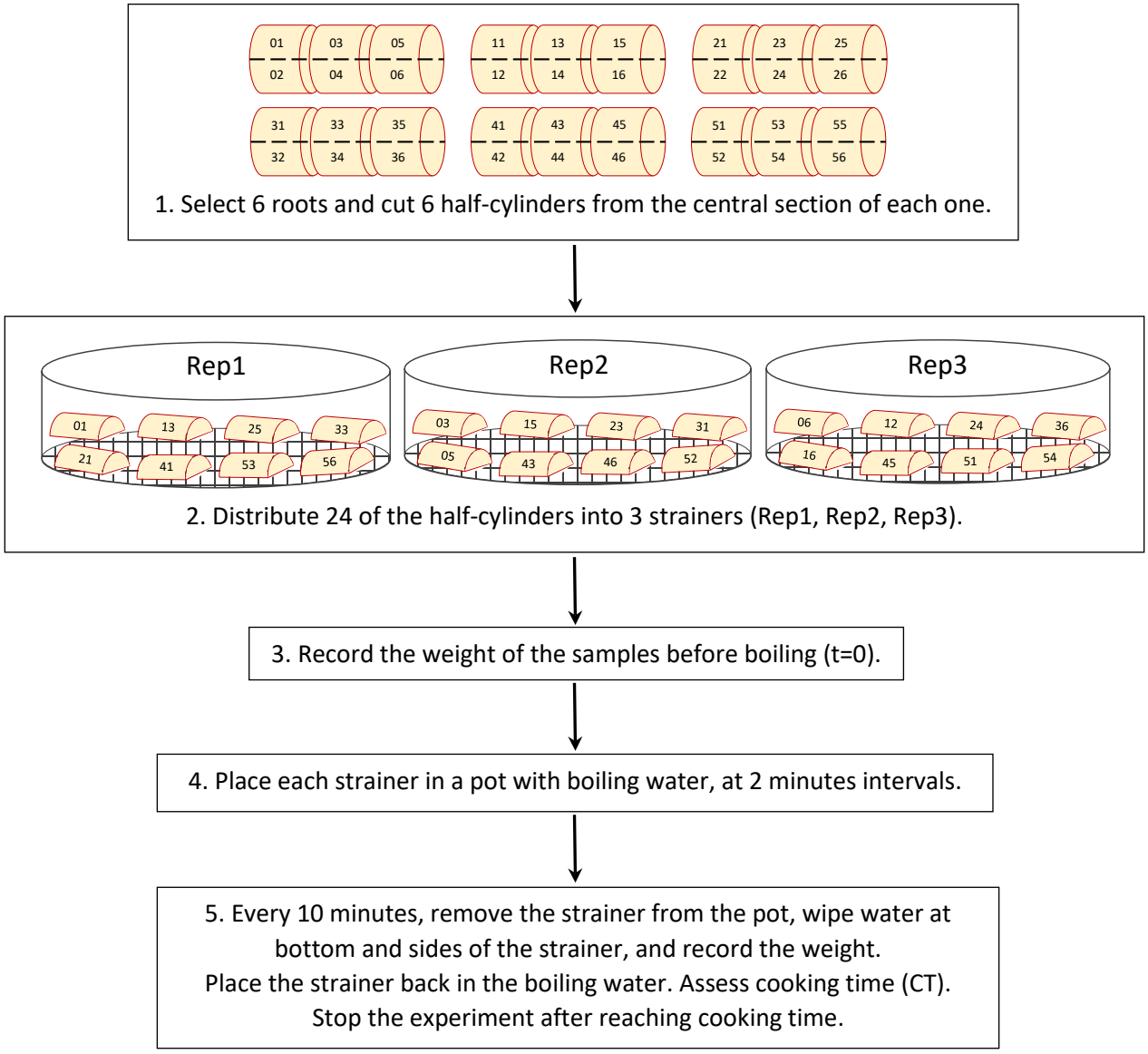

Figure 6: Flowchart of the experimental protocol for water absorption and cooking time

Most of the change in weight is caused by absorption of water (WAB) by the root pieces. However, at the beginning of boiling some soluble material can be released by the roots into the cooking water, sometimes resulting in negative WAB at t=10min. To take this into account, a mass balance can be carried out by measuring the dry matter of the roots before and after boiling, and the dry matter of the water after boiling (dry matter of the water before boiling is assumed to be zero or negligible). One source of inaccuracy of this WAB protocol is the convection of hot air above the balance during weighing the sample + strainer. Preliminary tests showed that the display of the balance varied over a maximum range of 20g due to hot air convection, which given the initial weight of the sample + strainer (ranging from 800 to 1100 g), corresponded to a loss of accuracy between 1.8 and 2.5%.

Measurement of CT: Monitor the pieces of roots in the three strainers during boiling by carefully probing their surface with a fork (fig. 7) and visually assessing their appearance, until becoming soft, i.e. acceptable for consumption. The time at which six out of the eight pieces contained in one

strainer become soft is recorded as CT of the strainer, after subtracting the cumulated time the strainer spent outside boiling water for the various WAB weighing. Calculate the average CT for the three replications (three strainers), and record this value as CT of the respective cassava genotype.

This CT protocol is semi-empirical and relies on the operator, with a precision between 1 and 2 minutes, and an accuracy of ±5 minutes. To reach this precision, the assessor needs to be trained to feel the changes in texture during boiling, so as to detect accurately and in a repeatable way the optimum cooking time (CT). During training, the operator 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 operator, 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 four roots. When reporting cassava boiling experiments for RTBfoods, the results of the operator's performance should be included in the test report.

Figure 7: Evaluation of optimum cooking time by fork test

7 DETERMINATION OF CLOSING ANGLE

After reaching CT, remove the three strainers from the boiling water, place the pieces of roots on plastic trays and let them cool to room temperature. During cooling, 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. 8). To identify the samples, include in the photographs labels with the full sample code (prepare the labels in advance before the experiments). If possible, use standard lighting conditions, in order to facilitate automated image analysis.

Figure 8: Examples of photographs of root pieces at optimum cooking time (CT)

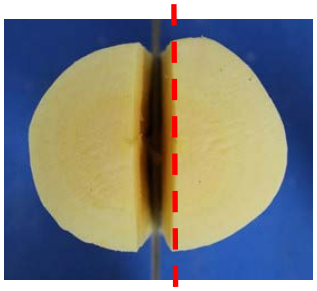

Figure 9: Closing angle before boiling is defined as 0°

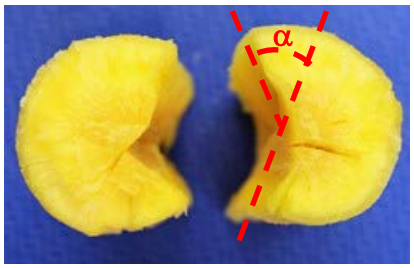

Figure 10: Closing angle □ after CT boiling is measured as indicated, using a protractor

In the template for reporting data of boiling experiments, record water absorption (WAB) at the various measuring times (t = 0, t = 10, t = 20, t = 30min, up to t = CT), the optimum cooking time (CT) and closing angle (table 3 in Annex).

Dimensions of the pieces of cassava root. The diameter and length of the half-cylinder pieces can be reported in the template for reporting data of cassava boiling experiment (table 3 in Annex). Unit to use: cm.

Water absorption kinetics (WAB). The water absorption at various boiling times (t = 0, t = 10, t = 20, t = 30min, up to t = CT) is recorded according to the protocol described above, and reported in the template for reporting data of cassava boiling experiment (table 3 in Annex). Unit to use: Percentage (%) of weight change compared to the initial weight of the raw roots.

Optimum cooking time (CT). The optimum cooking time is evaluated by a trained assessor using a fork, and is reported in the template for reporting data of cassava boiling experiment (table 3 in Annex). Unit to use: minutes.

Closing angle of the half-cylinder after boiling to optimum cooking time (CT) The angle of closing of the half-cylinder before boiling is defined as 0° (fig. 9). The closing angle □ at CT is measured using a protractor (fig. 10), and reported in the template for reporting data of cassava boiling experiment (table 3 in Annex). Unit to use: degrees.

8 CRITICAL POINTS AND NOTES ON THE PROCEDURE

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 3 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).*

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11 ANNEX

Table 3: Example of template for reporting data on Water absorption and Optimum cooking time during cassava boiling experiment.

Description: **Characterization of optimum cooking time and texture of boiled cassava by texture-extrusion**
Trial ID 20-xx
Type of sample: Boiled cassava
Test: Water absorption (WAB) and Optimum cooking time (CT)
SOP used (title): ...
Location: CIAT
Analyst: Andrés Escobar

Key	Code WP2	Date of analysis (yy.mm.dd)	Genotype	Age at harvest (month)	Repetition	WAB at 0 min (%)	WAB at 10 min (%)	WAB at 20 min (%)	WAB at 30 min (%)	WAB at 40 min (%)	WAB at 50 min (%)	WAB at 60 min (%)	Optimum cooking time (min)	Closing angle (deg)
1	20G01M10R01	20.01.29	CM7436-7	M10	R01	0	4.06	7.32	16.14	19.44			37.4	
2	20G01M10R02	20.01.29	CM7436-7	M10	R02	0	3.34	6.62	14.02	19.50			37.2	
3	20G01M10R03	20.01.29	CM7436-7	M10	R03	0	3.44	6.20	13.07	19.51			37.8	
4	20G02M10R01	20.01.24	COL2246	M10	R01	0	3.10	5.10	12.73	20.12	24.23		36.2	
5	20G02M10R02	20.01.24	COL2246	M10	R02	0	1.91	3.14	8.26	16.53	19.56		46.2	
6	20G02M10R03	20.01.24	COL2246	M10	R03	0	1.91	3.41	8.02	15.58	19.89		46.0	
7	20G03M10R01	20.01.29	GUA24	M10	R01	0	2.57	4.77	12.90	18.12			27.5	
8	20G03M10R02	20.01.29	GUA24	M10	R02	0	1.92	4.21	11.83	21.63			27.5	
9	20G03M10R03	20.01.29	GUA24	M10	R03	0	3.00	4.99	10.33	16.76			27.9	
10	20G04M10R01	20.01.28	MAL3	M10	R01	0	3.96	5.94	11.90	18.77	22.32	25.14	55.9	
11	20G04M10R02	20.01.28	MAL3	M10	R02	0	4.00	4.73	8.51	12.82	18.10	21.00	56.1	
12	20G04M10R03	20.01.28	MAL3	M10	R03	0	2.85	3.99	7.99	14.15	20.79	24.31	55.9	
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