

African Food Tradition rEvisited by Research
FP7 n°245025

Start date of project: **01/09/2010**

Duration: **45 months**

Deliverable number: D1.2.1.3

Title of deliverable: SOPs for sampling strategy for Group 3

Deliverable type (Report, Prototype, Demonstration, Other): Report

Dissemination level (PU, PP, RE, CO)*: RE

Contractual date of delivery: April 2011

Actual date of delivery: June 2011

Work-package contributing to the deliverable: WP 1

Organisation name of lead contractor for this deliverable: CSIR

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This document has been send to :

The coordinator by WP Leader	Date: June 2011
To the Commission by the Coordinator	Date: October 2011

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1. Sampling method for physico-chemical and microbiological analyses of calyx of *Hibiscus sabdariffa* in Senegal

Introduced in the country in the XIXth century, *Hibiscus sabdariffa* L. is grown throughout the territory of Senegal, particularly in the Kaolack, Djourbel, Thies, Saint-Louis and Louga regions. In these areas, a dozen varieties are grown including Vimto, Koor, Thai and CLT 92.

The main processing activities of the *H. sabdariffa* calyx are crushing and the production of drinks and concentrate. The manufacture of beverages, the main method of transformation, carried out under the direction of women's groups, has remained virtually traditional. In recent years, industrial companies (SIAGRO, ESTEVAL, Laiterie du berger, etc.) are in the production of beverages and concentrates based on *Hibiscus sabdariffa* calyx. However, quality problems are rated by consumers. For drinking, two criteria are essential for consumers, namely the red color of the drink and its acidity.

It is therefore necessary to perform physico-chemical and microbiological analyses on calyx of *Hibiscus sabdariffa* and products from these calyx before improving its quality.

This document therefore proposes a method for the sampling of *Hibiscus sabdariffa* for the purpose of microbiological and biochemical analyses. The operations for the collection, transport, packaging and preservation of samples are described.

1.1 The scope of sampling

The sampling strategy is based on the surveys (performed from 14 to 30 March 2011) which allowed the collection of information relevant to the different steps of the production process and the forms of consumption of *Hibiscus sabdariffa*.

This document describes the procedure for selecting producers and for collecting representative samples based on the results from the survey on *Hibiscus sabdariffa* processing and commercialization.

1.2 Variability of sampled products

Sampling will be done considering the variability identified during the prior surveys. The retained parameters are the following:

- a) The varieties of *Hibiscus sabdariffa*:
 - Vimto (v)
 - Koor (k)
 - Thai (t)
 - CLT 92 (c)
- b) The production zones :
 - Dakar (DK)
 - Diourbel (DB)
 - Kaolack (KK)
 - Louga (LG)
 - Thiés (TH)
- c) The types of actors :
 - Producer of calyx (P)
 - Retailer of calyx (R)
 - Producer of products (T) made from calyces of *Hibiscus sabdariffa* for sale (Pp) (Artisanal and industrial)

In our case artisanal production means a production undertaken by a person at home while industrial production is carried out by a group of processors or small and medium industry with a well defined process.

1.3 Sample size, selection of actors (Producers, retailers, processors), varieties and products

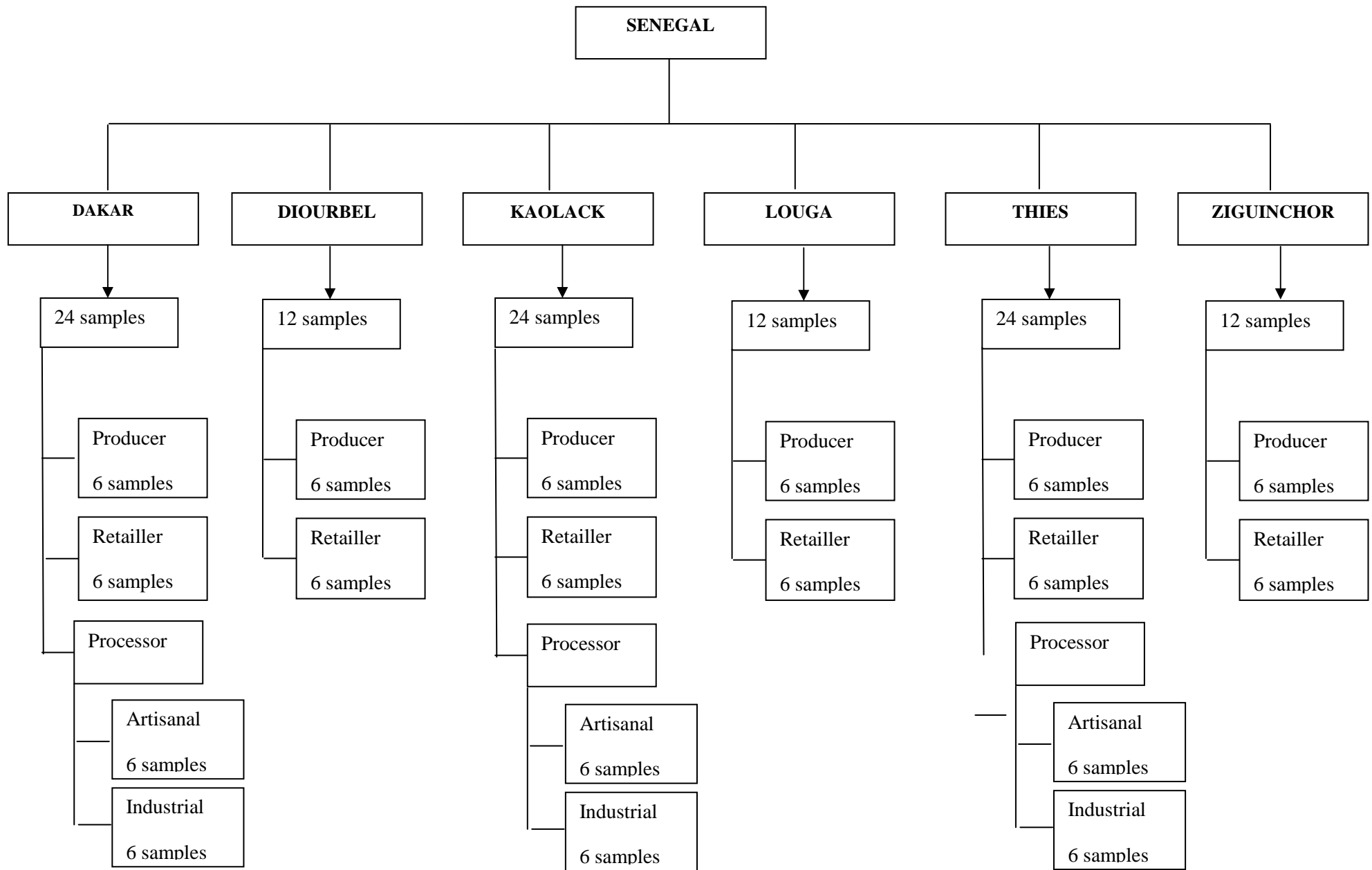
In each region:

- two varieties of *Hibiscus sabdariffa* will be selected based on the results of the surveys
- three (3) producers of calyx will be choose,
- three (3) retailers of calyx will be choose,

For products made from calyces of *Hibiscus sabdariffa*, drink and syrup will be considered.

3 artisanal producers will be selected

3 industrial-scale producers will be selected when it is possible



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The selection of eligible actors will be done randomly. However, in a given region, actors and/or products will be selected in different localities.

The samples will be taken at the point of sale and from producers targeted during the prior conducted surveys. The samples will all be of the final product. For calyx a sample of 1 kg will be taking. For drink and syrup sample of 1 liter are choose. In parallel, about 100 g of sample will be collected in sterile bag for microbiology tests. Samples will be packed in plastic bags, labeled with the code of the samples. For drink samples should be placed in a carboglaces cooler box and transported at laboratory;

1.4 Sampling coding

The code is constituted of the locality (the two initial), the types of actors, the code of actors, the type of product (calyx, drink, syrop), the first letter of the variety, and the processing variant (artisanal, industrial) for products only.

Example:

/DK-P-01-Ca-t The sample of calyx (Ca) of thai variety (t) from producer (P) n° 01 in Dakar (DK)

TH-T-03-Dr-v-Art The sample of drink from vimto variety with a artisanal process by the processor (T) n° 03 in Thies (TH)

1.5 Procedure for the preparation of samples in the laboratory

At laboratory, samples from each producer, seller and processor will be divided for physico-chemical, biochemical analyses:

- One part (250 g) for immediate analyses (pH, titratable acidity, water content, PSI etc.)

- One part (250 g) for freeze drying (sugar, pectins, organic acids, polyphenols, anthocyanins, antioxidant activities, vitamins, etc.)
- One part (250 g) for oven drying (ash, lipids, proteins, minerals etc.)
- One part (250 g) for freeze

For microbiology, as described above, 100 g will be used for microbiological analyses (Total flora, lactic acid bacteria, total coliform, fecal coliform, *Clostridium perfringens*, Enterobacteriaceae and yeast and moulds etc.).

2. Sampling method for physico-chemical and microbiological analyses of Baobab in Senegal

African baobab, a very long-living tree, has many potential nutritious and pharmaceutical properties. In many parts of Africa, tubers, fruits, seeds, leaves and flowers of this plant have been identified as common ingredients in traditional dishes in rural and urban areas. In Senegal, baobab is a good source of income for local people.

Baobab fruit pulp called also “Monkey bread” is widely used and consumed by Senegalese people in different forms: juice, pulp, etc. The pulp, because of its strongest economic potential compared to the remaining parts of the fruit, attracts more as foodstuff. The seed is used by many growing small enterprises for essential oil extraction and other cosmetic products. The exportation of the dried pulp to European areas is common nowadays because of many reasons.

Before any quality improvement, we found necessary to perform physico-chemical and microbiological analyses on different parts of baobab fruit and by-products.

Therefore, the present document highlights a method for the sampling of Baobab fruit for the purpose of microbiological and biochemical analyses. Also collection, transport, packaging and preservation of samples are described for operations to be used.

2.1 The sampling scope

The sampling strategy is based on surveys performed from March 11, 2011–May 5, 2011. These surveys allowed collection of relevant information from the different steps of the production process and the forms of Baobab fruit consumption. Only one variety of baobabs exists in Senegal (*Adansonia digitata*). Therefore, sampling should be conducted in three regions in Senegal.

2.2 Sampling areas

The sampling will be conducted in Tambacounda-Kédougou, Thiés, and Kaolack. The actors will be selected from these localities, and from three categories of actors (artisanal producers, half industrial producer, and retailers).

2.3 Variability of sampled products

Sampling will be done according to the variability observed during the previous surveys. The retained parameters are below:

- d) Two main types of baobab fruit by-products based on the raw material:
 - Baobab dried fruit pulp (BP)
 - Baobab pulp fruit drinks (BD)
- e) Three production zones :
 - Tambacounda-Kédougou (TK)
 - Thiés (TH)
 - Kaolack (KA)
- f) Three types of actors :
 - Artisanal producer (AP)
 - Haft industrial producer (HIP)
 - Retailer (R)

Table 1: Samples variability

Collection zones	Levels of collection	Raw materials	Production processes
TK : Tambacounda- Kédougou TH : Thiés KA : Kaolack	AP: Artisanal producer HIP: Haft industrial producer R : Retailer	BF: Baobab fruit	DEP : drying/pulp extraction /packaging RDP : ratio/drink processing/packaging

2.4 Diagram for sampling

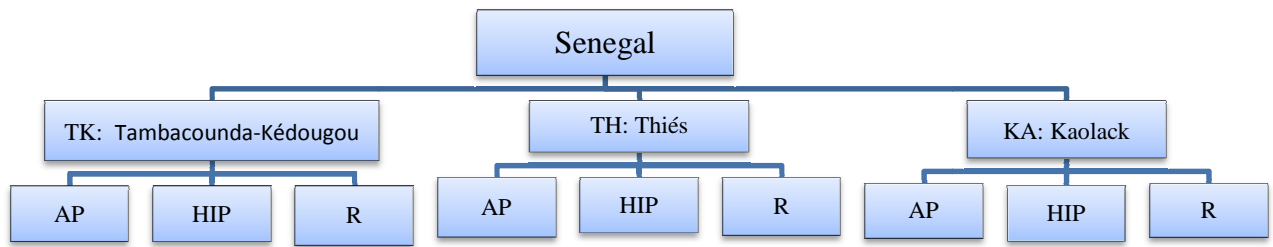


Figure 1: Diagram representing the sampling actors at different zones
Key diagram legend: refer to **Table 1**

2.5 Distribution of samples

Based on the hypothesis for selecting 2 producers/sellers and 1 retailer/seller per variant, the maximum expected number of actors is 35. These Thirty five samples will be taken for each type of baobab fruit production in the following manner: 10 from Tambacounda-Kédougou, 15 from Thiés and 10 from Kaolack. In each zone, different types of baobab fruit by-products will be collected according to the production process:

- pulp extraction/drying /packaging;
- ratio/drink processing/packaging.

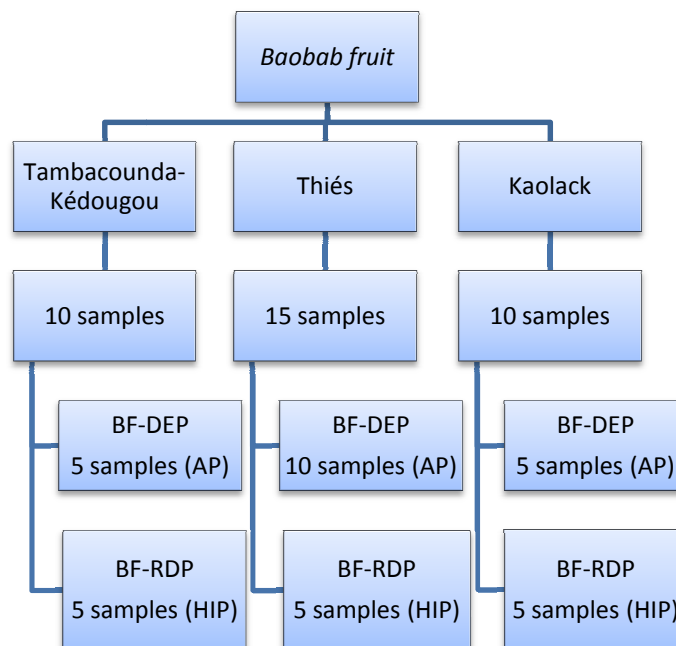


Figure 2: Diagram of distribution and number of samples

Key diagram legend: refer to **Table 1**

With a baobab fruit production of 3200 t/year in Senegal, the pulp is essentially consumed by traditional way. The Figure 2 shows the total sample in the study comprising of 3 actors (AP, HIP, and R). In this present sampling method, 20 samples will be AP, 15 samples HIP, and 10 samples R. In this retailer part, 5 samples will be from TH, and 5 samples from KA because of the more commercial and consumer developing regions compared to TK. The samples will be taken at the point of sale and from producers previously targeted during the previous conducted surveys.

The samples will be all collected from each type of baobab fruit by-product and according to different producers.

Samples will be collected also from retailers of baobab fruit dried pulp and baobab pulp fruit drinks.

2.6 Procedure of sample collection

Each sample weight will be 1000g. Sampling will be conducted in the morning around 8h00 GMT. After buying, the samples will be packed in sterile plastic bags, labelled with the code of the sample. The code should be traceable to the date, time and the sales point as well as indicating the product raw material, zone, type and the producer's name. Samples should be placed in a cooler around 4 to 5 °C and transported to the laboratory.

2.7 Sampling coding

The code is based on the locality (the two initial), the raw material (initial), the processing variant (as defined in **Table 1**), and the code of producer or retailer.

Example: /TK/_BF/_ RDP _/_10_/. The sample of baobab fruit by-product producer n°10 produces by using ratio/drink processing/packaging (RDP) of Baobab fruit (BF) in Tambacounda-Kédougou (TK);

And /TK/_BFB/_R/_5_/. The sample n°5 from retailer of Baobab fruit by-product in Tambacounda-Kédougou (TK).

2.8 Procedure for the preparation of samples in the laboratory

On arrival at the laboratory each sample of baobab fruit by-product will be stored in a refrigerator at 4 to 5 °C or in a desiccator according to products nature until the analysis. The sample size should be divided for analyses in the following manner:

- 300g will go for microbiological analyses (“Food Industries Control Laboratory (LFCIA) in ESP or in other laboratories): test for *Salmonella*, *Escherichia coli*, *Staphylococcus*, total microbial count and lactic acid bacteria, Enterobacteriaceae, Yeast and Moulds, Bacillus, etc... (cf. SOPs microbiological methods of analysis related to baobab).

- 350g will be subjected to physico-chemical analyses in “LFCIA laboratory in ESP” or other laboratories: water content, particles’ size... (cf. SOPs nutritional methods of analysis related to baobab)

- 350g will go for biochemical analyses in “LFCIA laboratory in ESP” or other laboratories: pH, water activity, Vitamin C, total phenols... (cf. SOPs nutritional methods of analysis related to baobab).

Samples for microbiological analyses should be processed on the day of sampling. Therefore, if culture medium has to be prepared before analysis, it should be placed in « stomacher bags » and stored in a refrigerator. Samples for physico-chemical analyses, whether analysed in “LFCIA laboratory in ESP” or others ESP laboratories, should be packed under vacuum in plastic bags and then immediately frozen at -18 °C.

3. Sampling method of *Jaabi* and its processed products for physico-chemical, biochemical and microbiological analyses

Jaabi or jujube is the fruit of jujube tree, a shrub found in the Far North region of Cameroon. Local populations use the fruit as a snack food or transform it into flour. The flour is cooked and compressed into small round cakes commonly called "*yaabaande*" in Fulfulde language

Yaabaande, the main form of local processing of the fruit, presents intertribal technology variations, which can lead to variability in the quality of the product. Although information on quality criteria indicate that *jaabi* and its processed product can be stored for a long period, hygienic and biochemical modifications of *yaabaande* during processing methods and storage are not known.

It is therefore necessary to carry out physicochemical and biochemical analysis of *jaabi* and *yaabaande*, so as to identify the determinants of product quality. This document proposes a method for the sampling of *jaabi* and *yaabaande* for physico-chemical analyses. Collection, packaging and preservation of samples are described.

3.1 Scope of sampling

The sampling is undergone in the production and processing areas identified during the survey (March 20 to April 10). The document describes the representativeness of samples, based on how actors involved in production, processing and marketing of *Jaabi* are chosen

3.2 Sampling areas and size

Samples of *Jaabi* and *yaabaande* will be collected from producers, processors and traders in three locations in the Far North Cameroon (Maroua, Mokolo, Mora), where the survey took place

Two types of samples will be collected from stakeholders in each locality, the *Jaabi* fruit and its transformation product, *yaabaande*. Three modes of heat treatments will be considered: steam cooking in Maroua, stifle cooking in Mokolo and solar drying in Mora

The sampling design is as follows:

- For *jaabi* fruit: 3 stakeholders x 3 localities
- For processed product, *yaabaande*: 3 processors x 1 product x 1 locality

3.3 Sample treatments and conditioning before analysis

Jaabi samples collected from producers / traders will be sorted, crushed in a mortar and packed in labeled plastic bags.

Yaabaande samples will also be packed in labeled plastic bags

3.4 Sampling coding

The samples will be coded as indicated in table 1 below:

Table 1: Coding of samples

Collection zones	Levels of collection	Nature of product
Mr : Maroua	P: Producer	J: Jaabi
Mo : Mora	C: Trader	Y: Yaabaande
Mk : Mokolo	T: Processor	

Example:

Jaabi collected from a trader in Maroua will be labeled: MrTJ

3.5 Procedure for the preparation of samples in the laboratory

In laboratory, samples from each stakeholder will be divided for physico-chemical, biochemical and microbiological analyses:

- 500 g will be subjected to physic-chemical analyses (pH, titratable acidity, water content, ash, lipids, proteins, sugars, pectins, minerals, etc)
- 500 g for biochemical and functional analyses (bioflavonoids, triterpenoic acids, polyphenols, vitamins, ABTS, DPPH, FRAP, etc.)
- 100 g of *yaabaande* will be used for microbiological analyses (lactic acid bacteria, *S. aureus*, *Clostridium perfringens*, Enterobacteriaceae, *Bacillus cereus* and yeast and moulds etc.)