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Authors: Danielle Rakoto (UT), Janvier Kindossi (UAC), Victor Anihouvi (UAC), Nicolas Ayessou (UCAD)

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# 1. Sampling method for physico-chemical and microbiological analysis of smoked kong in Senegal

The smoked fish called « kong » in Senegal is scientifically attributed to *Arius heudelotii* (Valenciennes, 1840). The smoking occurs all along the year and represents 7.5% of traditional transformed fish in Senegal. The smoking technology still be at traditional level. The finally quality depend on it with water content up to 61%. Unfortunately, this moisture influences the microbiological quality. In order to improve the smoking process and the kong's quality, it seems normal to determine both microbiological and chemical quality of smoked kong as it is sold in Senegal. This document proposes a method for the sampling of smoked kong for the purpose of microbiological and biochemical analyses. The operations for the collection, transport, packaging and preservation of samples are described.

This document contributes to deliverable D1.2.1.2 “SOPs of sampling strategy for Group 2” of the AFTER project.

## 1.1 The scope of sampling

The statistics data in Senegal shows the main regions of kong's fishing to be Thiès, Ziguinchor and Fatick. The surveys results brought a lot of informations about the process, retailers and consumers. According to that, the sampling strategy is based on both combined knowledges. Finally, sampling should be carried out in two regions according to areas of producers which are much closed to consumers's one. The first one gathered **Ziguinchor**, (including Karabane-Diogu  and Kafountine). The second group is localized in **Dakar** (see Figure 1)

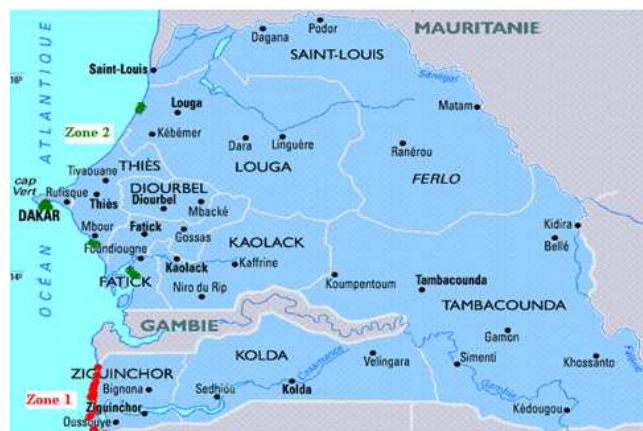


Figure 1: The map of important fishing's sites of *Arius heudelotii* in the Senegal

- Zone 1: Karabane-Diogu , Ziguinchor, Kafountine ; Zone 2: Dakar

## 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) Two principal types of smoked kong based on the degree of water content:
  - *Well dried smoked kong*
  - *Wet smoked kong*
- b) Two main production zones :
  - Urban zone of Dakar
  - Important kong fishing's zone (Ziguinchor's region)
- c) Two types of actors :
  - Producers (P)
  - Retailers (R)

Table 1: Variability of samples

<b>Collection zones</b>	<b>Area of sampling</b>	<b>Levels of collection</b>	<b>Number of samples</b>	<b>Final aspects</b>
Zone 1 : Ziguinchor	Ziguinchor	P and R	8	<i>Wet smoked kong</i>
	Kafountine	p	4	<i>Well dried smoked kong</i>
	Karabane	p	3	<i>Well dried smoked kong</i>
	Diogu�	p	5	<i>Well dried smoked kong</i>
Zone 2 : Dakar	Market 1	R	3	<i>Wet smoked kong</i>
	Market 2	R	3	<i>Wet smoked kong</i>
	Market 3	R	3	<i>Wet smoked kong</i>
	Market 4	R	3	<i>Wet smoked kong</i>
	Market 5	R	3	<i>Wet smoked kong</i>
		<b>TOTAL</b>	<b>35</b>	



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The half of the sample subject for physico-chemical analysis will be treated the same day to determine water content, pH, salt, protein, fat content. The second half of the chemical's sample should be packed under vacuum in plastic bags and then immediately frozen at -20°C. This part will be use for phenolic and HAP content.

## 2. Sampling method for physico-chemical and microbiological analyses of *kitoza* in Madagascar

*Kitoza*, as a traditional dish, forms an integral part of Malagasy nutrition and serves as a base for numerous culinary recipes. Its consumption continues to grow and production no longer only takes place at home, but it is available at butcheries, deli's and supermarkets. However hygienic conditions are not well controlled during the different steps of production and storage and this affects the microbiological quality of the final product. On the other hand, the effect of the sequence of production operations and parameters on neither the final quality nor the suitability for storage is well known. It is therefore necessary to perform physico-chemical and microbiological analyses on *kitoza* before improving its quality.

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

This document contributes to deliverable D1.2.1.2 “SOPs of sampling strategy for Group 2” of the AFTER project.

### 2.1 The scope of sampling

The sampling strategy is based on the surveys (performed from 11 March to 5 May 2011) which allowed the collection of information relevant to the different steps of the production process and the forms of consumption of *kitoza*.

Sampling should be conducted in three zones in Antananarivo : the urban zone, the peri-urban zone and the rural zone and according to three categories of actors : producers, retailers and consumers.

### 2.2 Variability of sampled products

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

- d) Two principal types of *kitoza* based on the raw material:
  - *kitoza* from zebu (Beef) (KB)
  - *kitoza* from pork (KP)
- e) Three production zones :

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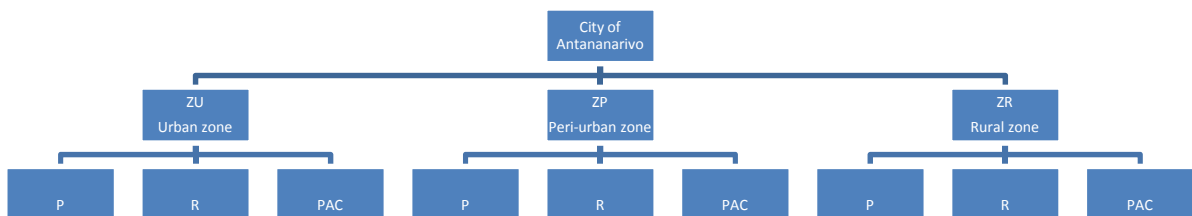
- Urban zone (ZU)
  - Peri-urban zone (ZP)
  - Rural zone (ZR)
- f) Three types of actors :
- Producer (P)
  - Retailer (R)
  - Producer for self-consumption (PAC)

Table of the variability of samples :

Collection zones	Levels of collection	Raw materials	Production processes
ZU : Urban zone ZP : Peri-urban zone ZR : Rural zone	P : Producer R : Retailer PAC : Producer for self-consumption	KB : <i>kitoza</i> from zebu (beef) KP : <i>kitoza</i> from pork	SF : salted/ smoked SS : salted/ dried

### 2.3 Diagramme for sampling

Figure 1 : Diagramme representing the study zones



Legend of diagram: refer to table of variability of samples above

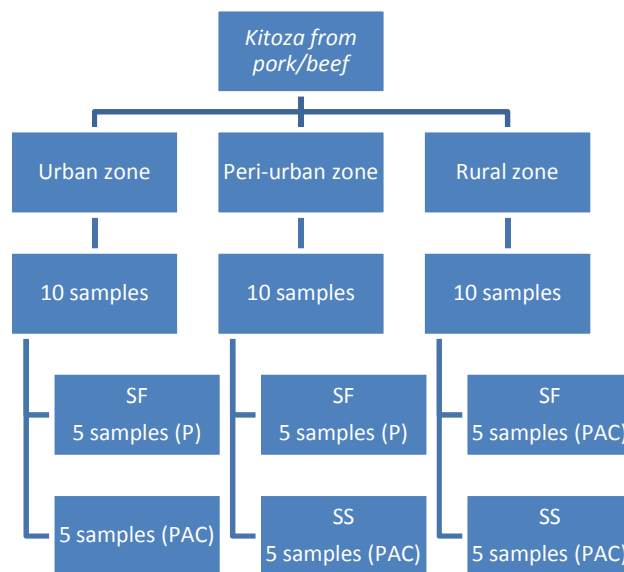


## 2.4 Distribution of samples

Thirty samples will be taken for each type of meat (pork or beef) in the following manner: 10 from the urban zone, 10 from the peri-urban zone and 10 from the rural zone. In each zone, different types of *kitoza* will be collected according to the production process:

- Salted/ smoked;
- Salted/ dried.

Figure 2: Diagramme of the distribution and number of samples



Legend of diagram: please refer to table of variability of samples above.

The samples will be taken at the point of sale and from producers for self-consumption previously targeted during the prior conducted surveys.

The samples will all be of the final product and collected at the different producers/retailers and the producers for own consumption for each type of *kitoza*.

No sample will be collected from retailers only as the producers of *kitoza* are also the sellers.

In the rural zone, there are no industrial scale producers of smoked *kitoza*, but some smoked *kitoza* is produced for own consumption. The *kitoza* is smoked in the traditional manner by suspending it on hooks over the fire.

## 2.5 Procedure of sample collection

Each sample will weigh 600g. Sampling will take place in the morning around 8h00 to 10h00

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After buying, the samples will be packed in sterile plastic bags, labeled with the code of the sample. The code should be traceable to the date, time and the sales point as well as indicate the product raw material, zone, type and the name of the producer. Samples should be placed in a cooler and transported to the laboratory..

## **2.6 Procedure for the preparation of samples in the laboratory**

On arrival at the laboratory each samples of *kitoza* will be cut and diced into pieces of a few centimetres with sterile scissors in a laminar flow hood. The pieces should be mixed and then divided for analyses in the following manner:

- 150g will go for microbiological analyses: 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 kitoza).

- 200g will be subjected to physico-chemical analyses in Madagascar: water content, collagen and proteins

- 250g will go for biochemical analyses in Réunion : pH, water activity, fat content (Folch), total phenols, salt, D- and L-lactic acid, polycyclic aromatic hydrocarbons (PAH), measure the oxidation of lipids (TBARS).

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 fridge. Samples for physico-chemical analyses, whether analysed in Madagascar or in Réunion, should be packed under vacuum in plastic bags and then immediately frozen at -20°C.

### **3. Sampling method for physico-chemical and microbiological analysis of Lanhouin in Benin**

Lanhouin, a traditional fermented fish based condiment processed in the coastal areas of Benin, is mostly used as taste enhancer and flavouring agent in many types of dishes including the European ones. However, its production is still artisanal; consequently the quality of the final product is unpredictable; in addition sale conditions are not likely to guarantee its harmlessness. Moreover, the most significant operations such as ripening and fermentation are not well defined, nor controlled whereas they determine the final quality of *lanhouin*. In order to improve the quality of lanhouin, it would be necessary to characterise this product on both microbiological and physico-chemical aspects. Due to the variability of the know-how and quality of the products in different production areas of Benin, the sampling strategy for quality assessment will be developed in such a way that it allows to assess the representative quality of the traditional lanhouin in those production areas.

Therefore this document proposes a method for sampling of lanhouin prior to characterization through microbiological and physico-chemical analyses. The operations for the collection, packaging, transport and preservation of samples are described.

#### **3.1 Scope of sampling**

The sampling strategy will be based on the survey (performed from 10 March to 10 April 2011) which allowed collecting information mainly on the production, commercialisation and consumption of lanhouin. Sampling will be conducted in Grand-Popo municipality (urban and rural zones) from large and small-scale processors, and traders (wholesaler, retailers).

#### **3.2 Variability of sampled products**

Sampling will be done by considering the variability identified during the survey. The selected parameters are:

- a) Three types of technology according to unit operations and material used for fermentation:
  - Fermentation in aerobic conditions with basket used as fermentation material (FA)

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- Fermentation in micro-aerobic conditions with container or basket with cement layer, and can used as fermentation materials (FAN)
- Fermentation in anaerobic conditions and without ripening (fish buried in the ground)

b) Two types of fish: lean fish and fatty fish

- Lanhouin from Kingfish/ Spanish mackerel (LK)
- Lanhouin from Cassava croaker (LC)

c) Two collection level

- Processing sites (PS) with Processor (P) as actors
- Market (M) with Wholesaler (W) and Retailer (R) as actors

The sampling will be also done in the market because the lanhouin samples collected at processors level are not yet sun-dried whereas those collected in the market are the dried ones. According to the technology, the sun-drying of lanhouin is the last step of the processing procedure and this step is done one or two days before, when the seller is ready to take his product to the market. In addition, the storage conditions are not the same at the processor and seller level. This means that the lanhouin samples collected at the two levels may not have the same final quality.

Table 1: Variability of samples

<b>Collection zones</b>	<b>Levels of collection</b>	<b>Raw materials</b>	<b>Processing methods</b>
PS : Processing site  M : Market	P : Processor  W: Wholesaler  R : Retailer	LK : Lanhouin from Kingfish (KF)/ Spanish mackerel  LC : Lanhouin from Cassava fish (CF) Cassava croaker (Cc)	FA : Fermentation in aerobic conditions  FMA : Fermentation in micro- aerobic conditions  FAN: Fermentation in anaerobic conditions without ripening

### 3.3 Diagram for sampling

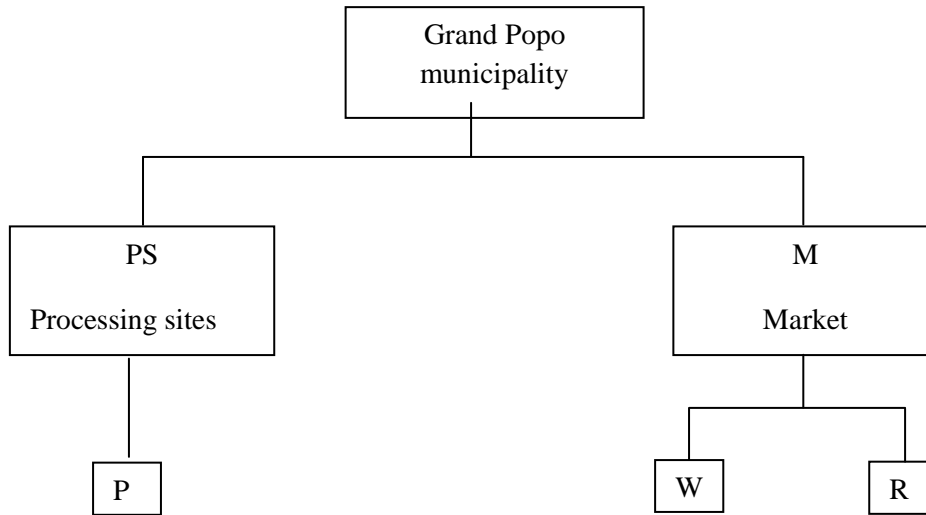


Figure 1: Diagram representing the sampling zones

Legend of diagram: refer to table of variability of samples above

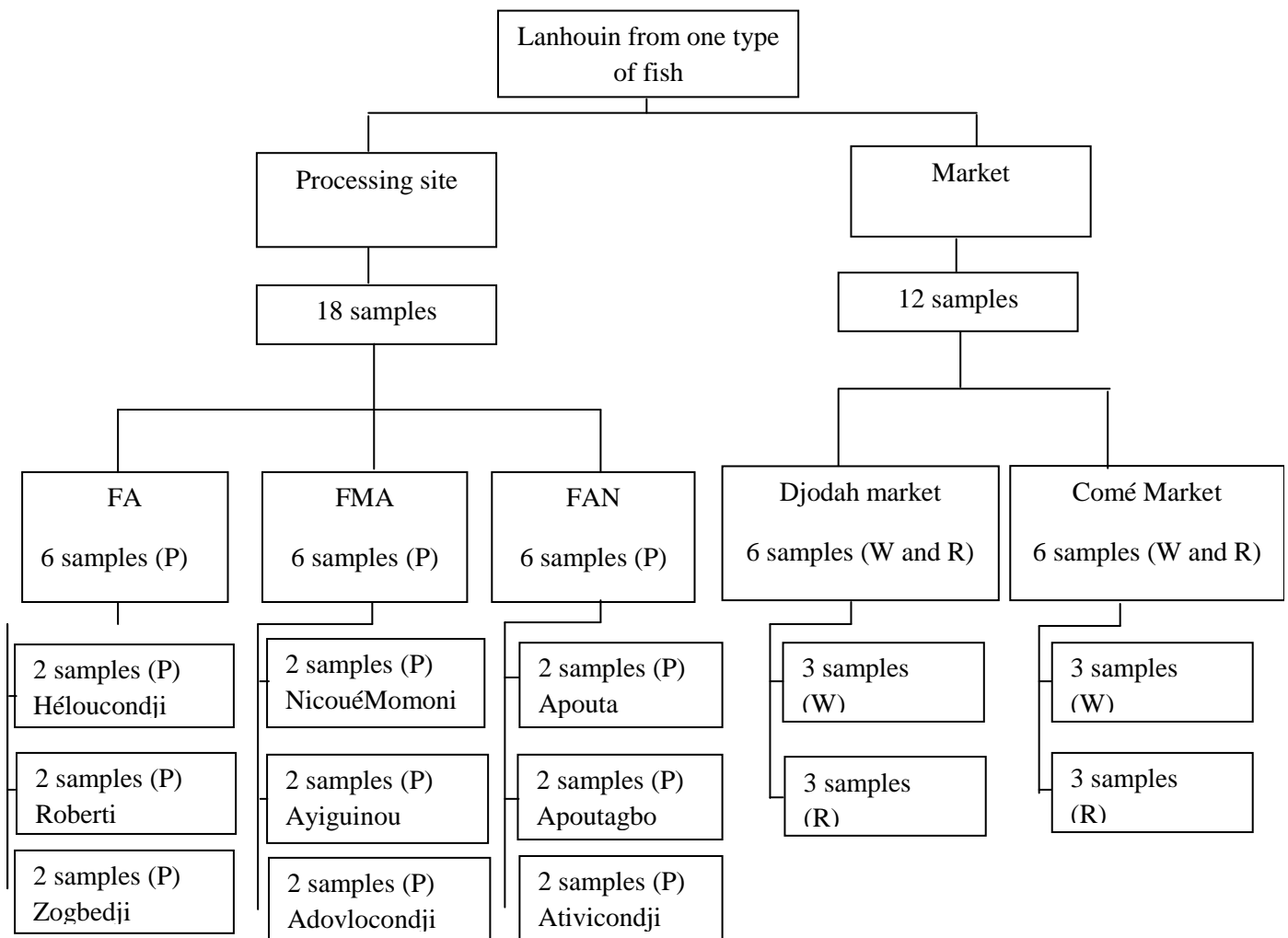
### 3.4 Distribution of samples

Thirty (30) samples will be taken for each type of fish and distributed as follows: eighteen (18) from the processing sites and 12 from market. On the level of the processing site, lanhouin samples will be collected according to the type of fermentation:

- Fermentation in aerobic conditions
- Fermentation in micro aerobic conditions
- Fermentation in anaerobic conditions without ripening

The choice of the processing sites is done according the technology mainly used in the zone. Based on this, the sampling will be done with three (3) processors randomly selected per type of fermentation and type of fish on the list of processors surveyed. Six (6) samples of Lanhouin will be collected per type of technology and type of fish; this means that two (2) samples per processor and per type of fish will be collected at processing site level. Three (3) samples of lanhouin will be collected per seller at market level. In short thirty six ( $36 = 6 \times 3 \times 2$ ) samples of lanhouin will be collected for the two types of fish and the three types of technology, and this from 18 processors, and twenty four ( $24 = 3 \times 4 \times 2$ ) samples from 8 sellers for the two types of fish (Figure 2); this gives a total of sixty (60) samples.

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**Figure 2:** Diagram of the distribution and number of samples per type of fish

Legend of diagram: refer to table of variability of samples above

### 3.5 Procedure of sample collection

After buying, the samples will be packed in sterile stomacher bags, labeled with the code number. The code should be traceable to the date, time and the sampling place, the product raw material and the processor’s number. Samples will be put in an ice chest filled with dry ice and transported to the laboratory.

### 3.6 Sample coding

The code is constituted of the name of producer/seller, the date and time of collection, the locality (the two initial), the raw material (initial), the processing variant (as defined in table

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1), the step of the flow diagram, the date of production and the code of producer in the database (ref. survey sheet).

- For lanhouin samples collected at market level the code will be for example:

Lanhouin/LK/ 05-10-11/15h00/Cm. This means Lanhouin sample processed with king fish, collected the 5 October 2011 at 15h from Comé market.

- For Lanhouin collected at the processing site level, the code will be for example:

Jeanne/\_/15-06-11/\_/13h00/\_/Gp (Apouta-Site)/ LK/\_FA/\_/10-06-11/\_/25\_/. This means, lanhouin sample from Jeanne representing n°25 (number of processor in survey database), collected the 15 June 2011 at 13h from Grand-Popo (Gp) mainly Apouta site. The sample is produced with king fish by Fermentation in Aerobic conditions the 10 June 2011.

- For samples collected during the characterization of the processing method, the code will be for example:

Jeanne/\_/15-06-11/\_/13h00/\_/Gp (Apouta-Site)/ LK/\_FA/\_/25\_/Diag1/Step3/Prod2. This means, sample from Jeanne representing n°25 (number of processor in survey database), collected the 15 June 2011 at 13h from Grand-Popo (Gp) mainly Apouta processing site. The sample is produced with king fish by Fermentation in Aerobic conditions using the flow diagram n°1, collected at the step 3 during the second trial.

### **3.7 Procedure for the preparation of samples in laboratory**

At the laboratory, the samples will be treated the same day; the remaining will be stored in a refrigerator and processed within 24 h. Each sample of lanhouin will be filleted with sterilized material, bones will be removed in aseptic conditions and the flesh blended using a lab blender. The glass jar cup of the blender will be autoclaved at 121°C for 15-20 min or cleaned with ethanol 70%. If ethanol is used, this should be completely dried before the glass jar cup is used.

- One hundred (100) g of blended sample will go for microbiological analyses : test for *Salmonella* –*Shigella* , *Micrococci*, *Bacillus cereus*, *Clostridium perfringens*, Coagulase positive and coagulase negative *Staphylococcus*, *Listeria monocytogenes*, *Vibrio/Aeromonas*, *E. coli*, yeast and moulds, lactic acid bacteria, *Bacillus* spp.

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- Each remaining global sample will be divided in three (3) parts according to the quantity needed for physico-chemical analyses (pH, moisture, crud protein, fat, water activity, salt, lead, mercury, cadmium, calcium, iron and phosphorus) and biochemical analyses, (histamine, putrescine, cadaverine, total volatile nitrogen, vitamin A, free fatty acids). Those samples for physicochemical and biochemical analyses will be packed under vacuum plastic bags or stomacher bag and then immediately stored in a freezer at - 20°C. Two parts will be kept in Bénin (one part will be used for analyses and the second one in reserve) and the third one sent to ADIV or CIRAD for biochemical analyses (total free amino acids, lysine, leucine aroma compounds, carbonyl, Polycyclic Aromatic Hydrocarbon and fatty acid profile).