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Hygienic Quality of *Mbala-pinda*, a Fermented Food Formulated from Local Products of Congo

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study is part of the framework for the food safety of locally consumed products in Congo. The aim was to study the hygienic quality of *Mbala-pinda*, a food made from fermented cassava paste and peanut paste, formulated with maize, taro, and plantain banana. To this end, five (5) types of *Mbala-pinda* were developed in the laboratory by modifying the traditional process and then analyzed by enumeration of total aerobic mesophilic flora, total and fecal coliforms, *Staphylococcus aureus*, yeasts and molds using the serial dilution technique. (NF.V 08-051-Feb.

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(1999), AFNOR V 08 013 and directive 2005/2073/EC). The presence of Salmonella and Shigella was also checked on specific SS medium after enrichment of the cultures. The results showed a microbial load in FMAT of 3.5. 10^{-3}, 3.1. 10^{-4} and 1.5. 10^{-3} CFU/g respectively for samples E1, E2 and E5. This microbial load is below the threshold set by Directive 10^{6} While samples E3 and E4 have zero FMAT load. All *Mbala-pinda* samples were free of bacteria from the total and faecal coliform groups, *Staphylococcus aureus* species, yeasts and molds, as well as Salmonella and Shigella. Thus, the formulated *Mbala-pinda* has a satisfactory hygienic quality. It is therefore important to promote this food by popularizing it among the population.

Keywords: Hygienic quality; fermented food; formulated food; Mbala-pinda.

1. INTRODUCTION

Fermented foods are of great importance in the diet, food security and social well-being of millions of people around the world. They contribute 20-40% of food worldwide [1]. Fermentation also contributes to the improvement of nutritional value, the development of compounds aromatics and the digestibility of the final product [2]. This method of food preservation has been used for centuries and many fermented foods are consumed around the world each nation has its own types and/or forms of fermented foods, representing the basic diet from the raw materials available [3]. Cassava and cereals such as maize, sorghum and millet are ground and fermented to obtain non-alcoholic products (pasta and drinks) and alcoholic beverages which are known by different names in the countries of East Africa. west [4].

In the Congo, these fermented products are eaten cooked in various forms, such as chikwangue (fermented cassava tuber), poto-poto (fermented corn), ntoba mbodi (fermented cassava leaves), *Mbala-pinda* (cassava rouie paste more peanut paste) [5, 3, 6]. Some of these products are eaten as main courses, others as appetizers. Food intended for human consumption cannot be consumed without an assessment of its quality. These are nutritional, organoleptic and hygienic aspects. The latter is used to study germs of hygienic interest, including total aerobic mesophilic flora, fecal and total coliforms, *Staphylococcus aureus*, Salmonella and Shighella [7]. With this in mind, we thought we'd check the hygienic quality of these *Mbala-pinda*. In fact, pathogenic microorganisms (those most commonly found in food) belong to the group of fecal coliforms, total coliforms, *Staphylococcus aureus*, Shigella and Samonella and are sometimes responsible for health problems [8,9]. *Mbala-pinda*, which is a Congolese food, must comply with the rules of hygiene necessary to preserve the health of consumers. This gap that the present study seeks to fill aims to assess the hygienic quality of formulated *Mbala-pinda*.

2. MATERIALS AND METHODS

2.1 Sample Type and Sampling

Five (5) samples of *Mbala-pinda* were analyzed in this study (Fig. 1). These were prepared at the INRSIIT Food Technology Laboratory according to the procedures shown in Figs. 2, 3, 4, 5 and 6. In this preparations, in addition to the classic formulation of *Mbala-pinda* composed of rouie cassava paste and peanut paste, the four other formulations were made up of corn, banana, taro and a mixture of these three ingredients (cassava, corn and taro). The samples were packed in plastic bags, placed in a cooler and taken to the Applied Microbiology laboratory of the National Institute for Research in Exact and Natural Sciences (IRSEN) for microbiological analysis.

2.2 Microbiological Analyses

2.2.1 Preparation of inocula

The surface and deep parts of a sample of *Mbala-pinda* were mixed under aseptic conditions using a sterile spatula in the sterile zone of the Bunsen burner. Then, 10 g of *Mbala-pinda* was collected and weighed around the flame of the Bunsen burner using a precision balance (GαG Electronic Scale T500, China). This mass was then aseptically introduced into a 250 mL bottle containing 90 mL of physiological water sterilized by autoclaving. The mixture was homogenized by manual stirring for approximately two minutes. This solution represents the mother solution diluted to 10^{-1}. A volume of 1 mL of the stock solution was then taken with a micropipette and inoculated into a test tube containing 9 mL of sterile physiological

water. This mixture has been homogenized as before and represents the 10^{-2} dilution. From this dilution, the process was repeated up to a 10^{-5} dilution. The different dilutions obtained are used as inocula [10].

2.2.2 Plating and Incubation

2.2.2.1 Total aerobic mesophilic flora

A volume of 100 µL of each dilution was removed with a micropipette and then applied to the surface of Plate Count Agar (PCA) medium (Oxoid; Basingstoke, UK). The culture dishes were incubated at 37°C for 48 hours in an oven (Memmert IN 110, Germany). Inoculations were performed in duplicate. After incubation, all colonies were counted manually.

2.2.2.2 Fecal coliform and total coliform

For coliforms, 100 µL of each dilution was taken with a micropipette and deposited on the surface of a violet red bile agar medium (VRBA; Oxoid, Scharleau, Spain). It was then spread over the entire surface of the agar medium in tight streaks. The dishes thus inoculated were incubated at 44°C for fecal coliforms and 37°C for total coliforms. At the end of incubation, only pink, red and purple colonies were counted manually. Two boxes were inoculated by dilution.

2.2.2.3 Staphylococcus aureus

A volume of 100 µL, dilutions 10^{-1}, $10^{-3}$and 10^{-5}, was spread separately in tight streaks over the entire surface of the Chapman agar using a pipette converted into a spreader by flambéing with a Bunsen burner. Dishes were incubated at 37°C for 48 hours and only yellow colonies were counted. Two boxes were inoculated by dilution.

2.2.2.4 Yeasts and mold fungi

One hundred (100) µL of each dilution was taken with a micropipette and applied to the surface of a chloramphenicol yeast glucose agar medium (NF.V 08-051-Feb.1999). The inoculum was then spread using a sterile Pasteur pipette converted into a spreader. After plating, the inoculated culture dishes were incubated at 37°C for 72 hours in a Mermmert oven (IN 110, Germany). Two Petri dishes were inoculated by dilution.

2.2.2.5 Salmonella and Shigella

Salmonella and *Shigella* were counted for presence or absence (AFNOR V 08 013 standard). Inoculations were performed as follows: A mass of 25 g of each *Mbala-pinda* sample was weighed on a precision balance as before and inoculated into 100 mL of the pre-enrichment broth. After incubation of this first culture, 1 mL is added to 10 mL of Rappoport broth in a tube. This second culture is incubated for 24 hours. At the end of this second incubation, 100 µL are taken with a micropipette and spread on the surface of the Salmonella and Shigella (SS) agar medium using the dial spreading method. The plates inoculated in this way are incubated for 24 hours at 37°C.

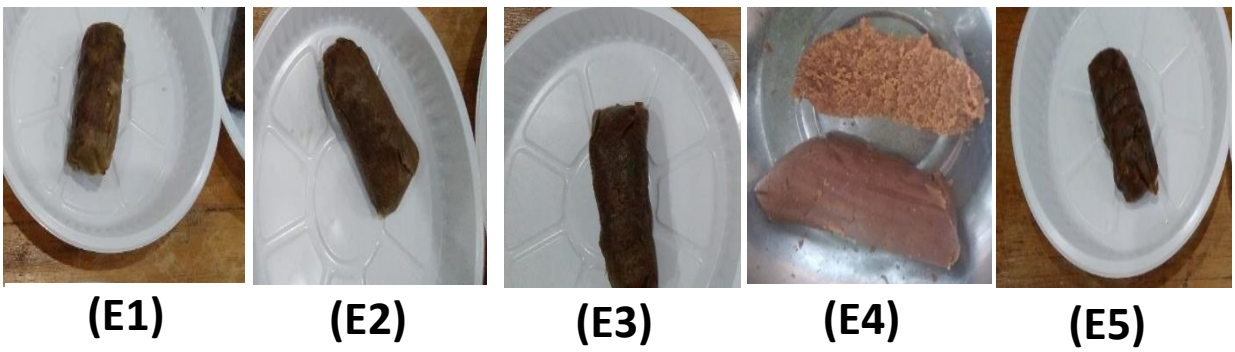

Fig. 1. Different samples of *Mbala-pinda* analyzed in this study: (E1) *Mbala-pinda* made from maize; (E2) cassava-based *Mbala-pinda;* (E3) *Mbala-pinda* made from the mixture*;* (E4) *Mbala-pinda* made from banana; (E5) *Mbala-pinda* made from taro

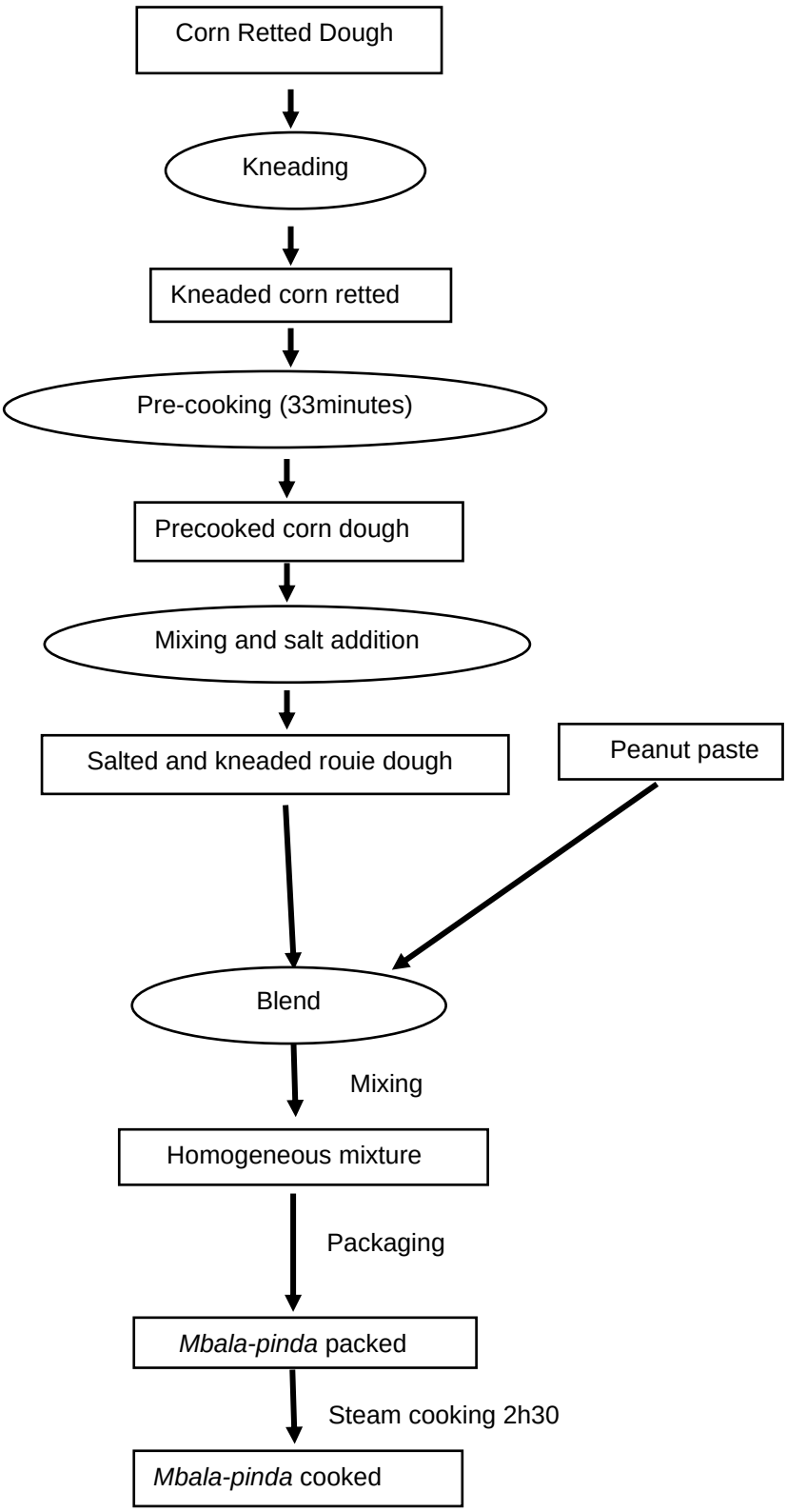

Fig. 2. Fabrication diagram for sample E1

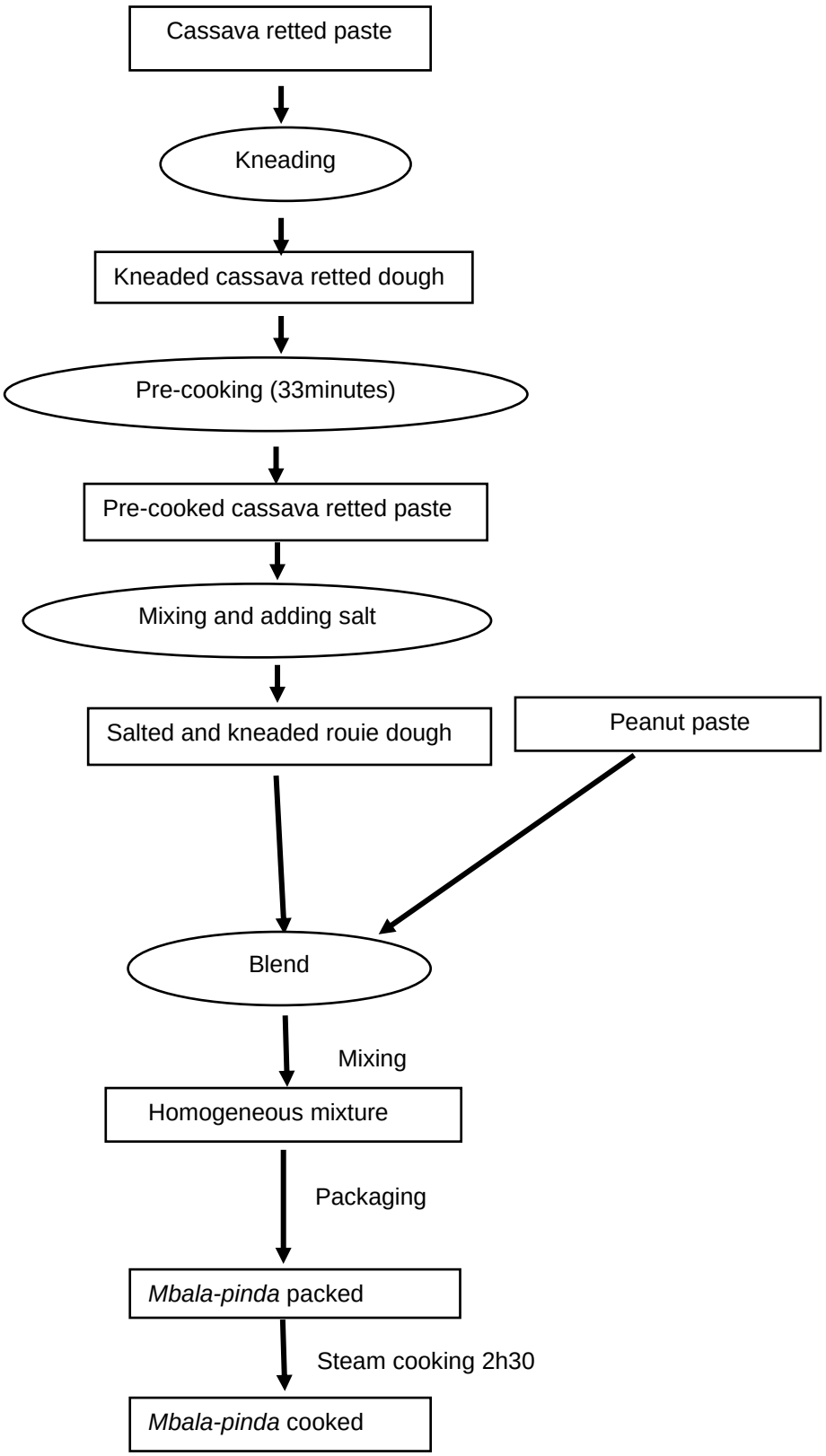

Fig. 3. Fabrication diagram for sample E2

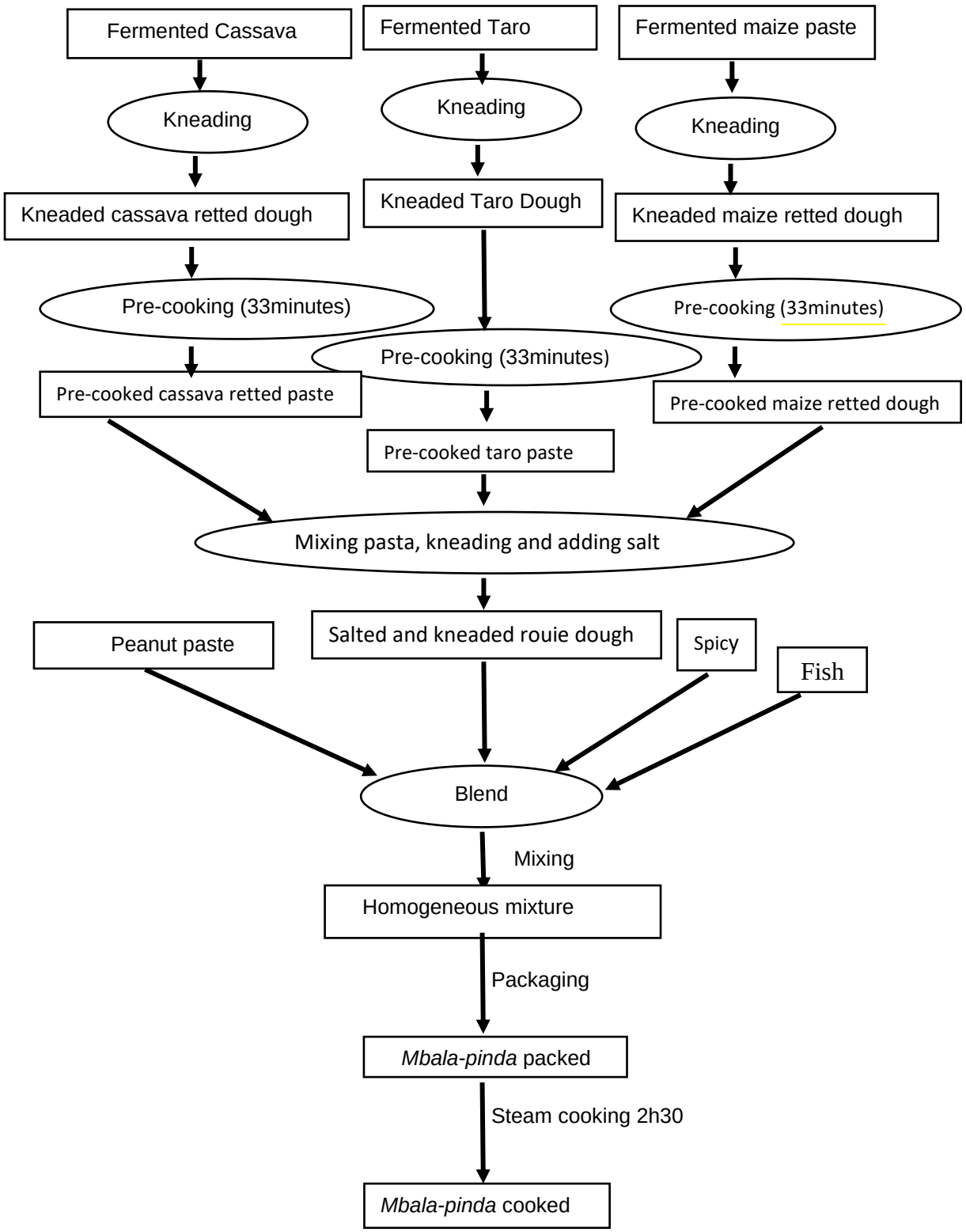

Fig. 4. Fabrication diagram for sample E3

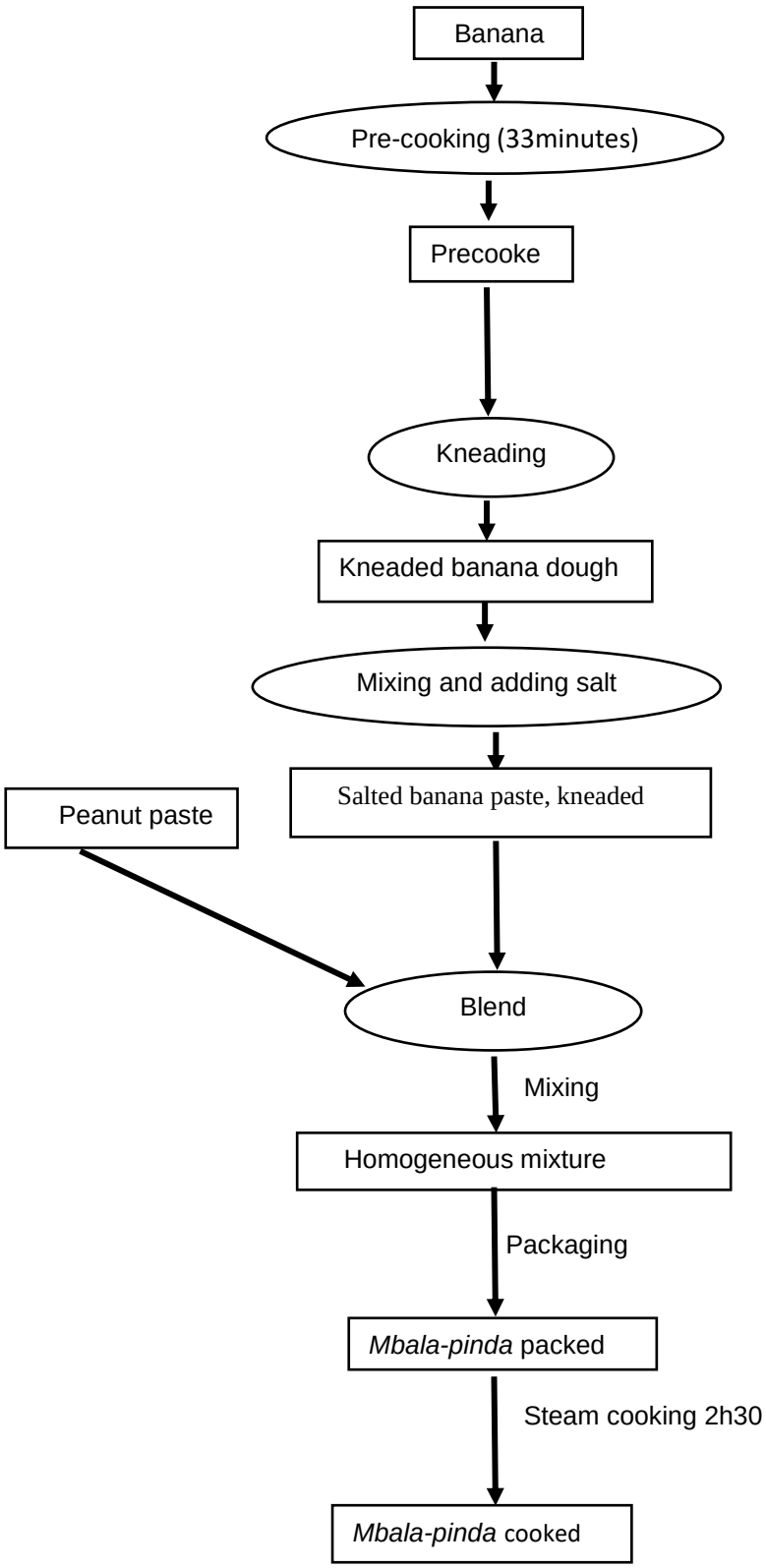

Fig. 5. Fabrication diagram for sample E4

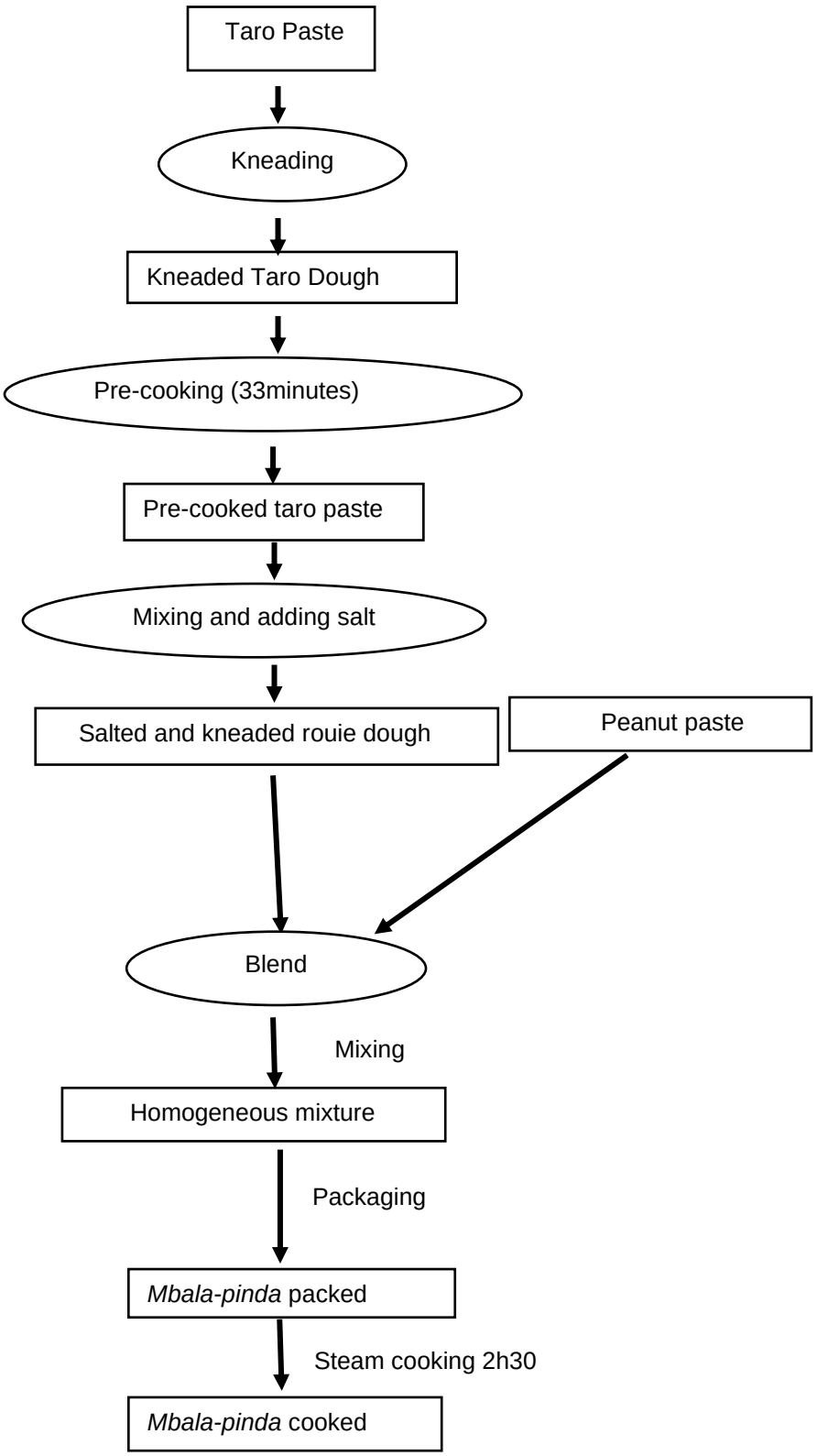

Fig. 6. Fabrication diagram for sample E5

2.2.3 Germs reading

At the end of the incubation, the culture dishes containing a number of colonies less than or equal to 300 were selected for the manual counting of the colonies. After counting, the number of bacteria or germs (N) in colony-forming units (CFU) per gram of sample was calculated using the following formula.

$$N\ (CFU/\ g\ sample) = \frac{n}{V_{I.D}} X \frac{V_{SM}}{V_M}$$

N= Bacterial count CFU/g
n= Colony average of the dilution taken into account
V= inoculum volume
D= dilution factor
V_{SM}= stock solution volume
V_M= Mass of the sample

2.2.4 Data handling

The data were processed using GraphPad Prism 7.00 for graphing, and the between-sample similarity dendogram was performed on Past 3.26 using the UPGMA matching algorithm and the Bray-Curtis similarity index with boot N of 1000

3. RESULTS

3.1 Total Aerobic Mesophilic Flora (FMAT)

Fig. 7 shows the number of microorganisms of the total aerobic mesophilic flora in the 5 samples of *Mbala-pinda*. Samples E1, E2 and E5 have a microbial load of 3.5. 10^{-3}, 3.1. 10^{-4} and 1.5. 10^{-3} CFU/g sample respectively, while samples E3 and E4 have a zero FMAT load, i.e. do not contain microorganisms of this group. All these samples have an FMAT load below the threshold set by Directive 2005/2073/EC.

3.2 Faecal and Total Coliform Load

None of the samples analyzed contained bacteria belonging to the group of total coliforms or faecal coliforms (Table 1). Thus, the microbial density is in compliance with the thresholds of Directive 2005/2073/EC, which are 10 and 10^3 CFU/g for faecal coliforms and total coliforms, respectively.

3.3 *Staphylococcus aureus* Load

Table 2 shows the results of the enumeration of bacteria belonging to the species *Staphylococcus aureus*. All samples analyzed are free from *Staphylococcus aureus*. And these samples comply with the Directive 2005/2073/EC. This shows their good quality with respect to this microbiological parameter.

3.4 Fungal Load

Table 3 shows the levels of fungi (yeasts and molds) in the 5 samples of *Mbala-pinda*. All samples tested are free of yeasts and molds.

3.5 *Salmonella* and *Shigella*

None of the five samples of *Mbala-pinda* analyzed showed colonies of Salmonella or Shigella after inoculation of the enrichment cultures on specific SS medium (Table 4).

3.6 Hierarchical Classification

Fig. 8 shows the similarity dendrogram between the samples. The latter was performed from the enumeration results. The samples were thus grouped into two classes based on their microbial load. A class formed by the pair *Mbala-pinda* E3 and *Mbala-pinda* E4, which are free of any microbial contamination with a similarity index greater than 0.9. And a second class formed by the rest of the samples. In this second class, samples E5 and E1, with a load of about 10^3 CFU/g, form a cluster (similarity index of about 0.6) associated with sample E2, whose microbial density is of the order of 10^4 CFU/g.

4. DISCUSSION

Fermented foods, whether they are consumed after cooking or not, are a staple part of the diet of people in the developing country [11]. This is because these foods are priced within the reach of low-income populations. However, the lack of control over the fermenting process means that the microbiological and nutritional quality of the final product varies and is not always satisfactory [12,13,3]. However, the consumption of food intended for human consumption cannot take place without an assessment of its quality. These are the nutritional, the organoleptic and the hygienic aspects. The objective of this work was therefore the evaluation of the hygienic quality of *Mbala-pinda*, a fermented food consumed in the Republic of Congo. The results of the microbiological analyses showed that all samples had an FMAT level below the threshold set by Directive 2005/2073/EC. In fact, samples E1, E2, E3, E4, and E5 have FMAT loads between 0 and

10^4 CFU/g sample. Studies carried out on traditional and industrial wheat flours show an average load of 4.10^4 CFU/g and $2.5.10^4$ CFU/g respectively. These levels are also below the threshold set by the Directive [7]. This low microbial density could be explained by adherence to good preparation or manufacturing practices. Failure to follow these rules could result in an overabundance of total mesophilic aerobic flora. The reasons for this abundance of FMAT are: the unhealthy environment in which the product is manufactured and the utensils used; non-compliance with hygiene regulations during the transformation of raw materials into products; Gusts of wind, dust and flies settling on the measurement ladle during the sale, in the case of liquid products sold without "Kindirmou" open-air packaging [12].

The count of bacteria belonging to the group of total and fecal coliforms did not reveal the presence of any colony of these bacteria in any of the *Mbala-pinda* samples analyzed. Thus, the microbial density complies with the threshold values of Directive 2005/2073/EC which are 10 and 10^3 CFU/g respectively for faecal coliforms and total coliforms. Our results are similar to those carried out on tomato purees, which showed no fecal coliform or total coliform colonies [14]. The absence of coliform bacteria

Table 1. Count fecal and total coliforms

Sample	fecal coliforms (CFU/g of sample)	Directive 2005/2073/EC (CFU/g)	Total coliforms (CFU/g of sample)	Directive 2005/2073/EC (CFU/g)
Mbala-pinda E1	0		0	
Mbala-pinda E2	0		0	
Mbala-pinda E3	0	10^1	0	10^3
Mbala-pinda E4	0		0	
Mbala-pinda E5	0		0	

Table 2. Count of *Staphylococcus aureus* in the 5 samples from *Mbala-pinda*

Sample	*Staphylococcus aureus* (CFU/g of sample)	Directive 2005/2073/EC (CFU/g)
Mbala-pinda E1	0	
Mbala-pinda E2	0	10^2
Mbala-pinda E3	0	
Mbala-pinda E4	0	
Mbala-pinda E5	0	

Table 3. Enumeration of yeasts and molds in the 5 samples of *Mbala-pinda*

Sample	Yeasts and molds (CFU/g sample)
Mbala-pinda E1	0
Mbala-pinda E2	0
Mbala-pinda E3	0
Mbala-pinda E4	0
Mbala-pinda E5	0

Table 4. Results of analyses of *Mbala-pinda* samples for absence or presence of *Salmonella* or *Shigella*

Sample	*Salmonella*	*Shigella*	Standard
Mbala-pinda E1	Absence	Absence	
Mbala-pinda E2	Absence	Absence	Absence / 25 g
Mbala-pinda E3	Absence	Absence	
Mbala-pinda E4	Absence	Absence	
Mbala-pinda E5	Absence	Absence	

could be explained by the fact that cooking prepackaged *Mbala-pinda* would destroy these microorganisms. The same is true for bacteria belonging to the *Staphylococcus aureus* species. In fact, these samples are also free of these germs and therefore comply with Directive 2005/2073/EC. Clearly, poor hand hygiene, the manufacturing environment, and faulty or contaminated equipment are likely sources of contamination. When these sources of contamination are controlled and brought under control, the final product will be of very good hygienic quality, as in the case of this study [15,16].

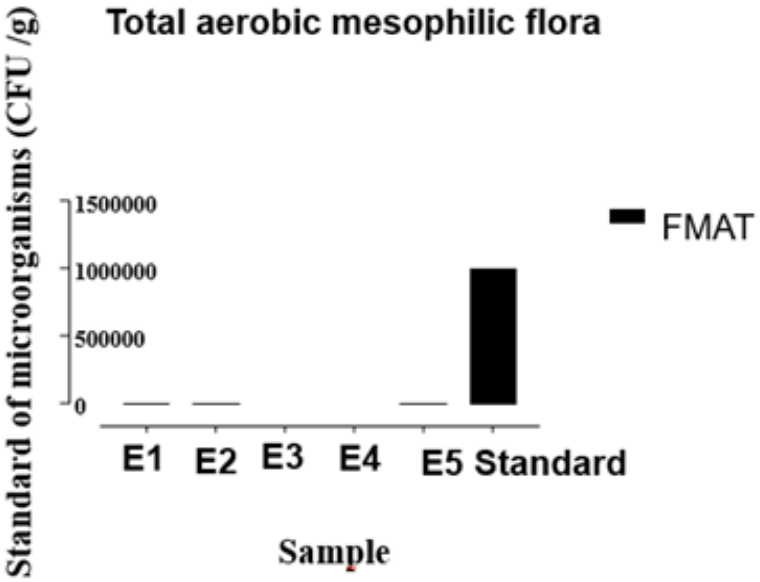

Fig. 7. Total aerobic mesophilic flora count

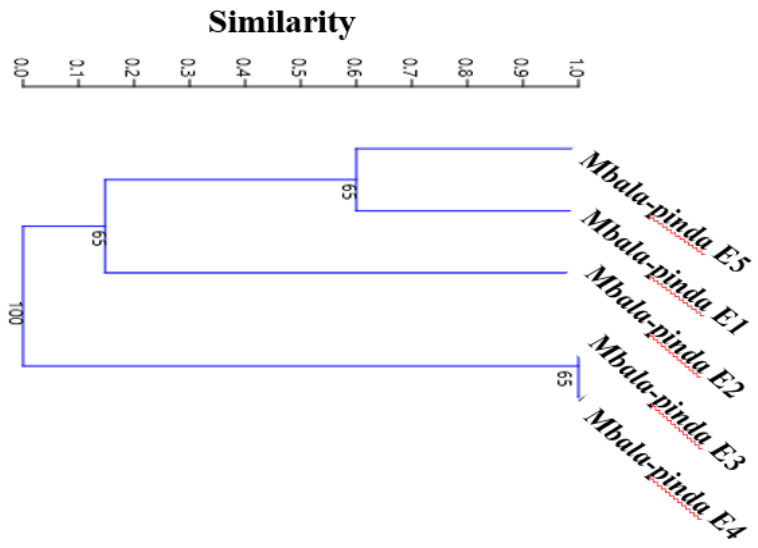

Fig. 8. Dendrogram of the similarity between the samples

With regard to yeasts and molds, the 5 *Mbala-pinda* samples tested were free of these fungi. Our results differ from those conducted on garba and maize bread or kandji [17,18]. This difference could be explained by the fact that *Mbala-pinda* is a packaged food and therefore protected from external contamination. On the other hand, the two foods analyzed in their studies [17,18] although packaged, are exposed during preparation and sold on the street after being unwrapped. Thus, these foods are within reach of any aerosols present in the ambient air.

Finally, the search for Salmonella species (sp.) and Shigella species (sp.) in enrichment cultures by plating on specific SS medium showed a total absence of these germs in all *Mbala-pinda* samples analyses. These results confirm those obtained on street food in Cotonou [19]. This absence of pathogenic bacteria can be explained by good manufacturing practices, such as sanitizing the food preparation environment and following asepsis rules.

5. CONCLUSION

The aim of this work was to assess the hygienic quality of Mbala-pinda and these formulations by incorporating plantain, taro and maize. The results of the microbiological analyses show that these samples comply with Directive 2005/2073/EC. They are therefore of very satisfactory microbiological quality and are likely to be subjected to sensory analysis. As Salmonella species (sp) is a very dangerous bacteria is responsible for typhoid fever, its presence in the samples would have raised serious questions. The absence of these germ in these samples of Mbala-pinda validates the manufacturing process and the formulations obtained.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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