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Microbiological properties of the pulp of different forms of shea (*Vitellaria paradoxa*) in the northern region of Côte d'Ivoire

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Abstract

Shea fruits (*Vitellaria paradoxa*) were harvested in the departments of Korhogo (Lataha, Nalourgokaha, Dahouakaha and Dikodougou), Boundiali and Ferké (Houphouekaha) in northern Côte d'Ivoire. The aim of this work is to contribute to food security. To this end, research and enumeration of pulp microorganisms were carried out in the different study areas. Microbiological analyses revealed that fruit pulp is colonized by microorganisms. The pulp of the rounded shape is more colonized by microorganisms than that of the ovoid shape. In addition, the microbiological analysis showed an unsatisfactory result with reference to recommendations for bacteria such as Aerobic Mesophilic Germs, Enterococci, Faecal Coliforms, Aerobic Sulphite-Reducing Germs.

Keywords: *Vitellaria paradoxa*; Pulp; Variability; Microorganisms

1. Introduction

In Côte d'Ivoire, many wild fruits are consumed by the rural population for their numerous nutritional and health benefits [1]. These include baobab, néré, tamarind, etc. However, there is another fruit that is richer in nutrients and found in sufficient quantities in the savannahs of Côte d'Ivoire. The shea tree produces a fruit consisting of pulp and nuts.

Studies carried out on shea fruit pulp from Mali, Burkina Faso, Cameroon, Uganda, Chad and Nigeria have shown that it contains a significant quantity of nutrients. It contains carbohydrates, proteins, fiber, minerals and vitamins [2,3,4]. The abundance of these nutrients enables the growth of numerous microorganisms.

Although shea pulp has been the subject of numerous studies in other countries, to our knowledge no studies have yet been carried out on this vegetative material in Côte d'Ivoire. Yet this pulp can be colonized by microorganisms with undesirable characteristics. It is these observations that have guided this study. The aim of this work is to contribute to food safety. The various aspects addressed by this study are the enumeration of Aerobic Mesophilic Germs (AMG), faecal coliforms, Anaerobic Sulphito-reducers (ASR) and enterococci.

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2. Materials and methods

2.1. Materials

2.1.1. Plant material

The plant material used was fresh shea (*Vitellaria paradoxa*) pulp harvested in the Korhogo (Lataha, Nalourgokaha, Dahouakaha and Dikodougou), Boundiali and Ferké (Houphouekaha) departments. Harvesting was carried out in plantations located in these departments.

2.1.2. Culture media

- The culture media used for enumeration and isolation were
- Plate Count Agar (PCA) for aerobic mesophilic germs (AMG);
- VRBG (crystal violet and red bile) agar for coliforms;
- TSN (tryptone sulfite neomycin) agar for sulfite-reducing anaerobes;
- BEA (Bile Esculin Azide) agar for the detection of enterococci;
- buffered peptone water (BPW) for various dilutions

2.2. Methods

2.2.1. Sampling

Sampling took place in the departments of Korhogo (Lataha and Nalourgokaha), Sinematiali (Dahouakaha), Dikodougou, Boundiali and Ferké (Houphouekaha) between June and July 2022. The departments and shapes of the shea fruit (*Vitéllaria paradoxa*) were selected on the basis of a preliminary survey of shea butter wholesalers in Natio, Korhogo. This direct interview survey revealed the abundance of shea fruits in these departments, and the predominance of ovoid and rounded shapes among the fruits. In these departments, sampling was carried out with the help of two (02) volunteer village guides. Fruits were harvested at maturity from wild shea plants in each of these localities. Two (02) batches of fruit were collected from twenty (20) shea trees in each locality, taking into account the shape of the fruit. Ten (10) trees were used to collect round-shaped fruit and the other ten (10) for ovoid-shaped fruit. Sixty (60) samples of each fruit shape were collected from the six (06) sites at a rate of 10 samples per site for the first sampling session. Three (03) sampling sessions were carried out for a total of 360 samples (180 samples of the ovoid shape; 180 samples of the rounded shape) (Table I). The fruit collected was placed in labelled stomacher bags and transported to the laboratory using a cooler containing ice.

Table 1 Sampling summary

		Shea fruit shape		
Localities	**Sampling session**	**Ovoid**	**Rounded**	**Total**
Lataha	3	30	30	60
Nalourgokaha	3	30	30	60
Dahouakaha	3	30	30	60
Dikodougou	3	30	30	60
Boundiali	3	30	30	60
Houphouekaha	3	30	30	60
Total	3	180	180	360

2.2.2. Pulp extraction

Aseptically separated from the kernel, the pulp obtained was ground using a blender for 3 minutes. The crushed pulp was packaged in sterile jars and stored in a freezer for analysis of the various parameters (figure 1).

Figure 1 Pulp crushed in sterile jars

2.2.3. Preparation of stock suspension and dilutions

Fresh pulp samples were ground under aseptic conditions (stomacher bags) prior to preparation of the mother suspension. The mother suspension was prepared by adding 10 g of freshly ground shea pulp to 90 mL of sterile peptone water (EPT) (AES Laboratory, COMBOURG France). The resulting mixture constitutes the 10^{-1} dilution stock suspension. From this suspension, several successive decimal dilutions were made. To prepare the 10^{-2} dilution, 1 mL of the stock suspension was removed aseptically from a Bunsen burner flame using a sterile pipette, and placed in a test tube containing 9 mL of sterile buffered peptone water (BPW). Using the same technique, dilutions ranging from 10^{-3} to 10^{-8} were obtained.

2.2.4. Research and enumeration of microorganisms

Enumeration of Aerobic Mesophilic Germs (AMG)

AMG enumeration was carried out in accordance with AFNOR standard NF V08-051. The medium used for the enumeration of mesophilic aerobic germs is PCA gelose (Plate Count Agar). Inoculation was performed by incorporation. To do this, 1 mL of the mother suspension or decimal dilutions were deposited in empty Petri dishes. Then 12 to 15 mL of pre-prepared PCA gelose, supercooled to 45°C, were poured into the dishes containing the inoculum. The mixture was homogenized by slow agitation. After solidification, a second layer of 4 to 5 mL of PCA agar was poured onto the first layer to prevent the plates from being invaded by certain germs. Inoculated Petri dishes were incubated at 30°C, followed by reading 24 to 72 hours later. Plates containing 30 to 300 colonies were selected.

Enumeration of Fecal coliform

Fecal coliform were counted in accordance with AFNOR standard NF ISO 4832 July 1991. The medium used for coliform enumeration was crystal violet and red bile lactose agar (VBRG). Inoculation was performed by incorporating 1 mL of the stock suspension or retained dilutions into sterile Petri dishes. Next, 12 to 15 mL of the supercooled medium was poured at 45°C into the Petri dishes containing the inoculum, then the mixture was homogenized by gentle agitation. After solidification, a second layer of 4-5 mL of the same medium was poured onto the first layer. Incubation took place at 44°C for 24 h. Counting was done by counting colonies. Plates containing between 15 and 300 colonies were retained.

Enumeration of Anaerobic Sulfite-reducing germs

Sulfite-reducing anaerobes were enumerated using the method of [5]. The medium used was tryptone sulfite neomycin agar (TSN). For inoculation, 1 mL of the mother suspension and/or decimal dilutions were taken and inoculated in duplicate into the mass of TSN agar previously melted and kept in supercooling tubes at 45°C. Once the agar had solidified, the tubes were incubated in an oven at 30°C. Large black colonies in the tube were counted. Plates containing between 15 and 150 colonies were selected.

Enumeration of enterococci

Enterococci enumeration was carried out in accordance with ISO 7899. BEA (Bile Esculin Azide) agar was used for enterococci enumeration. These microorganisms hydrolyze esculin, which appears as a black halo of esculin associated with iron around the colonies. On the solidified medium in the Petri dishes, 0.1 mL of the mother suspension or of the dilutions retained was deposited and spread with a rake. Petri dishes were incubated in an oven at 30°C for 24 to 48 hours. Colonies appeared as blackish dots. Plates containing 30 to 300 colonies were selected.

2.2.5. Expression of microorganism load results.

The germ load was determined according to the ISO 7218 formula

$$\mathbf{N\ (UFC/g)} = \frac{\mathbf{\Sigma\ C}}{\mathbf{V(\ n1\ +0.1n2)d}}$$

Σ C : sum of colonies on counted boxes
V: volume of inoculum
n_1 : number of Petri dishes inoculated with the first dilution
n_2 : number of Petri dishes inoculated with the second dilution
d : dilution retained
UFC/g: Colony Forming Unit per gram

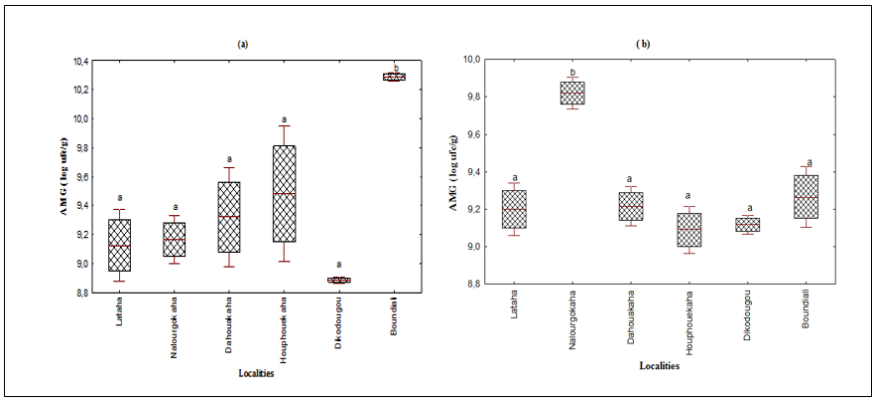
2.3. Statistical processing of data

Results are presented as mean followed by standard deviation. Single-factor analysis of variance (ANOVA) and the Newman Keuls test with a threshold of 5% were used to allocate homogeneous groups for microbiological parameters, using STATISTICA version 99 software. The choice of this test was made after verification of the normality and homogeneity of the variances.

3. Results and discussion

3.1. Mesophilic aerobic germs (MAG)

Figure 2 shows the mesophilic aerobic germ load. Aerobic mesophilic germ loads ranged from 8.86±0.02 log ufc/g to 10.29±0.03 log ufc/g for the rounded form, and from 9.10±0.13 log ufc/g to 9.82±0.08 log ufc/g for the ovoid form. The highest (10.29±0.03 log ufc/g) and lowest (1.59±0.16 log ufc/g) loads were recorded in the rounded form from Boundiali and Dikodougou respectively (P<0.05). These microbial loads are well above the ISO: 7218 standard of May 1996 (<310^3 ufc/g) for foodstuffs. The microbiological quality of shea pulp in both forms is unsatisfactory in terms of ISO: 7218 (May 1996). The results of this study are superior to those of [6], who observed an average total load of 3.67 log ufc/g of Aerobic Mesophilic Germs in the different samples of baobab *(Andonsonia digitata*) pulp. This difference can be explained by the amount of sugar present in the pulp of these fruits. Indeed, microorganisms use the sugar contained in food for their growth [7, 8]. The high loads observed in our various samples are probably due to exposure of the shea fruit to the open air, and to the action of insects attracted by the high sugar content of the shea fruit pulp.

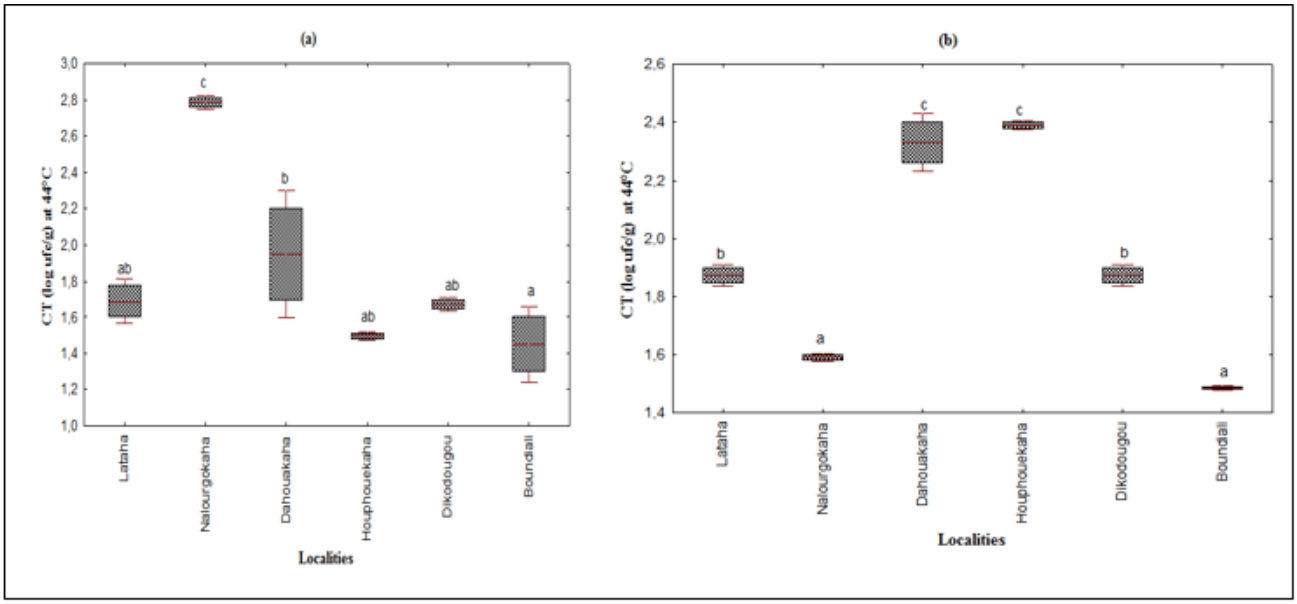


a: rounded shape; b: ovoid shape.
Sprouts from localities with similar letter do no indicate a significant difference P<0.05

Figure 2 Average AMG loads in different localities, by shape

3.2. Faecal coliforms

Fecal coliform loads ranged from 1.45 ± 0.21 log ufc/g to 2.79 ±0.04 log ufc/g for the rounded form, and from 1.49 ± 0.01 log ufc/g to 2.39 ±0.01 log ufc/g for the ovoid form. With an average load of 1.45 ± 0.21 log ufc/g, Boundiali recorded the lowest load in pulps of the rounded shape (P<0.05). The highest load (2.79 ±0.04 log ufc/g) is obtained in the rounded shape of the Nalourgokaha locality (P<0.05) (Figure 3). The microbiological quality standard for faecal coliforms is 0.3 log ufc/g. A high fecal coliform load can lead to product spoilage and constitute a risk for the presence of pathogenic germs [9]. Most people eat or handle fruit without washing their hands. This attitude is contrary to OMS guidelines, which recommend hand washing with soap or any other detergent containing an antiseptic agent to eliminate any contaminants [10]. Failure to wash hands with soap or any other disinfectant after defecation is associated with widespread faecal contamination of hands [11]. In the course of the study, it was observed that the majority of farmers used manure from livestock to fertilize crop soils. The presence of faecal coliforms in the pulp could be due to indirect contamination by faecal bacteria from the animals during the fertilization process using manure, or through direct contact with humans during harvesting [12,13].



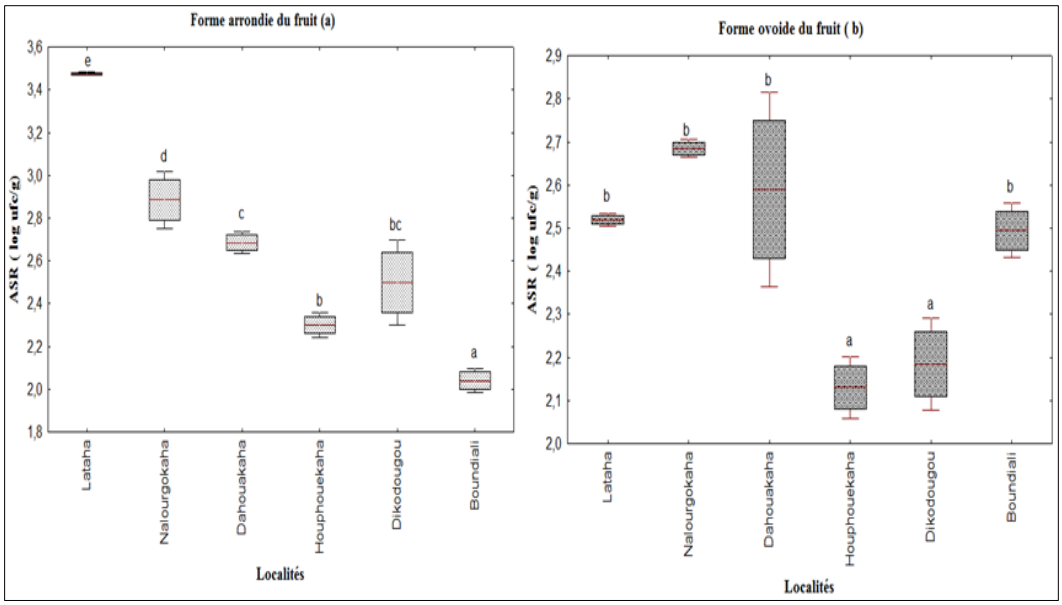
a: rounded shape; b: ovoid shape.

Sprouts from localities with similar letter do no indicate a significant difference P<0.05

Figure 3 Average faecal coliform loads in different localities according to form

3.3. Anaerobic sulfite-reducing germs

Figure 4 shows the results of sulfite-reducing anaerobic bacteria (SRSA) counts for the rounded and ovoid forms from the various locations studied. The counts ranged from 2.04 ±0.06 log ufc/g to 3.47±0.01 log ufc/g for the rounded form, and from 2.13 ±0.07 log ufc/g to 2.68±0.02 log ufc/g for the ovoid form. The average anaerobic-sulfite-reducing germ load was highest at Lataha (P<0.05) and lowest at Boundiali, in the rounded form respectively. These results obtained in the course of this study exceed the ISO: 7218 food standard of May 1996, which stipulates counts of less than 1.47 log ufc/g. The presence of these pathogens in shea pulp from *Vitéllaria paradoxa* could be linked to the contact of these fruits with the soil [14]. Collection equipment, handling and the quantity of water present in the shea pulp are parameters that could also be at the origin of the microbial loads obtained.



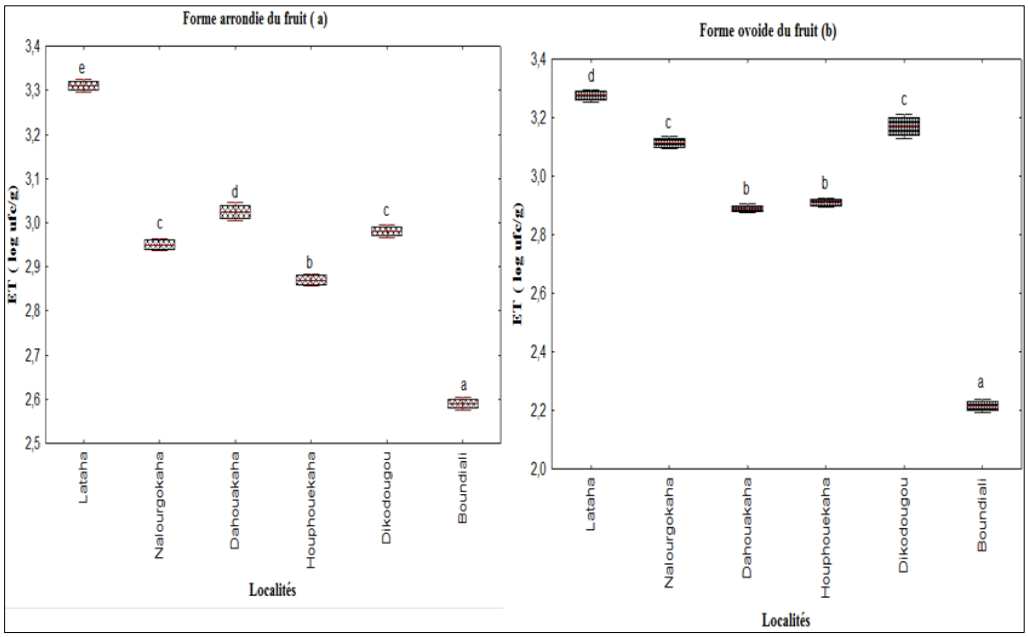
a: rounded shape; b: ovoid shape.

Sprouts from localities with similar letter do no indicate a significant difference P<0.05

Figure 4 Average ASR loads in different localities, by shape.

3.4. Enterococci

Figure 5 shows the enterococci loads recorded in shea pulp. Enterococci load ranged from 2.59 ± 0.01 log ufc/g to 3.31 ± 0.01 log ufc/g for the rounded form and from 2.22 ±0.02 log ufc/g to 3.28 ± 0.02 log ufc/g The highest value (P<0, 05) was obtained in the rounded form at Lataha (3.31 ± 0.01 log ufc/g), while the lowest (P<0.05) was obtained in the ovoid form at Boundiali (2.22 ±0.02 log ufc/g).



a: rounded shape; b: ovoid shape.

Sprouts from localities with similar letter do no indicate a significant difference P<0.05

Figure 5 Average enterococci load in different localities according to form.

4. Conclusion

This work is part of a food safety program. Its aim is to assess the quality of shea pulp sold on markets and consumed by farmers during field work. The study revealed that round *Vitéllaria paradoxa* pulp is more contaminated with microorganisms than ovoid pulp. The microorganisms investigated and detected in shea pulp during this study were aerobic mesophilic germs, enterococci, faecal coliforms and sulphite-reducing anaerobes. Microbiological analysis showed an unsatisfactory result with reference to the recommendations for bacteria such as aerobic mesophilic germs, enterococci, faecal coliforms and sulphite-reducing anaerobes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author's contributions

All authors participated in the redaction of this document.

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