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Indigenous Perception and Characterization of *Yanyanku* and *Ikpiru*: Two Functional Additives for the Fermentation of African Locust Bean

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Indigenous perception, processing methods, and physicochemical and microbiological characteristics of Yanyanku *and* Ikpiru*, two additives used to produce fermented African locust bean condiments,* Sonru *and* Iru*, were evaluated. According to producers, these additives accelerate the fermentation and soften the texture of the condiments.* Yanyanku *is produced by spontaneous fermentation with either* Hibiscus sabdariffa *or* Gossypium hirsutum *or* Adansonia digitata *seeds, whereas only* Hibiscus sabdariffa *seeds are used for* Ikpiru. *Both additives, with pH values ranging between 6.2 and 10 and* Bacillus *spores varying between 5.5 and 8.9 Log_{10} (CFU/g), could be considered as softening additives or enrichment inocula to produce condiments.*

KEYWORDS yanyanku*,* ikpiru*, additives,* sonru*,* iru*,* parkia biglobosa

Food condiments obtained from the traditional processing of leguminous seeds are largely used in the diet of many poor people in West Africa (Ndir et al. 2000; Parkouda, Diawara, and Ouoba 2008). Processing methods applied involve alkaline fermentation which brings diversity into the kinds of foods available, makes otherwise inedible foods edible, enhances the nutritional value, decreases toxicity, preserves food, flavors dishes, and

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decreases cooking time and energy requirement (Steinkraus 1996). As far as nutritional value is concerned, several studies have reported that such condiments are good sources of protein and lipid, with increasing levels of free amino acids, increasing concentration of cysteine, methionine, leucine, isoleucine, tyrosine, phenylalanine, and even lysine, which is limiting in plant foods. It has been shown also that fermentation microorganisms involved in the process hydrolyze non digestible and flatulence producing carbohydrates into sugars which are readily digestible by humans and in addition, positively affect the texture of the product by softening the tissue. The presence of linoleic and oleic acids, which can be converted into polyunsaturated fatty acids, enhancing then the nutritive value of the products, has also been reported (Parkouda et al. 2008). Furthermore, Omafuvbe, Shonukan, and Abiose (2000) and Sarkar and Tamang (1995) reported that some fermented condiments are a good source of mineral elements such as iron, potassium, calcium, magnesium, sodium, and zinc, while Sarkar and colleagues (1998) recorded increases in vitamin B_2 (riboflavin) and vitamin B_3 (niacin).

These physicochemical and nutritional changes in the seeds during alkaline fermentation are the results of the activities of microorganisms naturally present in the production environment. In West Africa, the natural fermentation of African locust beans has been reported as an "uncontrolled fermentation," which is performed under uncontrolled environmental conditions, by the microorganisms present in the production environment. Consequently, the quality of the products is highly variable (Gutierrez et al. 2000; Azokpota, Hounhouigan, and Nago 2006; Parkouda et al. 2008) and failure of fermentation can occur resulting in the loss of the products. A major factor, which could stabilize the quality of products from one production batch to another, is the development and use of starter cultures for controlled fermentation (Achi 2005). However, recent studies on the traditional process showed also that some practices have been developed to get the fermentation of the seeds under control. It is the case of *Yanyanku* and *Ikpiru* used as functional additives to produce food condiments like *Sonru* and *Iru* in Benin (Azokpota et al. 2006). While those traditional food condiments were widely documented, *Yanyanku* and *Ikpiru* remain poorly investigated. The characterization for further possible improvement of these traditional additives is necessary to overcome the problem of variability of the condiments quality. This work aims to (1) collect information related to the producers' perception (softening texture, disintegration behaviour, enhancing taste and brightness) on *Yanyanku* and *Ikpiru* used as functional additives to produce African locust bean food condiments in Benin, their major quality attributes as well as their processing technologies and (2) to determine the basis of their functional properties through their microbiological and physicochemical characteristics.

MATERIALS AND METHODS

Survey and Samples Collection

A survey was conducted in seven municipalities of Benin where locust bean condiments are highly produced, including Pobè, Adja-Ouèrè, Kétou (Southern Benin), Abomey and Bohicon (Centre), and Parakou and N'dali (Northern Benin). In each municipality, producers in three to seven villages were selected in collaboration with the staff of the Regional Agricultural Production Office, on the basis of their experience in the production of traditional additives and African locust bean condiments. A total of 171 producers of traditional additives/condiments were interviewed using a semi-structured questionnaire. Data related to the nature and roles of additives used, the processing technologies, the quality criteria as perceived by the users, and the methods of storage and the shelf life of additives were collected. Samples of traditional additives were collected in stomacher bags, kept in ice-boxes for transport and then stored at 4°C for further analyses. Fifteen samples (15) of ash filtrate used as ingredients for additives preparation were also collected for pH determination.

Microbiological Analysis

Ten grams of *Yanyanku* or *Ikpiru* were added to 90 mL sterile peptone-physiological salt solution (5 g peptone, 8.5 g NaCl, 1000 mL distilled water, pH 7.0 ± 0.2) and homogenized for about two (2) minutes using a Stomacher Blender (Seward Laboratory Stomacher 400, England) at normal speed. Ten-fold dilutions were then prepared and the micro-organisms were enumerated by drop plate method (Herigstad, Hamilton, and Heersink 2001). Enumeration of aerobic mesophilic bacteria was carried out on Plate Count Agar (OXOID, CM, 0463, Basingstoke, Hampshire, England) incubated at 30°C for 72 hours; lactic acid bacteria on de Man Rogosa and Sharpe (OXOID, CM 0361, Basingstoke, Hampshire, England) incubated in anaerobic jar at 30°C for 48 hours; total and faecal coliforms on Violet Red Bile Agar (OXOID, CM, 0107, Basingstoke, Hampshire, England) incubated at 37°C for 48 hours and 44°C for 24 hours, respectively. Yeasts and molds were enumerated after incubation at 25°C on MYGP supplemented with chloramphenicol and chlortetracycline according to Jespersen et al. (1994). *Bacillus* were enumerated by counting *Bacillus* spores as reported by Harrigan and McCance (1976). For *Bacillus* spores count, 5 ml of the primary dilution was heated at 80°C for 10 minutes. Colonies were examined by microscopy (X1000 ZEISS AXIOSTAR plus, Germany). *Bacillus* species were determined as Gram positive catalase positive rods which mostly bore phase bright spores (Terlabie, Sakyi-Dawson, and Amoa-Awua 2006).

Physicochemical Analysis

The dry matter, crude protein and ash content of additives were determined by Association of Official Analytical Chemists (AOAC) methods 27.005, 27.007 and 27.009, respectively (Association of Official Analytical Chemists 1984). The pH of the additives and ash filtrate was measured according to Nout and colleagues (1989).

Statistical Analysis

Data from survey were analyzed using a descriptive and multivariate method. Data related to physicochemical and microbiological analysis were subjected to analysis of variance using Statistica 7 (StatSoft, Tulsa, Oklahoma, USA) followed by Student-Newman-Keuls test. Significance was determined at $p < .05$.

RESULTS AND DISCUSSION

Distribution of African Locust Bean Condiments and Associated Functional Additives Used

Five types of condiments obtained by natural fermentation of African locust bean (*Parkia biglobosa*) were identified in the communities investigated: *Tchakara*, *Afitin*, *Sonru*, *Sonru-babaru* and *Iru* (table 1), while two additives locally called *Yanyanku* (or *Yinyinku*) and *Ikpiru* (or *Igberu*) were identified. Only three of these food condiments (*Sonru*, *Sonru-babaru*, and *Iru*) are produced using these additives. A highly significant association was observed among food additives, condiments, and producers' communities since condiments and additives used, if any, are specific to the production zones. *Sonru* and *Sonru-babaru*, requiring the use of *Yanyanku*, are specific to Parakou and N'dali (in the North of Benin), whereas *Iru* is produced particularly in Kétou, Pobè, Adja-Ouèrè (in the South of Benin) using *Ikpiru* as additive.

Producers' Perception of the Functional Quality of Traditional Additives

Use of traditional additives for locust bean condiments fermentation

Sonru and *Iru* processing includes a first long cooking (about 12 hours) of the seeds, dehulling and a short cooking (about 2 hours) of the cotyledons; *Yanyanku* (for *Sonru*) and *Ikpiru* (for *Iru*) are used at the end or during this second short cooking before fermentation (Azokpota et al. 2006). Indeed,

TABLE 1 Processes and Some Characteristics of Beninese Traditional Condiments

Traditional condiment	Additives	Food use	Process unit operations	Number of intact cotyledon[a] in 100 g of condiment [range]	Number of intact dehulled locust bean seeds in 100 g of condiment
Tchakara ($n = 10$)	–	- Consumed alone or with hot pepper - Vegetable sauce	Cleaning, cooking (about 12 hours, 100°C), dehulling, sorting, washing, coloration, cooking (about 2 hours, 100°C), fermentation (20–24 hours)	[540–610]	30–110
Afitin ($n = 10$)	–	- Vegetable sauce - Slimy sauce	Cleaning, cooking (about 12 hours, 100°C), dehulling, sorting, washing, cooking (about 2 hours, 100°C), fermentation (18–24 hours), grinding, blending	[310–470][b]	0
Sonru ($n = 10$)	*Yanyanku*/ *Yinyinku*	- Consumed alone - Vegetable sauce - Slimy sauce	Cleaning, cooking (about 12 hours, 100°C), dehulling, sorting, washing, cooking (about 2 hours, 100°C), fermentation (36–48 hours)	[330–410]	0–5
Sonru-babaru ($n = 10$)	*Yanyanku*/ *Yinyinku*	- Vegetable sauce - Slimy sauce	Cleaning, cooking (about 12 hours, 100°C), dehulling, sorting, washing, cooking (about 2 hours, 100°C), fermentation (36–48 hours), sun drying, pounding	[200–210]	15–20
Iru ($n = 10$)	*Ikpiru* /*Igberu* or plant stem	- Vegetable sauce - Slimy sauce	Cleaning, cooking (about 12 hours, 100°C), dehulling, sorting, washing, cooking (about 2 hours, 100°C), fermentation (36–48 hours) Salting, Blending	[130–260]	5–10

[a]Whole and partially cut (more than half) in 100 g of each condiment type.
[b]Range.

67% (39/58) of respondents used these additives at the end of the second cooking, before draining. In this respect, after adding additives and stirring, the hot cooking water is drained off immediately and the cotyledons are then spread into calabash trays, wrapped with clothes and left to ferment for 48 h. For 47% of the respondents, the cotyledons could also be cooked for about 10 min after adding the additives and stirring, before draining off the hot cooking water. There was a great variation in the proportion of *Yanyanku* or *Ikpiru* used. About 8.4 to 120.9 g of *Yanyanku* or *Ikpiru* (mean value of 59.1 ± 45.4 g; $n = 22$, representing 38% of the users interviewed) were used for 100 kg of seeds. Indeed, according to producers, the proportion of additives used depends on its effectiveness during usage.

Role and quality of *Yanyanku* and *Ikpiru*

The semantic of the local names of *Yanyanku* and *Ikpiru* explains the perception of the producers on the role of these additives during the processing of African locust bean to produce *Sonru*, *Sonru-babaru* or *Iru* in Benin. *Yanyanku* (or *Yinyinku*) means "that makes the product soft" in Bariba language, while in Nagot language, *Ikpiru* (or *Igberu*) means "that helps to rise." All the interviewed *Sonru* and *Sonru-babaru* producers (100%) used *Yanyanku* and about 58% of them prepared this additive themselves whereas the others (42%) buy it at the local market. From a total of 41 *Iru* producers (i.e., 24% of the interviewed producers), only 32% used *Ikpiru* to prepare *Iru* while the others (68%) use plant (*Jatropha curcas* or *Cissus arguta*) stem.

Sonru, *Sonru-babaru* and *Iru* undergo more than 24 h of fermentation with the use of additives. *Tchakara* and *Afitin* are processed without additives and undergo 18–24 hours of fermentation. Consequently, their texture is less soft and less pasty, with more individualized cotyledons, compared to *Sonru*, *Sonru-babaru*, and *Iru*, which is illustrated by the lower number of intact cotyledons per 100 g of these condiments (310–610 in *Tchakara* and *Afitin* against 130–410 in *Sonru*, *Sonru-babaru* and *Iru*; table 1). *Afitin* producers generally grind a part of the fermented cotyledons to get a slightly soft texture appreciated by *Afitin* consumers, which is noticed through the lower number of intact cotyledons in 100 g of the condiment (310–470) compared to that of *Tchakara* (540–610).

Although all the additives investigated are used during the second cooking operation before fermentation, it seems evident for 96% of producers using *Yanyanku*, 67% of those using *Ikpiru,* and 40% of those using plant stems that they play a role in the softening of the cotyledons during the fermentation (table 2). Whereas there is no evidence that the additives studied affect the taste and aroma of the condiments even if some producers (7% of producers using *Yanyanku*) affirmed this, 30% of respondents consider that

TABLE 2 Producers' Perception on the Functional Properties of the Additives Used for the Fermentation of African Locust Bean

	Yanyanku		*Ikpiru*		Plant stems[c]	
Functional properties	*n*[a]	%[b]	*n*	%	*n*	%
Softening the cotyledons of locust bean during fermentation	44	96	8	67	10	40
Softening the cotyledons of locust bean during cooking	–	–	2	17	5	20
Enhancing the brightness of the cotyledons during fermentation	14	30	–	–	1	4
Improving the condiment taste and aroma	3	7	–	–	2	8

[a]Number of respondents who quoted a particular functional property.
[b]Percentage of respondents using the additive.
[c]*Jatropha curcas* or *Cissus arguta*.

Yanyanku enhances the color of the products. This color change is probably associated with the development of microorganisms on the surface of the cotyledons during fermentation. According to 60% (35/58) of respondents using the additives, the quality of *Yanyanku* and *Ikpiru* is assessed through their ability to soften the texture of the condiments during fermentation.

Yanyanku and *Ikpiru* processing techniques

The *Yanyanku* and *Ikpiru* making processes involve cooking, draining, fermentation, pounding/crushing, moulding (if necessary) and sun drying (figures 1 and 2). However, variations were observed between and within these steps according to the know-how of the producers. *Hibiscus sabdariffa, Gossypium hirsutum or Adansonia digitata* seeds are the raw materials used for *Yanyanku,* whereas only *Hibiscus sabdariffa* seeds are used for *Ikpiru* preparation. The flow diagrams of both products are similar to the one used for *Bikalga* preparation in Burkina Faso (Parkouda et al. 2008). Thus, *Ikpiru* seems more similar to *Bikalga* since the raw material (*Hibiscus sabdariffa*) and the technology of production are similar (Parkouda et al.). However, *Bikalga* is used as food condiment while *Ikpiru* is used as functional additive for the fermentation of African locust bean in Benin. Similarly, whereas *Yanyanku* is exclusively used as functional additive in Benin, similar products such as *Botso*, *Datou*, *Furundu*, and *Mbuja* are used directly as condiments for preparation of sauces in Burkina Faso, Mali, Niger, Nigeria, Cameroon, Sudan (Parkouda et al.).

During the production of *Yanyanku*, ash solution (0.5 g/mL) from specific woods (*Vitellaria paradoxa*, *Parkia biglobosa*) is filtered and mixed with seeds during cooking or pounding/milling steps (100% of respondents

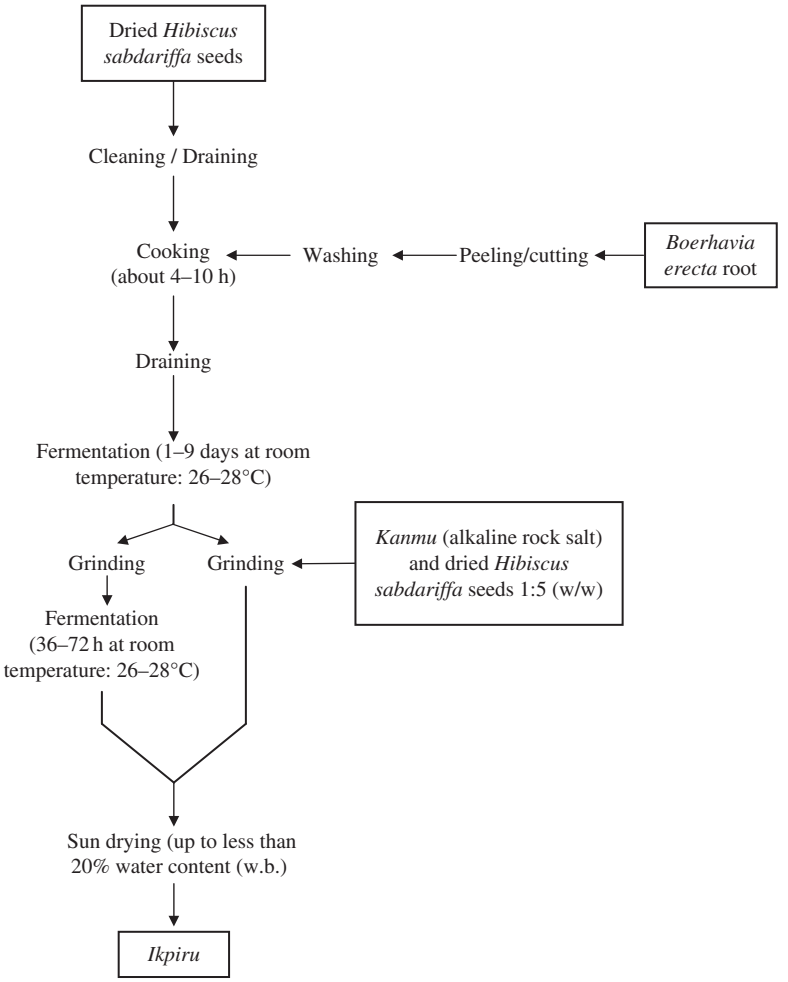

FIGURE 1 Flow sheet of *Ikpiru* processing.

of *Yanyanku* producers). The ash filtrate can be added before or after fermentation of the cotyledons, depending on the technology used (figure 2). Ash from shea wood (*Vitellaria paradoxa*), as reported by 72% of *Yanyanku* producers, provides the best quality of ash solution. About 62% of *Yanyanku* producers use filtrates from African locust bean wood (*Parkia biglobosa*) as it is also thought to yield ash of good quality. Similarly, cotton (22%), cassava (22%) and millet (15%) stalks can also be used to obtain the ash filtrate. The quality of the later depends on its alkali concentration which is roughly estimated by the processor by tasting the filtrate. Instead of ash filtrate, *Kanmu*, also known as *Kanwa*, is used in the case of *Ikpiru*. *Kanwa* is a naturally occurring alkaline rock salt, mainly composed of sesquicarbonates (Na_2CO_3,

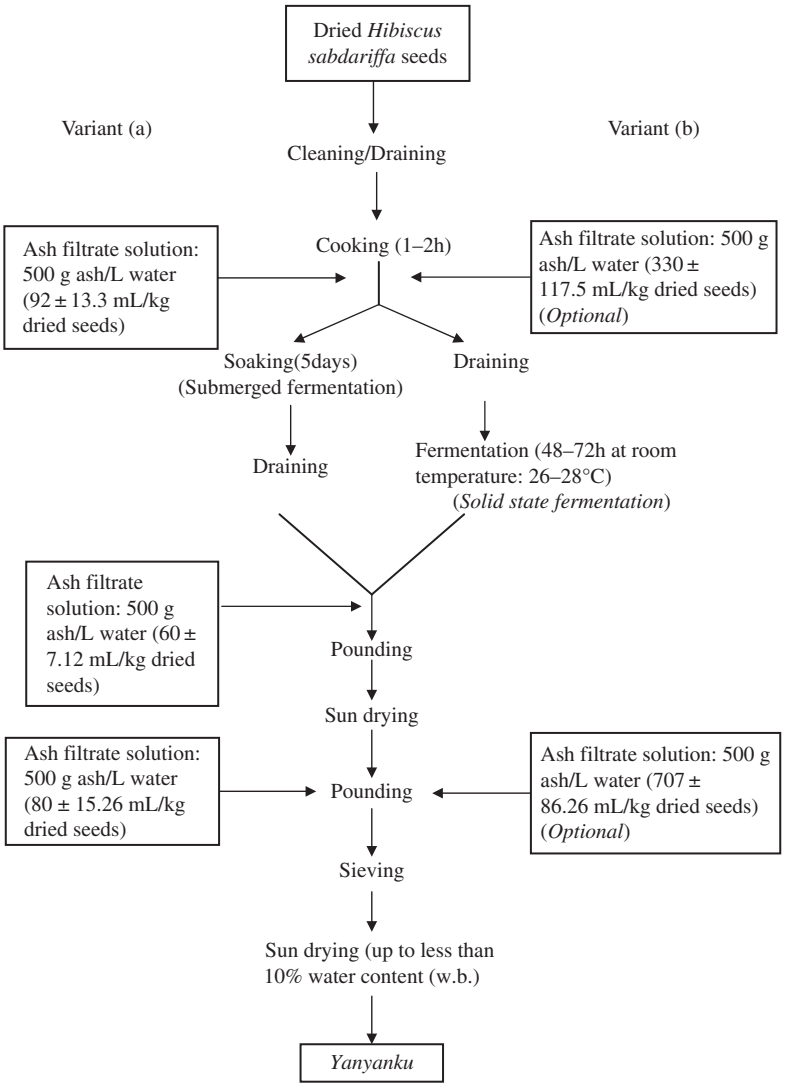

FIGURE 2 Flow sheet of *Yanyanku* processing.

$NaHCO_3.xH_2O$) containing various elements such as Ca, Fe, S, Cl, Si, P, K and Al (Zanabria et al. 2006).

STORAGE AND SHELF LIFE OF *YANYANKU* AND *IKPIRU*

Yanyanku and *Ikpiru* are kept in plastic boxes (33% of interviewed), in plastic bags (21% of interviewed) or in plastic cans (17% of interviewed) but closed properly to avoid that the additives become damp. They can be kept as small balls or reduced to powder. According to the users, *Yanyanku*

and *Ikpiru* can be stored for two months (2% respondents) to 10 years (9 % respondents), with an optimum at 24 months (29% respondents). This also depends on storage containers and conditions.

For some processors (12/58 representing 21% of respondents), the efficacy of these traditional functional additives increases with the storage duration. Thus, the longer the storage duration, the lower the proportion of additives used during the condiment preparation. Accordingly, only the moisture can reduce *Yanyanku* and *Ikpiru* efficacy during storage (6% of respondents using theses additives).

Regarding the plant stems, they could be used for several batches of condiment productions and could be replaced if after fermentation the condiment is not soft enough.

Microbial and physicochemical composition of *Ikpiru* and *Yanyanku*

Table 3 presents the microbial and physicochemical composition of *Yanyanku* and *Ikpiru*. There was an overall absence of molds, total and fecal coliforms in different samples of additives, except for one sample of *Yanyanku* which contained about 6.08, 5.65, and 5.58 Log_{10} (CFU/g) of molds, total and fecal coliforms, respectively. The presence of coliforms and molds in this sample could be explained by a probable contamination during production or storage.

The total aerobic mesophilic bacteria and *Bacillus* spores counts were similar for both types of additives but numbers of lactic acid bacteria were significantly ($p < .05$) higher in *Ikpiru* than in *Yanyanku* (table 3). *Bacillus* count was significantly higher ($p < .01$) than the count of lactic acid bacteria; therefore *Bacillus* spores predominated in *Yanyanku* and *Ikpiru* and their number ranged between 5.6 and 8.9 Log_{10} (CFU/g). *Bacillus* spp. were reported to be the main microorganisms responsible for the fermentation of African locust beans, *Hibiscus sabdariffa* seeds and soya beans to produce African or Asian fermented condiments: *Dawadawa*, *Soumbala*, *Netetu*, *Afitin*, *Iru*, *Sonru, Bikalga* (Odunfa, 1981; Ndir et al. 1994; Diawara and et al. 1998; Ouoba et al. 2004; Azokpota et al. 2006; Parkouda et al. 2008) and *Natto* (Beaumont 2000; Wang and Fung 1996).

The pH of *Ikpiru* and *Yanyanku* samples ranged from 6.7 to 10 and 6.2 to 9.7 with an average of 9.4 and 7.5, respectively (table 3). Those pH values are similar to that reported by Parkouda et al. (2008) in food condiment from *Hibiscus sabdariffa* seeds in Burkina Faso. Accordingly, the pH of these additives ranged in the group of the alkaline products like the condiments for which they are used. The high pH value in the final product could be explained by the use of *Kanmu* (1:5 w/w) of dried *Hibiscus sabdariffa* seeds (figure 1) or high ash concentration filtrate (about 0.5 g/mL) (figure 2) during *Ikpiru* and *Yanyanku* preparation. Indeed, the pH of ash filtrate ready to be used for *Yanyanku* ranged between 10 and 13 and varied depending

TABLE 3 Microbiological and Chemical Characteristics of *Yanyanku* and *Ikpiru*

	Microbial count (Log10 (CFU/ g))				Physicochemical characteristics		
Additives samples	Total count	*Bacillus* spores	Lactic acid bacteria	pH range	Dry matter (%, wet basis)	Ash (%, dry basis)	Protein (%, dry basis)
Ikpiru ($n = 12$)	7.6 ± 0.3 a[a]	7.5 ± 0.5 a	6.4 ± 0.5 a	[6.7–10.0][b]	[79.8–89.0]	[14.5–20.1]	[15–21.8]
Yanyanku ($n = 25$)	7.3 ± 1.0 a	7.4 ± 1.1 a	4.5 ± 2.1 b	[6.2–9.7]	[79–94.7]	[6.1–33.6]	[11.2–27.8]

[a]Samples number means ± standard deviation; means with the same letter in the same columns are not significantly different ($p > .05$).
[b]Range.
n = Samples number.

on the wood used for ash production. Particularly, ash from shea (*Vitellaria paradoxa*) wood had higher pH value, ranging between 11.9 and 13, than others (9.8–11.9 for African locust bean wood, cassava stem, millet, etc.). The reason behind the traditional know-how, using ash filtrate or *Kanmu* for the preparation of seed fermentation broth, could be, to create an alkaline environment, more favourable for *Bacillus* development than for lactic bacteria and yeasts. Because of the presence in high concentration of *Bacillus* spp. responsible of fermentation of *Iru*, *Sonru*, and *Sonru-babaru*, these functional additives could be considered as enrichment inocula. However, further study is necessary to validate this hypothesis.

The dry matter (DM) content in *Yanyanku* and *Ikpiru* varied between 79.2% and 94.7%, due to the fact that the sun drying of these additives was subjected to the fluctuation of the weather. Protein content of both additives varied between 11.2% and 27.8%. Considering the proportion used during condiment production (mean value of 59.1 ± 45.4 g of *Yanyanku* or *Ikpiru* /100 kg of seeds), this protein content would be too low to affect positively the protein content of the condiments. Similar observation can be made for total mineral (ash) content. *Yanyanku* ash content varied between 6.19% and 33.6% and is higher than values from *Bikalga*, as reported by Parkouda and colleagues (2008). This great variation could be explained by the variability of the components and the concentration of ash filtrate used. Indeed, three different seeds (*Hibiscus sabdariffa, Gossypium hirsutum*, or *Adansonia digitata*) can be used to produce *Yanyanku*; furthermore, the source and the concentration of ash to get the filtrate can vary from one processor to another. Lower ash variation (between 14.5% and 20.1%, dry basis) was observed in the case of *Ikpiru* for which only *Hibiscus sabdariffa* seeds are used.

CONCLUSION

Yanyanku and Ikpiru are considered by producers as softening additives for the fermentation of African locust bean to produce *Sonru* and *Iru*, respectively. Due to their high level of *Bacillus* spores, *Yanyanku* and *Ikpiru* also could be considered as "enrichment inocula." However future investigation is needed to assess the effectiveness of those traditional additives, with the aim to upgrade their performance for small-scale enterprises.

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