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Research Article

Assessment of Microbiological and Nutritional Quality of "akandji", A Traditional Corn (Zea mays L.) Bread Produced in Benin

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Abstract

Background and Objective: Traditional corn-based bread, *akandji* is a Beninese foodstuff the conditions of its production and its properties remain little studied. The present study aimed to evaluate the technological production parameters and the microbiological and nutritional properties of *akandji*. **Materials and Methods:** The *akandjii's* production monitoring has been carried out in Abomey and Ouidah, where samples were gathered for analysis using reference methods. The physiochemical and nutritional measured parameters were pH, titratable acidity, protein, carbohydrate, lipid, fiber, ash, moisture, nitrogen, total sugar and mineral (iron, calcium and magnesium). Microbiological analysis included the detection and enumeration of aerobic mesophilic germs, total coliforms, *Staphylococcus aureus*, fungal flora, sulfite-reducing anaerobes and salmonella. Analysis of variance and multiple mean comparisons were performed. **Results:** The Abomey had longer fermentation and cooking times (11.50 ± 0.50 hrs and 60.00 ± 0.00 min) than Ouidah (1.67 ± 0.17 hrs and 43.33 ± 1.67 min), but lower cooking temperature (100.00 ± 0.00 vs 178.33 ± 4.41 °C). Abomey also used malting, which lasted 107 hrs while Ouidah did not. Yeasts were the prevalent microflora in both locations with a higher count in Abomey (6.17 log10 UFC/g) than Ouidah (4.31 log10 UFC/g). No moulds, *Staphylococcus aureus*, *Salmonella* or Sulfite-reducing anaerobic bacteria were detected. The water, carbohydrates, total sugars, protein, ash, Mg and Ca contents and energy values were similar in both types of *akandji*. Nevertheless, iron, fiber and lipid contents differed. **Conclusion:** In view of its nutritional composition and absence of pathogenic germs, *akandji* produced especially in Abomey plays an important role in health and is recommended for people of all ages, preferably the most vulnerable.

Key words: Traditional corn based-bread, akandji, fermented foodstuff, malt process, hygienic, nutritive quality

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Maize, rice and wheat are the three main cereal crops produced worldwide, with a cumulative annual harvest of around 2.5 billion ton¹. Cereal growing has shaped the geographical, food and cultural landscapes in the world. Cereals are the pillars of global food security, particularly those of developing countries. Among them, maize (*Zea mays* L.), the second most cultivated cereal after wheat plays a vital role in the diet of impoverished rural and urban populations in Sub-Saharan Africa¹ where is most widely consumed.

The annual production of maize production was estimated to be 1.1 million tons in 2012/2013². Average consumption in Benin is estimated at over 85 kg/capita/year, placing it among the top consumer countries in West Africa³. Whether used for human or animal consumption or as the object of traditional cults and rituals, maize forms the basis of the diet of a large section of the Beninese population⁴. It is the most widely used raw material for local dishes in the artisanal food sector, where traditional food processing technologies enable local agricultural products to be valorised⁵.

However, the role of maize in people's diets is shrinking considerably, to the benefit of other food crops, mainly wheat, rice, potatoes and yams. Indeed, the galloping urbanization of the living environment and the significant economic boom experienced by African countries in recent decades⁶ have induced profound changes in lifestyles and working patterns, but also in eating habits⁷. Benin's population, like that of Africa, is now more focused on imported foodstuffs to the detriment of local dishes. As a corollary, we are witnessing a worrying disappearance of many corn-based dishes, once considered delicacies, hence a reduction in the diversity of derived products. Among the wide range of products generated by maize processing⁸, have identified several endangered dishes including akandji. Known as akandji in the Fon language, Kandji in Yoruba and Goun or bolou in Adja. It is a royal and historic food prepared especially for the royal family during the demonstrations ("Adjagbé, ritual of exit of the twins", "Ninsounhwéhoun", funeral rites, consultation of the oracle) but also given as food to vodoun (divinities) Dan and Tohossou and served as provisions during wars9. The akandji is a type of traditional bread made from maize flour¹⁰. Very little known, is the consumption of akandji limited to a given segment of the population explained by a lack of knowledge or popularization of the nutritional qualities of this food. Found in Southern Benin, mainly in the communes of Ouidah and Abomey, it is sold in markets, in the streets and along roadsides. It is therefore exposed to all kinds of hazards

and poses a public health problem. In view of the above, there is an urgent need to enhance the value of *akandji* by studying its various components, to contribute to the preservation of our culinary and cultural heritage and to solve the problem of food insecurity and public health posed by its marketing. Hence, the study aimed to evaluate the technological production parameters and the microbiological and nutritional properties of *akandji*.

MATERIALS AND METHODS

Study area: This study was performed at the Faculty of Sciences and Technology of Abomey-Calavi University, Benin. Samples were collected from the towns of Abomey and Pahou, where kandji is produced. The microbiological analysis were conducted at the Central Laboratory for Food Safety (LCSSA) in Cotonou at Benin. Nutritional analysis were conducted at both the LCSSA and Laboratory of Biochemistry and Food and Medicinal Formulations, National School of Biosciences and Applied Biotechnologies, National University of Sciences, Technologies, Engineering and Mathematics in Dassa-Zoume at Benin. The study spanned 7 months and two weeks, from 7 July, 2020 to 19 February, 2021.

Corn variety and leaves used as packing: The corn variety employed is the white maize (*Zea mays* L.), locally designated as Adjakouin or Nikkikouin (in Fon language), selected and validated for this objective by the *akandji* producers. The 10 kg of corn was purchased at the Pahou and Abomey markets, near from traditional production area to monitor the production of the two *akandji* manufacturing technologies. The *akandji* was packaged using leaves of banana (*Musa sapientum*), Senegal arrowroot (*Thalia geniculata*) and Senegal iris (*Lasimorpha senegalensis*), which are known in Fon as kokoéman, toungoman and afléman, respectively.

Monitoring *akandji* **production** and **measurement of technological parameters:** The *akandji* production was followed step by step in the towns of Abomey and Ouidah, using the two production technologies identified⁹. The two methods differ from each other in the crushing, sieving, preparation of the maize porridge, non-spontaneous fermentation (addition of leaves) and baking operations carried out by the Ouidah producers⁹ and the malting (soaking, germination, sun-drying), spontaneous fermentation and steaming operations carried out by the Abomey producers⁹. For each technology, three growers with at least 10 years experience were selected for production in two replications. The parameters measured were essentially the duration and temperature of the divergent unit operations.

Physicochemical and microbiological properties of *akandji* samples:

Sampling plan: Samples were taken at random from three female producers and three female retailers on three different occasions (regular 7-day periods) in Abomey and Ouidah. On each occasion, 12 samples were transported to the laboratory in their original packaging, in a cool box containing cold accumulators. A total of 36 *akandji* samples, including 18 from Abomey and 18 from Ouidah, were collected and sent to the laboratory for physicochemical and microbiological analysis.

Physicochemical analysis: The physicochemical and nutritional characterization of *akandji* samples was carried out by determining pH, titratable acidity, protein, carbohydrate, lipid, fiber, ash, moisture, nitrogen, total sugar and mineral (iron, calcium, magnesium) contents. The pH and titratable acidity were determined using the method of Nout *et al.*¹¹. Protein, carbohydrate, lipid, fiber, ash, water and nitrogen contents were determined by standard methods¹². Protein content was calculated by converting nitrogen content, determined by the Kjeldahl method $(6.25 \times N)^{12}$. Carbohydrate content was determined by the differential method using data on protein, lipid, fiber, water and ash content according to the equation¹³:

Carbohydrates (g/100 g of sample) = 100 - ((water+lipids+ proteins+ashes+raw fibers) g/100 g)

Lipids were extracted in a Soxhlet extractor using hexane¹⁴. The number of fibers was determined on 2 g samples that had been previously boiled with a diluted Sulphuric Acid (H₂SO₄) solution (0.3 N). Subsequently, the mixture was filtered and washed with 200 mL of boiling water and NaOH (0.5 N). The residue was then subjected to a second extraction, washing with boiling distilled water and acetone and after drying at 105°C until a constant weight was achieved. The treated samples were heated at 550°C/3 hrs, after which the weight was recorded. The water content was determined by drying the samples in an oven at 105°C until a constant weight was obtained. The ash content was determined by calcining the samples in a muffle furnace at 550°C/4 hrs. The total sugars were determined using the method of DuBois et al.15. The energy value was estimated using Atwater's coefficients¹⁶, which express the quantities of kilocalories supplied by proteins (4 kcal/g), carbohydrates (total sugars 4 kcal /g) and lipids (9 kcal/g). Minerals (iron, calcium and magnesium) were determined by atomic absorption spectrophotometry using the method of Hernández et al.¹⁷. All analysis were repeated three times and the average of the values obtained was considered.

Microbiological analysis: The microbiological analysis were carried out not only for pathogenic microorganisms, but also for indicative germs of good hygiene practices in food products. Culture media were prepared according to the manufacturer's instructions and maintained in undercooling until inoculation. To prepare the stock solution, 10 g of each sample was taken with a sterile spatula from a sterile stomacher bag and 90 m of Buffered Peptone Water was added and the mixture was homogenized. Successive decimal dilutions were made from the stock solution. Inoculum was added to the agar using the mass inoculation technique, except for the dichloran rose bengal chloramphenicol (DRBC) agar used to test for fungal flora, which was surface inoculated. Aerobic mesophilic germs (AMG) were counted on PCA agar after incubation at 30°C for 72±2 hrs¹⁸; total coliforms on VRBL agar after incubation at 37°C for $24\pm2\,hrs^{19}$; staphylococci on Baird Parker agar supplemented with PLF (Rabbit Plasma and Fibrinogen) after incubation at 37°C during 18-24 hrs²⁰; yeasts and moulds on DRBC agar after incubation at 25°C for 5 days²¹. Sulfite-reducing anaerobic (SRA) bacteria were counted on Tryptone-Sulfite-Cycloserine (TSC) agar after incubation in anaerobic jars at 37°C during 48±2 hrs²². Salmonella testing was carried out in accordance with standard²³. Microbial germ counts were carried out in accordance with standard²⁴. All analysis were repeated three times and the average of the values obtained was considered.

Statistical analysis: The data collected were analyzed using SPSS (Statistical Package for Social Sciences) version 20.0²⁵ to determine descriptive statistics in terms of percentage and mean. Quantitative data were then subjected to Analysis of Variance (ANOVA) using SAS (Statistical Analysis System) software version 9.2 according to Balogoun *et al.*²⁶ and MiniTab 2014. Multiple mean comparisons were performed using the Student Newman-Keuls test²⁷. The significance level retained was 5% (p<0.05).

RESULTS AND DISCUSSION

Technological parameters for akandji production: From the raw material of maize to the production of akandji, production time varies according to the production method used. As depicted in Table 1, it was 6 days for the production method identified in Abomey and around 6 hrs for the Ouidah production method (p<0.05). This difference is mainly due to the malting process which is exclusive to the Abomey method and lasted 107 hrs. Malting involving soaking, germination and drying, is a process of biochemical transformation of the grains and synthesis of enzymes^{28,29}.

In addition to saccharifying starch and solubilising nitrogenous matter, malting reduces toxic compounds and anti-nutritional factors to low levels, as highlighted by the work of Aloo et al.30. The germination of corn seeds promotes the solubilization of certain amino acids (lysine and tryptophan), increases the content of certain vitamins (riboflavin, niacin, ascorbic acid) and iron, as well as the degradation of phytates and the proper assimilation of iron and zinc by the body³¹. This unitary operation could therefore improve the nutritional quality of akandji produced in Abomey. Table 1 also reported that the fermentation temperature did not vary significantly at a 5% level from one region to another (p<0.05). However, fermentation and cooking times were significantly higher in Abomey than in Ouidah (p<0.05). The fermentation time is 10 times longer in the Abomey production technology than in the Ouidah method. The difference between the fermentation processes of akandji in Ouidah and Abomey is related to the use of sourdough in the former and the absence of it in the latter. Sourdough acts as a starter culture that enhances the fermentation rate in Ouidah, while Abomey relies on the natural microflora of the environment, which may have inhibitory effects on the fermentation. To understand the microbial diversity and functionality of akandji fermentation, it would be beneficial to isolate and

characterize the microorganisms involved. Another difference between the two production methods is the higher cooking temperature of *akandji* in Ouidah compared to Abomey (p<0.05). The factor that influences the cooking temperature of *akandji* is the type of cooking used in the production process. In Ouidah, where a traditional oven is employed, the cooking temperature is higher than in Abomey, where steam cooking is preferred. This results in a shorter cooking time for the Ouidah *akandji*. However, a drawback of the traditional oven is that it damages the vegetable wrapping that protects and preserves *akandji*. This could compromise the microbiological quality of the product.

Physico-chemical and nutritional properties of *akandji*: The physicochemical properties of *akandji* samples were presented in Table 2. Samples showed a pH ranging from 4.20 (Ouidah) to 4.60 (Abomey) which corresponds, respectively to titratable acidity of 54.70 meq/L and 47.43 meq/L. Thus, *akandji* produced in Ouidah was slightly more acidic than that produced in Abomey (p <0.05). The low pH of *akandji* produced in Ouidah compared with that of Abomey could be justified by the fact that fermentation is induced in Ouidah thanks to leavens, whereas in Abomey it was spontaneous. The pH value of

Table 1: Technological parameters for akandji production in Benin's two main production areas

	Or	_	
Distinctive parameter	Abomey	 Pahou	p-value
Fermentation temperature (°C)	27.50±2.50 ^a	31.33±0.88a	0.18
Fermentation time (hrs)	11.50±0.50ª	1.67±0.17 ^b	0.0002
Baking temperature (°C)	100.00±0.00 ^b	178.33±4.41ª	0.0008
Baking time (min)	60.00 ± 0.00^{a}	43.33±1.67 ^b	0.0045
Production time (days)	6.00 ± 0.00^a	0.23 ± 0.02^{b}	< 0.0001

^{a,b}Means with different letters in the same line indicate significant differences (p<0.05)

Table 2: Physicochemical and nutritional properties of sampled "akandji"

	Ori	gins		
Physicochemical properties	Abomey	Ouidah	Average	p-value
pH	4.60±0.00ª	4.20±0.00 ^b	4.4±0.0	< 0.001
Titratable acidity (Meq/L)	47.43±10.45°	54.70±3.61ª	51.06±7.03	0.57
Moisture content (g/100 g)	65.12±1.60 ^a	65.21±1.09 ^a	65.17±1.34	0.97
Fibers (g/100 g)	0.89 ± 0.03^{a}	$0.65 \pm 0.00^{\rm b}$	0.77±0.14	0.01
Total sugars (g/100 g)	0.41 ± 0.09^{a}	0.43 ± 0.04^{a}	0.42 ± 0.06	0.84
Crude proteins (g/100 g)	4.32±0.12 ^a	4.09±0.19ª	4.20±0.16	0.44
Lipids (g/100 g)	0.61 ± 0.00^{b}	1.14±0.03ª	0.88±0.31	0.02
Carbohydrates (g/100 g)	27.91±1.87ª	28.86±1.09ª	28.39 ± 1.36	0.44
Energy (Kcal/100 g)	141.66±9.02ª	139.63±6.13ª	140.65±6.40	0.81
Ash content (%)	0.60 ± 0.18^{a}	0.57±0.21ª	0.58±0.19	0.92
Iron (mg/100 g)	130.81±17.14°	27.91±6.75 ^b	79.36±11.94	0.03
Calcium (mg/100 g)	32.00 ± 3.36^{a}	32.59±0.83ª	32.29±2.09	0.88
Magnesium (mg/100 g)	95.19±2.21 ^a	88.90 ± 3.38^a	92.04±2.79	0.26

^{a,b} Means with different letters in the same line indicate significant differences (p<0.05)

Table 3: Microbiological characteristics of akandji collected from women producers and sellers in Benin's two main production areas

Microorganisms sought (Log₁₀ UFC/g)

Origin	Sample type	AMG	TC	S. aureus	Yeasts	Moulds	SRA	Salmonella
Abomey	Producer	7.40±0.06 ^a	Abs ^b	Abs	4.33±0.06 ^b	Abs	Abs	Abs
	Saleswoman	7.47 ± 0.00^{a}	1.60 ± 0.01^{a}	Abs	6.17 ± 0.00^{a}	Abs	Abs	Abs
Ouidah	Producer	4.10±0.10 ^b	Abs ^b	Abs	3.37±0.77 ^b	Abs	Abs	Abs
	Saleswoman	5.52±0.44a	3.53±0.05°	Abs	4.31±0.01a	Abs	Abs	Abs

AMG: Aerobic mesophilic germs, TC: Total coliforms, *S. aureus*. *Staphylococcus aureus*, SRA: Sulfite-reducing anaerobic, Abs: Absent and ^{a,b} Means with different letters in the same column indicate significant differences (p<0.05)

Abomey akandji was higher than those of Ablo (pH = 4.2)³², which are also traditional products of corn fermentation. However, the pH of akandji produced in Ouidah were similar to the pH values obtained by Banon et al.32. The analysis of this table reveals that water, total sugars, carbohydrates, crude protein and ash content and energy value did not vary significantly from one locality to another at the 5% threshold. The dry matter contents of the akandji sampled were lower than those (39.47% DM) of *Ablo* obtained by Nago *et al.*⁵ and similar to those (36.6% DM) of uncooked gowé obtained by Adinsi et al.33. In contrario, the protein content of the akandji sampled (Table 3) is lower than that of cooked (9.18%) and uncooked (8.75%) gowé³³ and millet-based *Ablo* and sorghum-based Ablo (7%)34. They are close to those of protein in gnomy (4.7 g/100 g) according to the work of Zita et al.35. In term of energy provided by akandji produced in Abomey and Ouidah, values are higher than those of fermented (90.85 kcal) and non-fermented foura (60.67 kcal)³⁶. It is also higher than the energy values determined by Kagambèga et al.37 for all cereal-based porridge samples analyzed. Of an average carbohydrate content of 28.39 g/100 g, the akandji samples contain only 0.42 g/100 g total sugars; thus, essentially made up of starch, which is considered a "low carbohydrate" as it is often slowly digested and absorbed. akandji would therefore be a low glycemic index (GI) food. Published meta-analysis confirm a favorable effect of low GI diets in type 2 diabetic patients^{38,39}. Other cohort studies show that diets with a high glycemic index are associated with a higher risk of type 2 diabetes^{40,41}. The meta-analysis of Schwingshackl *et al.*⁴² provides evidence of a beneficial effect of a low glycemic index diet in overweight and obese children and adolescents.

However, *akandji* from Abomey contained higher levels of fibers and lipids than *akandji* from Ouidah. Dietary fibers are carbohydrate polymers that are not hydrolysed by endogenous enzymes in the human small intestine. Increased dietary fiber intake can lower the risk of cardiovascular disease, type 2 diabetes and some cancers, such as colon-rectum and breast cancers. Therefore, *akandji* consumption can provide a significant

health benefits due to its fibers content. The variation in fiber and lipid content between Abomey and Ouidah *akandji* may be explained by the different cooking methods used. It is possible that the Abomey *akandji* production which involves the germination of maize kernels, which could potentially reduce its lipid content in comparison to Ouidah *akandji*. Indeed, the germ is the part of corn with the highest concentration of lipids. Studies have reported a 1.2 to 2.3-fold increase in levels of lipase and lipoxygenase activity during the germination of cereal grains⁴³. An 8 to 15% decrease in lipids content occurs in millet that has germinated for 3 days at room temperature⁴⁴.

Table 2 also indicated that while the Ca and Mg levels displayed no significant differences among localities at the 5% threshold, the iron content proved higher in akandji generated in Abomey as opposed to Ouidah. The Mg content of the sampled akandji closely resembles that of cooked gowé (84.72 mg/100 g)⁴⁵. The iron content of akandji produced in Abomey was higher than not only that of akandji produced in Ouidah but also higher than that of cooked gowé, uncooked gowé and Ablo, according to the research of Banon et al.32 and Célestin et al.45. This is likely due to the combined effects of germination and fermentation in the Abomey production process. Similarly, Kayodé et al.31, have demonstrated that germination enhances iron content and improves its assimilation. Célestin et al.36 found that fermentation significantly increases the bioavailability of minerals such as iron, calcium and magnesium. Despite this, akandji products as a whole are still high in Fe, Ca and Mg.

Microbiological properties of *akandji*: Table 3 shows the findings of the microbiological tests performed on *akandji* samples collected from Abomey and Ouidah. The data indicate that the main microbial population of *akandji* was composed of yeasts, with a maximum count of 6.17 Log10 CFU/g (Table 3), as did the dominant microflora of *Ablo*³⁴. On the other hand, *akandji* sampled from resellers had a higher yeast microbial load compared to those sampled from producers. Table 3 indicates no significant difference in AMG and total coliforms in *akandji* sampled at

both production and reseller's premises at the 5% threshold. Depending on the locality, the total coliform load did not vary significantly at the 5% threshold. However, *akandji* produced in Abomey exhibited higher AMG and yeast loads than those produced in Pahou. The notable disparity, meeting the 5% threshold, between the microbial quantities of AMG and yeast in *akandji* from Abomey and Pahou (Table 3) is attributed to the elevated temperature employed in traditional baking for the latter. Additionally, *akandji* retailed on the streets exhibited a higher yeast count than the ones directly from production.

This study also informs that no significant difference in total coliforms was observed among the various samples of akandji. On the other hand, total coliforms (TC) were found in the akandii samples obtained from retailers in both localities, except for akandji samples taken directly from producers in both localities (Table 3). Banon et al.³² and Zita et al.³⁵ reported a similar trend for as respectively in Abloand gnomy produced where total coliforms were absent. Célestin et al.36 added that total coliforms were present in the four sold on the market and absent in the foura produced, whether fermented or not. As coliforms are indicative of faecal contamination, their presence in the akandji samples concerned suggests that the sellers did not follow proper the hygiene practices. Regarding the AMG, no significant difference was noted between the akandii sampled at the producer's and the akandii sampled at the retailer's in Abomey, at the 5% threshold.

In Abomey, depending on the locality and sampling site, there was no significant difference at the 5% threshold in terms of aerobic mesophilic germs. This was not the case in Pahou, where akandji sampled from resellers had a higher AMG load than akandji sampled from growers (Table 3). This dissimilarity can be explained by the fact that akandji in Abomey was fully covered by the packaging, whereas akandji in Pahou was not. This corroborated the findings of Baba-Moussa et al.46 who noted in their work that foods that are exposed to air pollution, dust and more are more contaminated. This is also true for akandji sold along the tracks in Pahou. Furthermore, Célestin et al.45 point out that the presence of germs is due to the fact that the environment is not controlled. This could therefore explain the higher microbial load of AMG, total coliforms and yeasts among resellers compared to producers in the two surveyed localities (Table 3).

From a hygienic point of view, acidification is a major advantage. Indeed, these pH and titratable acidity levels induced by the fermentation during the *akandji* production are microbiologically favorable as inhibiting the growth of both spoilage and most pathogenic microorganisms; and ensuring the preservation of the fermented product⁴⁷.

Thus, no microbial load of *Staphylococcus aureus*, SRA or *Salmonella* was observed in any of the *akandji* sampled. The absence of *Salmonella* was also observed in the gnomy³⁵. The same applies to mould loads.

CONCLUSION

The results of this study indicate that yeast is the dominant microflora in the *akandji*. Microbiological analysis of the finished product showed the absence of moulds, total coliforms, *Salmonella* and Sulfite-reducing anaerobic. Physicochemical and nutritional parameters assessment demonstrate that *akandji* is a good source of protein, calcium and magnesium and is especially rich in iron, with Abomey having the highest content. Additionally, *akandji* contains fibers that are good for health. The consumption of *akandji* can therefore be recommended to people, particularly children, pregnant women and the elderly, to supplement any micronutrient deficiencies.

SIGNIFICANCE STATEMENT

This study aimed to fill the gap in scientific data on akandji, an endangered fermented foodstuff in Benin. The study revealed that technological parameters of the akandji production process vary between Abomey and Ouidah with regard to malting, cooking and fermentation. Microbiological analysis showed the absence of moulds and pathogens (Staphylococcus aureus, Salmonella or Sulfite-reducing anaerobic bacteria) in both varieties of akandji. However, from a nutritional point of view, iron content in Abomey akandji is significantly higher than that of Ouidah akandji although they all contain iron, calcium and magnesium.

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