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Technological variations, microbial diversity and quality characteristics of maize *ogi* used for *akpan* production in Benin

A.K. Carole Sanya [a,b], Yann E. Madode [b], Sijmen E. Schoustra [c,e], Eddy J. Smid [d], Anita R. Linnemann [a,*]

[a] Food Quality and Design (FQD), Wageningen University and Research, The Netherlands, 6700 HB Wageningen, the Netherlands
[b] Laboratoire des Sciences des Aliments (LSA), Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, Calavi, Benin
[c] Laboratory of Genetics, Wageningen University and Research, 6700 HB Wageningen, the Netherlands
[d] Food Microbiology, Wageningen University and Research, 6700 HB Wageningen, the Netherlands
[e] Department of Food Science and Nutrition, School of Agricultural Sciences, University of Zambia, Lusaka, Zambia

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ABSTRACT

Fermented maize starch, called *ogi* in Benin, is used for preparing *akpan*, a traditional yoghurt-like food that contributes to the food and nutrition security of its consumers. Current *ogi* processing technologies used by two socio-cultural groups of Benin, namely the Fon and the Goun, and aspects of the quality of the fermented starches were studied to assess the current state-of-the-art, explore changes in key product characteristics over time and identify priorities for follow-up research to increase product quality and shelf life. A survey on processing technologies was conducted in five municipalities in south Benin and samples of maize starch were collected, which were analysed after the fermentation required to obtain *ogi*. Four processing technologies were identified, two from the Goun (G1, G2) and two from the Fon (F1, F2). The main difference between the four processing technologies was the steeping procedure used for the maize grains. The pH of the *ogi* samples ranged between 3.1 and 4.2, with the highest values for G1 samples, which also contained relatively higher concentrations of sucrose (0.05–0.3 g/L) than F1 samples (0.02–0.08 g/L), and lower citrate and lactate concentrations (0.2–0.3 and 5.6–16.9 g/L, respectively) than F2 samples (0.4–0.5 and 14–27.7 g/L, respectively). Fon samples collected in Abomey were particularly rich in volatile organic compounds and free essential amino acids. Members of the genera *Lactobacillus (8.6–69.3%), Limosilactobacillus (5.4–79.1%), Streptococcus (0.6–59.3%) and Weissella (2.6–51.2%)* dominated the bacterial microbiota of *ogi* with a significant abundance of *Lactobacillus* spp. in Goun samples. Sordariomycetes (10.6–81.9%) and Saccharomycetes (6.2–81.4%) dominated the fungal microbiota. The yeast community of *ogi* samples mainly consisted of the genera *Diutina*, *Pichia*, *Kluyveromyces*, *Lachancea* and unclassified members of the Dipodascaceae family. Hierarchical clustering of metabolic data showed similarities between samples from different technologies at a default threshold of 0.05.

No obvious trend in the composition of the samples' microbial communities reflected the clusters observed for the metabolic characteristics. The results indicate that beyond the general impact of the use of Fon or Goun technologies on fermented maize starch, the individual contribution of processing practices warrants study, under controlled conditions, to determine the drivers of difference or similarity between maize *ogi* samples to further contribute to improving product quality and shelf life.

1. Introduction

Fermentation is a well-known, affordable technique in food production, which has widely been used around the world for thousands of years. Fermentation has several benefits such as the improvement of nutritional and sensorial quality, digestibility, and antioxidant and anti-inflammatory properties (Apaliya et al., 2022; Gabriele & Pucci, 2022; Lindner & Bernini, 2022). In addition, fermentation contributes to the preservation of food and to the health of its consumers (Marco et al., 2017). The range of foods made through fermentation is highly diverse,

* Corresponding author.
E-mail addresses: carole.sanya@wur.nl (A.K. Carole Sanya), yann.madode@uac.bj (Y.E. Madode), sijmen.schoustra@wur.nl (S.E. Schoustra), eddy.smid@wur.nl (E.J. Smid), anita.linnemann@wur.nl (A.R. Linnemann).

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often inexpensive and thus accessible to individuals with limited purchasing power (Marshall & Mejia, 2011). Moreover, fermentation, being a not-so-demanding economic activity combinable with domestic responsibilities, offers entrepreneurial opportunities with income generation for local processors and sellers, mainly women (Materia, Linnemann, Smid, & Schoustra, 2021). Cereals are used abundantly as raw materials for the production of fermented foods (Brandt, 2014). Particularly maize, rice, millet and sorghum (Achi & Asamudo, 2019) are used to produce a variety of food products. Maize is a major staple food in Africa, with a long list of foods and beverages as end-products from which many are fermented (Ekpa et al., 2018, 2019). A popular food produced in Benin, West Africa, is known as "*akpan*". It is a traditional yoghurt-like product that is consumed in urban as well as rural areas (Akissoé et al., 2015; Sacca et al., 2012). *Akpan* is made from fermented maize starch, called "*ogi*", and has potential prebiotic effects on gastrointestinal health according to Gullón et al. (2015). Unfortunately, the unpredictability of the results of traditional processing methods and spontaneous fermentation during maize transformation into *ogi* cause uncertain quality and a short shelf life of a couple of days in *akpan*. Standardisation of the *ogi* production process by defining best practices is expected to offer improved and stable quality *akpan* to consumers.

Previous studies have explored ways to standardise traditional maize *ogi* production in Benin, mainly by evaluating its physicochemical and microbiological characteristics. Variable values for pH and titratable acidity (Greppi et al., 2013; Nago, Hounhouigan, Akissoe, Zanou, & Mestres, 1998a; Sacca, 2015) were obtained for samples collected from different localities. Starch, proteins, ethanol and organic acids content (Nago, Tétégan, Matencio, & Mestres, 1998b), as well as other physicochemical parameters such as colour indexes and pasting behaviour (Nago et al., 1998a), were also evaluated on samples from different maize varieties, localities or produced following a laboratory-scale technology. The main microorganisms enumerated in traditional maize *ogi* were lactic acid bacteria (LAB) and yeasts, with log CFU g^{-1} counts of 9 and 7, respectively, found by Nago et al. (1998a), and 5.8 and 4.1, respectively, recorded by Sacca (2015). *Enterobacteriaceae* were below the detection limit (<1) in both studies. Extended identification of isolates by Nago et al. (1998a) revealed three dominant species of the genus *Lactobacillus* (i.e., *Lactobacillus cellobiosus*, *Lactobacillus brevis* and *Lactobacillus fermentum*), and three yeast species (i.e., *Candida humicola*, *Candida krusei* and *Geotrichum spp.*). Of these, *L. cellobiosus* and *L. fermentum* are reclassified as *Limosilactobacillus fermentum*, and *L. brevis* as *Levilactobacillus brevis* (Zheng et al., 2020). This implies that there are two main *Lactobacillus* species in *ogi* rather than three. Greppi et al. (2013) later revealed a different yeast threesome (i.e., *Candida krusei*, *Clavispora lusitaniae* and *Saccharomyces cerevisiae*) as the most abundant in maize *ogi* through a culture-independent method.

Ogi production generally follows sequential steps usually common to each processor but how these steps are performed can vary, and therefore affect the final product. To illustrate, Nago et al. (1998a) described three processing technologies based on the steeping procedures, which they referred to as "cold", "Fon" and "Goun" technologies (Fig. S1). In the cold steeping procedure, the maize grains are directly put in water at ambient temperature and steeped for 72 h, with a daily change of the steeping liquid. In the Fon steeping procedure, hot water is poured onto the grains, which are then steeped for 24 h. Lastly, in the Goun steeping procedure, maize grains are put in boiling water on the fire, then cooked for 10 min before off-fire steeping for 12–48 h. The variations in these steeping procedures can lead to differences in the microbiota in *ogi* (Nago et al., 1998a).

Currently available studies are all based on the Goun steeping procedure or report on samples without ample information on the applied processing technology. Moreover, the duration of spontaneous fermentation ranges from one to three days, but its impact on Beninese *ogi* characteristics is still unknown. Except for the yeast community, the microbial composition of Beninese *ogi* was only evaluated following a culture-dependent method. Nowadays, improved approaches for the efficient analysis of fermented foods microbiota are used such as high-throughput sequencing (HTS). Moreover, information is needed on the volatile organic compounds in Beninese *ogi* to describe its overall aroma, as well as on nutritional properties such as amino acid and vitamin content.

The present study fills the existing knowledge gaps by exploring the current state-of-the-art in Benin in terms of *ogi* processing technologies, the microbial composition, physicochemical characteristics and metabolites in the product when it is ready to make *akpan*. This will allow the identification of potential differences between traditional maize *ogi* samples resulting from different processing technologies, as a first step towards designing improved quality *ogi* for *akpan* production, thereby supporting the food security of consumers as well as the livelihoods of food processors, and establishing key priorities for follow-up research.

2. Materials and methods

This study focused on rural and urban areas of southern Benin where *ogi* and *akpan* are frequently consumed. The sampling areas were Porto-Novo, Bohicon, Abomey, Abomey-Calavi and Cotonou. The survey and sampling took place during the long dry season (November–March) when the ambient temperature was about 30 ± 3 °C.

2.1. Fieldwork and sampling

2.1.1. Survey and questionnaire on traditional technologies and practices

A survey was conducted in five municipalities with 40 maize-based *akpan* processors who were all females aged 27–67 years. The respondents were recruited based on their willingness to participate in the research, following convenience and snowball sampling techniques. Strategic places for selling *akpan* were visited, including town halls, ministries, stadiums and sports centres, schools, churches, markets, and bus stations. Interviews focused on the processing practices applied by the processors.

Next, a follow-up was conducted with 18 processors from the five localities using a questionnaire (Supplementary data 2) that was filled with enumerator observations and the responses from the processors. Data were collected on maize type, processing steps, timing, tips, experience with *akpan* production, seasonality of the activity, as well as socio-demographic backgrounds. The time course of the temperature during heat treatments was recorded using an ibutton device (temperature data logger; 0–125 °C; MAXIM, Philippines). Finally, 14 processors were chosen for sample preparation and collection using criteria that included their production technology, experience (≥ 5 years), and hygiene during production and on the production site.

2.1.2. Starch collection and ogi sampling

All 14 processors were asked to produce maize starch slurry according to their usual processing practices. The starch slurries were collected and transferred to the laboratory for fermentation and sampling at the ready-to-use time as indicated to be appropriate by each processor for her own technology. The time between the starch slurry extraction and ready-to-use moment corresponded to the fermentation time needed to turn the starch into *ogi*, prior to preparing *akpan* to be served to consumers.

2.2. Analysis of microbial composition

Total DNA was extracted directly from *ogi* samples using Qiagen DNeasy Blood & Tissue Kit (Qiagen, Venlo, The Netherlands). An optimised DNA extraction protocol was followed (Supplementary data 3), which is an adaptation from the spin-column protocol for the purification of total DNA from animal tissues provided by Qiagen (2006). *Ogi* samples were stored at −20 °C until analysis.

The eluted DNA was subsequently purified by ethanol precipitation.

The quality and concentration of purified DNA were pre-checked and the samples were sent for V3-V4 region 16S rDNA and ITS1 amplicon sequencing to the company Novogene (HK). The amplicon sequence variants (ASVs) abundance table was obtained at the level of the kingdom, phylum, class, order, family, genus, and species, with annotation information. Samples metadata file, ASVs and taxonomy tables were uploaded to Microbiome Analyst web (https://www.microbiomeanalyst.ca/) for a first screening. Taxa of "chloroplast", "mitochondria" and "archaea" considered either as part of the raw material DNA (maize grains being a plant matrix) or contaminants were deleted. The Phyloseq R package was used for this purpose, generating an improved version of ASVs and taxonomy tables. These new tables were used for further analysis.

2.3. Physicochemical and metabolite analyses

2.3.1. pH and titratable acidity

The pH and titratable acidity (TTA) of the ready-to-use *ogi* samples were determined in duplicate, according to ISO 1842:1991 (ISO, 1991) and ISO 750:1998 (ISO, 1998) methods, respectively. Dry matter content was calculated from the moisture content determined according to the AACC 44-15A method (AACC, 2000), and used to calculate the TTA expressed as g of lactic acid per 100 g of dry *ogi*.

2.3.2. Sugars, organic acids, ethanol and free amino acids

Glucose, fructose, sucrose, lactate, pyruvate, citrate, acetate, and ethanol were quantified via High Pressure Liquid Chromatography (HPLC), as described by van Mastrigt, Mager, Jamin, Abee, and Smid (2018), and expressed in g per litre of dry *ogi*. AccQ•Tag method (Waters) was used for free amino acids (FAAs) quantification via Ultra-performance liquid chromatography (UPLC), as described by Lanzl, van Mastrigt, Zwietering, Abee, and den Besten (2022). The concentration of each free amino acid in mg per litre of dry *ogi* was calculated from their peaks, after annotation and data normalisation to the internal standard. For the two types of quantification, at least 2 g of sample was taken in a 2 mL Eppendorf tube and centrifuged at 16,400 rpm for 15 min. The supernatant was collected in a 1.5 mL tube and centrifuged again. The supernatant from the second centrifugation was used further. The tubes were stored at −20 °C until analysis, and defrosted on the day of analysis to reach room temperature.

2.3.3. Gas chromatography analysis of volatile compounds

Volatile organic compounds (VOCs) were determined in 2 g of *ogi* sample directly weighed in a 5 mL gas chromatography vial and stored at −20 °C until analysis. A headspace solid-phase microextraction gas chromatography (HS – SPME – GC) coupled with mass spectrometry (MS) was applied using Thermo Fisher equipment as described by Moonga et al. (2021).

The peaks of the compounds were annotated and integrated, and peaks areas were determined. From this, the relative abundance expressed as a percentage of VOC groups in each *ogi* sample was calculated. The relative abundance of each VOC was further calculated using formula (1) by van Rijswijck, Kruis, Wolkers – Rooijackers, Abee, and Smid (2019) as follows:

$$Relativeabundance(y)ofcompound(x) = \log_2\left(\frac{MSquantitation(xy)}{Median(MSquantitation(x))}\right) \quad (1)$$

2.4. Statistical data analysis

Circular diagrams and histograms were plotted using Microsoft Excel v2202 (Office 365). Tukey HSD posthoc test of one-way analysis of variance, or Conover's all-pairs test of Kruskal Wallis non-parametric ANOVA, were performed in R v4.1.0. Multifactorial analysis (MFA) followed by ascending hierarchical classification (AHC) was computed using FactoMineR and Factoshiny R packages. Hierarchical cluster analysis by the means of heatmaps was performed using Clustvis, a web-based application (https://www.biit.cs.ut.ee/clustvis/). For the analysis of the microbial community, the T-test statistical method was used for alpha diversity, while PCoA with Bray-Curtis distance and ANOSIM method were used for beta diversity.

3. Results

3.1. Traditional technologies for ogi production applied by akpan processors

In our survey, the *ogi* production technology using a cold steeping procedure (steeping grains in water at ambient temperature for 72 h with a daily change of the steeping liquid), as reported by Nago et al. (1998a), was not mentioned as a current practice (Table 1). Some processors (~18%) declared to be aware of this cold steeping procedure used by maize *ogi* processors in Nigeria, which borders Benin to the east. This cold steeping technology was not appreciated for it is said to lead to products with an undesirable smell and low starch yield as argued by the processors.

Remarkably, none of the processors in Porto-Novo, who belong to the Goun social-cultural group, knew the Goun technology as described in the literature (Nago et al., 1998a). However, a processor in Bohicon, without any ties to the Goun social-cultural group, used a similar technology. No processor in any of the other locations was found to be familiar with the Goun technology described in literature. More than 50% of all processors in Bohicon, Abomey, Abomey-Calavi and Cotonou used a Fon technology for making *ogi*.

Several variations of traditional technology were encountered during the survey. These were either modified versions of Fon or Goun technologies, or combination of a new variant of cold and Fon technologies regarding the steeping procedures. Processors in Porto-Novo all used a similar variant of the Goun technology, namely by first putting the maize grains in water and then heating them to a temperature between 83 °C and 99 °C before off-fire steeping. Newly identified variants of the Fon technology include cold steeping of the grains (~30 ± 3 °C) for a certain period (1–6 h) followed by hot water steeping (92−97 °C), and hot water steeping followed by the renewal of steeping liquid every day for three days (Fig. 1). Consequently, four types (G1, G2, F1, F2) of technology were distinguished based on differences in steeping procedures. Within each of these technologies also variations exist in heating temperature, steeping duration, and absence or presence of a kneading step of the maize wet flour after grinding, followed by a rest period before filtration (Fig. 1).

3.2. Ogi production characteristics for the encountered technologies

Akpan processors have certain preferences for the materials to produce maize *ogi*. They only use white maize grains as the white colour is characteristic of the final *akpan*. They prefer "well-dried and old stock" maize grains (in Fon known as *gbade xoxo*) coming from harvests of past seasons. This type of maize is usually available during the whole year, but in larger quantities during the dry seasons. During rainy seasons, processors also use "not well-dried new stock" of maize grains (in Fon called *gbade yôyô*, or *kponmou,* and in Goun *tchikiti*), either on their own or in combination with *gbade xoxo*. In our study, all processors used clean "well-dried and old stock" maize grains.

Processors distinguish three types of maize according to their origin, namely "Nikki" (from north Benin), "Fon koui" (from south Benin) and "maize from unknown origin". "Nikki" is the most commonly used and the most preferred because of the medium to the large size of its oval grain. Processors unanimously claim that "Nikki" maize yields more starch than the other types. Besides that, "Nikki" is also more often available as *gbade xoxo*. "Maize from unknown origin" is usually a mixture of grains from different locations.

Table 1
Previously reported *ogi* traditional technologies encountered during the survey‡.

	Porto-Novo Saint Pierre et Paul, Sedjeko, Gbodjè, Malé, Hounsa, Djegan-kpèvi, Gbinkouè, Agboto, Attaké	**Bohicon** Gankon, Sèhoueho, Agbadjagon, Kpokon, Zakanmè, Zakpo	**Abomey** Adjahito, Dezeumè, Djègbé, Gbèkon-honli, Adandokpodji	**Abomey-Calavi** Hêvié, Gbegnigan, Zoca, Godomey, Gbodjo, Arconville, Atrokpocodji, Calavi-Kpota, Sainte Marie	**Cotonou** Cadjehoun, Zogbo, Sedjro, Agla, Ahogbohouè, Gbegamey
Cold technology	–	–	–	–	–
Goun technology	–	+	–	–	–
Fon technology	–	+	+	+	+

‡ Processors were interviewed at different places within a same territory as listed below each municipality. Absence or presence of a traditional technology is marked by a minus (-) or plus (+) sign, respectively.

Plastic containers are the most commonly used type of container for steeping maize grains as observed during the survey. However, some processors (39%) also steep the grains in cast aluminium pots directly after heat treatment and others (22.2%) use aluminium basins. The duration of steeping varies among processors (Fig. 2A) and may depend on unexpected events, which consequently increase or decrease the period of steeping. A steeping period, defined as off-fire steeping, between 12 h and 24 h, is the most adopted (84%).

The plastic containers used for steeping are usually also used for fermentation or for storage of the *akpan*, but some processors have specific plastic fermentation containers that they never use for anything else. The fermentation of maize starch into *ogi* is always performed at ambient temperature, around an average day/night temperature of 30 $\pm$ 3 °C as measured during the survey. It usually lasts 6–24 h depending on processor practices (Fig. 2B), after which the obtained *ogi* is ready for processing into *akpan*. Table 2 shows the estimated fermentation period until ready-to-use according to the processors of the 14 *ogi* samples collected for analysis.

Other *ogi* production practices were mentioned by processors applying the usual technologies illustrated in Fig. 1. First, when in a hurry to make a new batch of *ogi*, for instance, to prevent stock shortage or to cope with a last-minute order, experienced processors use the supernatant of a previous batch of *ogi* for steeping maize grains. This procedure was said to shorten the duration of starch fermentation into *ogi*. Alternatively, the supernatant of a previous batch was also said to be used for cooking *akpan* while skipping the fermentation step. About 67% of processors said they accelerate fermentation by exposing the starch slurry to sunlight; this also speeds up starch sedimentation. In our study, none of the processors of the 14 *ogi* samples collected for analysis applied these methods. After production, *ogi* is stored and portions of it are continuously used to prepare *akpan* for many days up to weeks. To prevent over-acidification during storage, processors replace the supernatant on *ogi* with fresh water after 12 h (16.7%) of fermentation or every one (44.4%) to two days (27.8%) after production.

3.3. Microbial diversity in ogi at ready-to-use time

3.3.1. Abundance and diversity of bacteria and fungi

Total DNA was extracted from 14 *ogi* samples representing all relevant processing variations of the four traditional technologies encountered to determine the impact of processing technology on the microbial community composition of *ogi*. Next, 16S rDNA encoding V3-V4 region and ITS1 were amplified with the appropriate primers and the amplicons were subjected to high-throughput sequencing. A total of 1656 and 4406 amplicon sequence variants (ASVs) of bacterial and fungal sequences, respectively, were found after the final cleaning performed with Phyloseq R. The read counts ranged between 12,218 and 17,572 for bacterial sequences and between 34,288 and 35,667 for fungal sequences (Table 3). The predicted number of bacterial species in samples from Fon F2 technology was higher than that of all other technologies. The number of observed species (i.e., species richness) and the α-diversity index Chao 1 for bacteria in all *ogi* samples were much lower than those for fungi, except for sample "Cot" showing the opposite trend. This indicates that *ogi* samples at ready-to-use time generally have fewer bacterial than fungal species. The Shannon index, which accounts for both richness and evenness (i.e., the relative abundances of taxa), indicated a uniform diversity ($P > 0.05$) of both bacterial and fungal species within *ogi* samples regardless of the steeping procedure. Beta diversity profiling (Fig. 3) showed a significant dissimilarity (R: 0.30515; p-value < 0.016) between the bacterial microbiota of *ogi* samples, mostly differentiating Goun and Fon samples, except for the Goun *ogi* PoN2 and Boh3, which seemed similar to some Fon *ogi*. The fungal microbiota of all samples was quite similar (R: 0.12634; p-value < 0.071).

3.3.2. Bacterial and fungal community composition

In general, the bacterial microbiota in ready-to-use *ogi* were

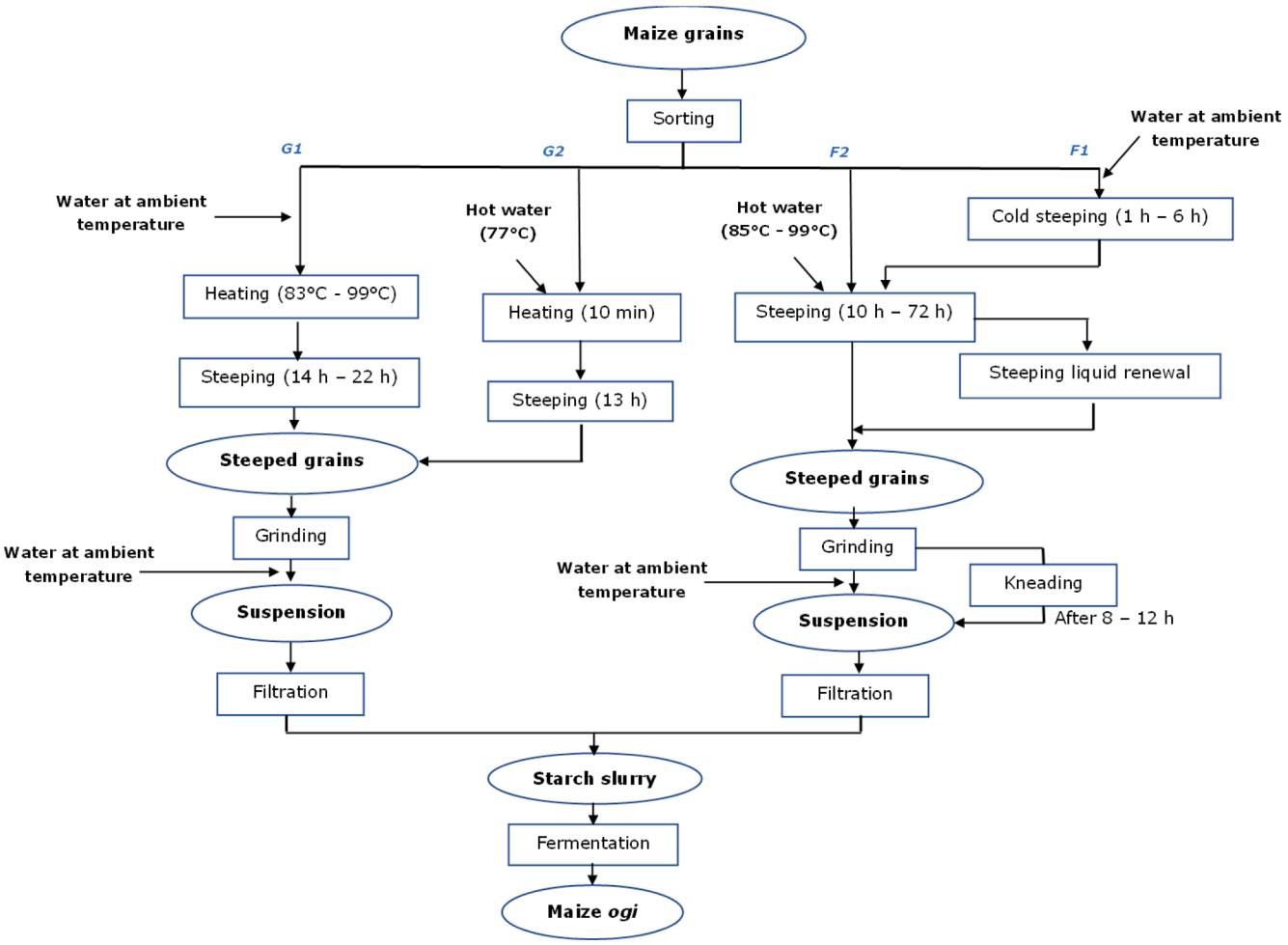


Fig. 1. Four different technologies (G1, G2, F1, F2) for *ogi* production as observed in the survey in Benin. G1: Identified Goun technology, G2: Previously described Goun technology, F1: Identified Fon technology, F2: Previously described Fon technology. The ambient temperature during the survey was about 30 ± 3 °C.

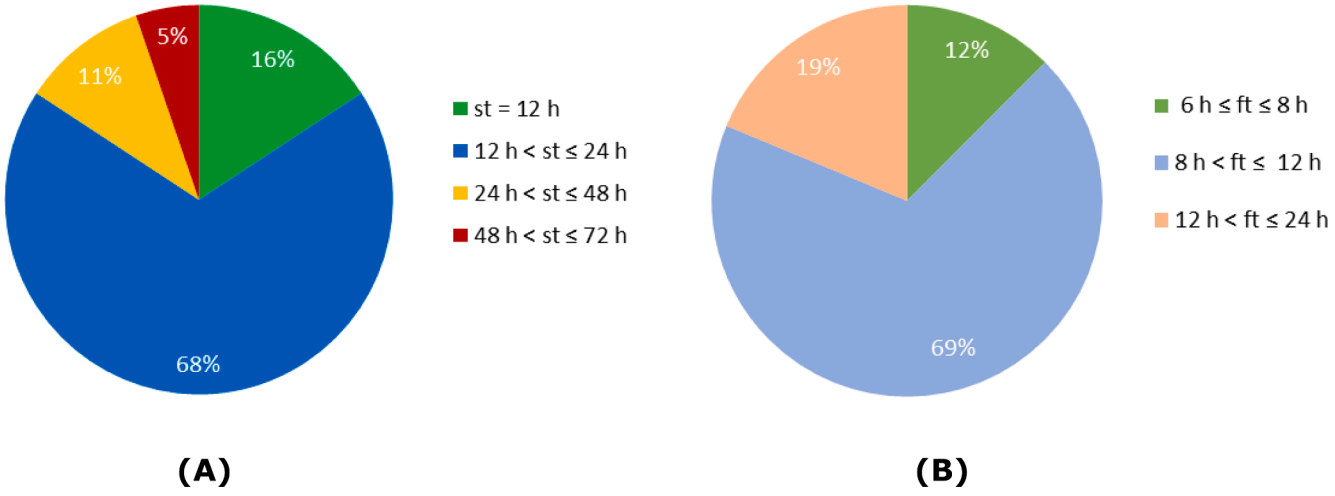


Fig. 2. Steeping duration "st" (A) and fermentation time "ft" (B) commonly adopted by processors.

dominated by members of the Firmicutes phylum (98.9%), whereas the dominant fungal phyla were Ascomycota (87.4%) followed by unclassified fungi (7.7%) and Basidiomycota (4.5%). Four bacterial genera, identified as *Limosilactobacillus* (5.4–79.1%), *Lactobacillus* (8.6–69.3%), *Streptococcus* (0.6–59.3%) and *Weissella* (2.6–51.2%), were predominant in all samples (Fig. 4). Members of a fifth bacterial genus, namely *Ligilactobacillus*, were present at a high relative abundance in the Fon *ogi* "Cal" and "Cot" (16.2% and 21.5%, respectively). A general observation was that species of the genus *Lactobacillus* were most abundant in the bacterial communities in Goun *ogi* except for sample PoN2, which was predominated by the genus *Streptococcus* (59.3%). In the bacterial communities of Fon *ogi*, *Limosilactobacillus* species were most abundant

Table 2
Description of the 14 ready-to-use *ogi* samples collected for analysis.

Applied technology	Samples	Sampling location	Ready-to-use time **
G1: Identified Goun technology	PoN1	Porto-Novo	6 h
	PoN2	Porto-Novo	12 h
	PoN3	Porto-Novo	12 h
	PoN4	Porto-Novo	24 h
	PoN5	Porto-Novo	12 h
	PoN6	Porto-Novo	8 h
F1: Identified Fon technology	Boh1	Bohicon	12 h
	Boh2	Bohicon	12 h
	Abo2	Abomey	12 h
	Abo3	Abomey	12 h
	Ca	Calavi	12 h
G2: Previously described Goun technology	Boh3	Bohicon	12 h
F2: Previously described Fon technology	Abo1	Abomey	24 h
	Cot	Cotonou	24 h

** Estimated fermentation period needed to turn starch into *ogi* that can already make *akpan*.

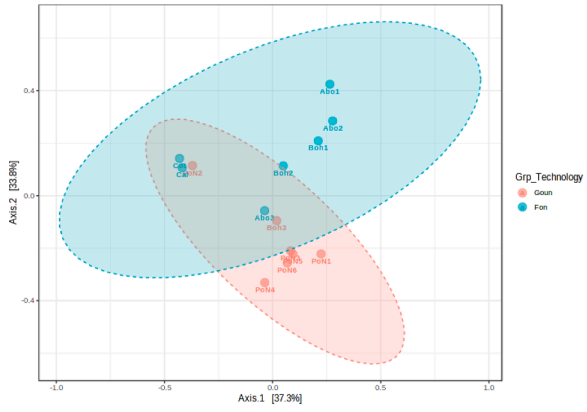
in four samples ("Boh1", "Boh2", "Abo1", "Abo2"), *Streptococcus* species in the samples "Cot" and "Cal" (34.8% and 40.2%, respectively) and *Weissella* species in sample "Abo3" (51.2%). Furthermore, a significant difference was found between samples from Fon and Goun technologies showing a higher abundance of *Lactobacillus* species in Goun samples than in Fon samples (Fig. 5A).

In all ready-to-use *ogi* samples, the fungal microbiota were generally sub-dominated by species belonging to the class Sordariomycetes (10.6–81.9%), Saccharomycetes (6.2–81.4%) and a few belonging to Eurotiomycetes (1.1 and 11.2%) (Fig. 4). However, sample "Cot" was sub-dominated by unclassified Ascomycota species at 42.1%. Members of *Fusarium* and *Sarocladium* genera were the main Sordariomycetes in all the samples. The class Saccharomycetes was mainly represented by *Diutina*, *Pichia*, *Kluyveromyces*, and *Lachancea* genera, as well as unclassified members of the Dipodascaceae family. Some *ogi* samples contained up to 8.3% *Aspergillus* species belonging to the class of Eurotiomycetes, and up to 11% unclassified members of the Ceratobasidiaceae family belonging to the phylum Basidiomycota. The main significant difference between samples of products made with the Fon and Goun technologies regarding their fungal communities was that *Cladosporium* species were abundant in Goun *ogi*, while the genus *Kurtzmaniella* prevails in Fon *ogi* (Fig. 5B,C).

Table 3
Sequence abundance and microbial diversity of *ogi* samples.

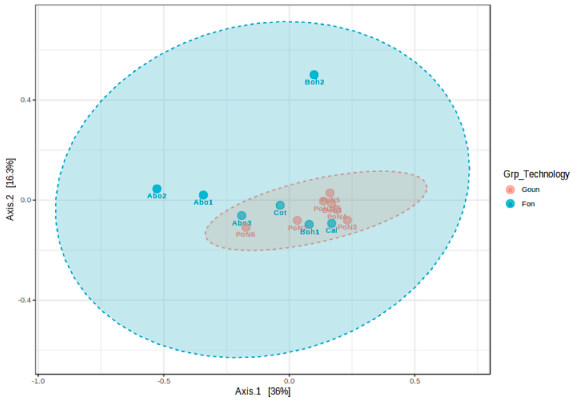
Sample	Technology	Number of unique types		Chao 1 index		Shannon index	
		Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
PoN1	G1	46	142	49	142.5	1.7	2.75
PoN2	G1	53	156	53.25	156.2	1.45	2.92
PoN3	G1	58	132	61	132.38	1.88	2.4
PoN4	G1	61	100	62.5	100.11	1.78	2.41
PoN5	G1	62	121	62.43	121.38	2.49	2.41
PoN6	G1	57	142	57.25	143.11	2.01	2.22
Boh3	G2	56	94	56.25	94.14	2.1	2.3
Boh1	F1	69	160	69.75	160.2	2.17	2.62
Boh2	F1	59	97	59.75	97.5	2.01	1.55
Abo2	F1	45	91	45.5	91.6	1.14	1.51
Abo3	F1	52	150	57	153	1.97	2.36
Cal	F1	65	152	65	152.11	2.1	2.51
Abo1	F2	85	115	85	116.5	1.61	2.52
Cot	F2	100	77	100.75	79.5	2.5	1.84

G1: Identified Goun technology (Goun *ogi*: PoN1, PoN2, PoN3, PoN4, PoN5, PoN6), G2: Previously described Goun technology (Boh3), F1: Identified Fon technology (Boh1, Boh2, Abo2, Abo3, Cal), F2: Previously described Fon technology (Abo1, Cot).



[ANOSIM] R: 0.30515; p-value < 0.016

(A) Bacteria



[ANOSIM] R: 0.12634; p-value < 0.071

(B) Fungi

Fig. 3. Beta diversity at features (ASVs) level of *ogi* bacterial (A) and fungal (B) microbiota affected to Fon and Goun technology. Goun *ogi*: PoN1, PoN2, PoN3, PoN4, PoN5, PoN6 and Boh3; Fon *ogi*: Boh2, Boh3, Abo1, Abo2, Abo3, Cal and Cot.

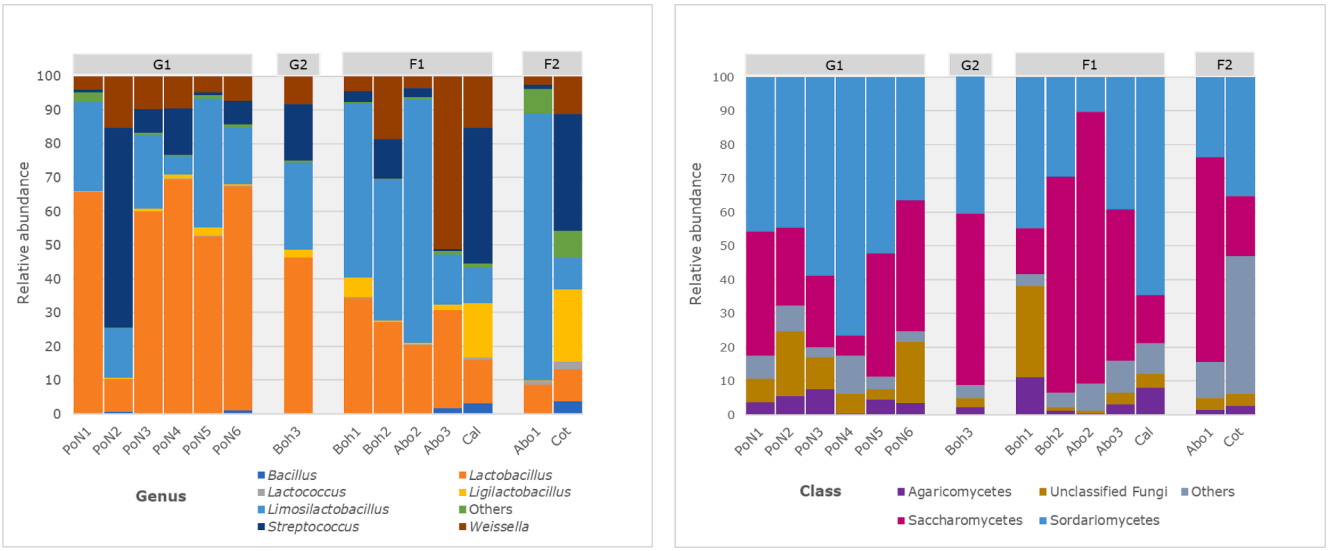


Fig. 4. Relative abundance at genus and class levels respectively for bacterial and fungal microbiota in *ogi*. G1: Identified Goun technology, G2: Previously described Goun technology, F1: Identified Fon technology, F2: Previously described Fon technology.

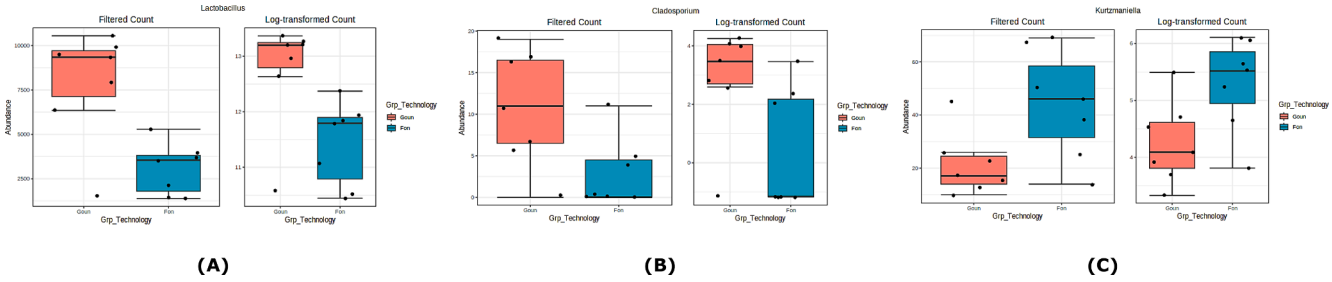


Fig. 5. Differential abundance at genus level respectively for bacterial (A) and fungal microbiota (B, C) in Fon and Goun *ogi*.

3.4. Quality characteristics of ogi at ready-to-use time

3.4.1. pH, acidity and non-volatile compounds

The pH of the *ogi* of the 14 collected samples ranged between 3.1 and 4.2 at ready-to-use time and the titratable acidity between 0.6 and 1.9% lactic acid (Fig. 6). For the two parameters, there was no significant difference ($P > 0.05$) between the Goun G1 and G2 technologies, or between the Fon F1 and F2 technologies. However, some samples within a same technology were significantly different ($P < 0.05$). The *ogi* samples from Goun G1 technology had significantly higher pH values ($P < 0.05$) than the samples from Fon F1 and F2 technologies. As expected, the pH and titratable acidity of analysed *ogi* samples were negatively correlated ($r = -0.446$, p-value $= 0.017$).

The main sugars in *ogi* at ready-to-use time were glucose (0.23–1.89

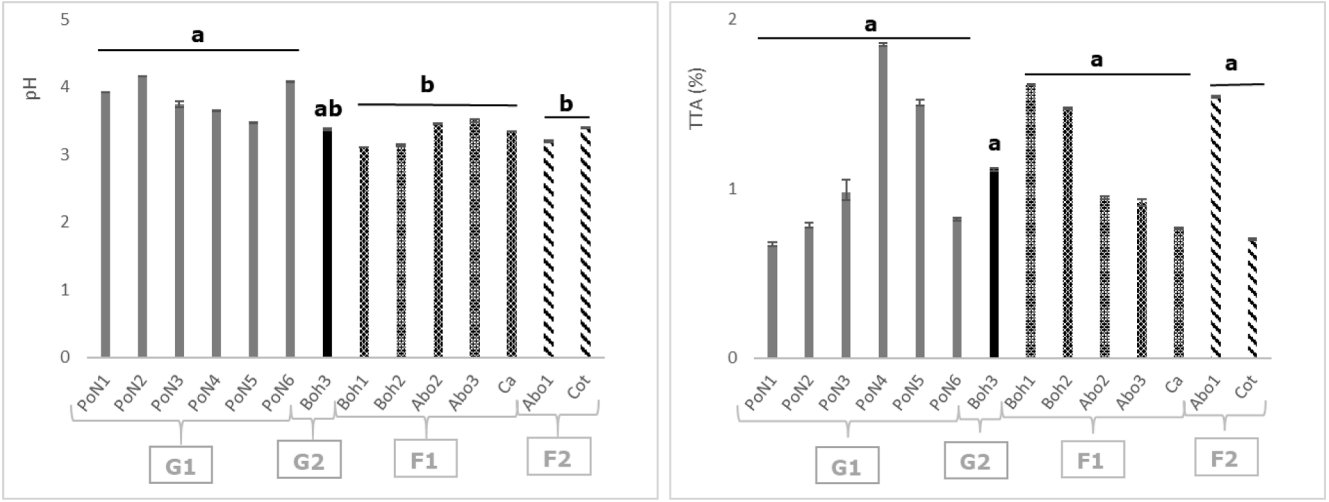


Fig. 6. pH and titratable acidity (TTA) of 14 *ogi* samples affected to their technology. G1: Identified Goun technology, G2: Previously described Goun technology, F1: Identified Fon technology, F2: Previously described Fon technology. Technologies sharing a same letter (a, b) are not significantly different at 5%.

g/L) and fructose (0.03–1.99 g/L), while sucrose was present in a very low amount (≤0.3 g/L), see Table 4. Sucrose showed significantly higher concentrations in samples from Goun G1 technology than in samples from Fon F1 technology. However, total sugar content, which varied between 0.3 and 3.2 g/L in all samples did not differentiate the four technologies.

Lactate was the most dominant organic acid in all *ogi* samples with concentrations ranging from 5.6 to 27.7 g/L. Lactate and citrate significantly differentiated the technologies, showing lower concentrations of each of the two compounds in samples from Goun G1 technology compared to samples from Fon F2 technology. These two technologies also differed significantly for the total organic acids content, which varied from 6.4 to 29.1 g/L of *ogi*. The citrate content of the samples ranged between 0.1 and 0.5 g/L. The ethanol content in *ogi* samples ranged between 0.8 and 3.2 g/L. The lowest and highest values were obtained within the same technology, namely Fon F1 technology. Finally, there was no significant difference ($P > 0.05$) for the ethanol content between the samples from the four technologies.

3.4.2. *Volatile organic compounds and free amino acids*

A total of 23 volatile organic compounds (VOCs) were identified in ready-to-use *ogi* samples and classified into eight groups (Table 5). The full profiles of *ogi* samples based on these groups are shown in Fig. 7. Alcohols, esters and acids were the most abundant groups. The dominant compounds were ethanol, acetyl acetate, propanedioic acid (malonic acid), 2-heptanone and hexanal, respectively, for alcohols, esters, acids, ketones and aldehydes. Samples "Abo1", "Abo2" and "Abo3" collected at Abomey contained the highest concentrations of most VOCs. Large variations were found in VOCs between samples, but these were not significant between technologies except for alkanes ($p = 0.04$) and other ($p = 0.012$) compound groups.

Most *ogi* samples at ready-to-use time had low concentrations of free amino acids (FAAs) as shown by the heatmap profile in Fig. 8. Only the samples "Abo1", "Abo2" and "Abo3" collected in Abomey, as well as "PoN4" and "Boh3" collected in Porto-Novo and Bohicon, respectively, contained relatively high amounts of FAAs. Sample "PoN4" from Goun G1 technology differed from all others by its richness in lysine and free ammonia. Except for lysine, the samples collected in Abomey contained the highest amounts of free essential amino acids, namely methionine, leucine, isoleucine, tryptophan, valine, threonine, phenylalanine and histidine. The total content of free essential amino acids in *ogi* samples collected from processors in Abomey was significantly higher (52.2–70.4 mg/L) than that of all other samples, which had amounts varying between 20.2 and 39.8 mg/L.

Table 5
Volatile organic compounds in *ogi*: retention times (RT) and characteristics.

Name	RT (min)	Formula	MW (g/mol)	Group
1-Butanol	8.03	C4H10O	74.12	Alcohols
Ethanol	4.58	C2H6O	46.07	
1-Octanol	13.97	C8H18O	130.23	
1-Pentanol	9.69	C5H12O	88.15	
Cyclohexanol	12.02	C6H12O	100.16	
1-Hexanol	11.20	C6H14O	102.17	
Ethylbenzene	7.86	C8H10	106.16	Benzenes
1-ethyl-3-methyl-Benzene	11.30	C9H12	120.19	
o-Cymene	11.65	C10H14	134.22	
1,2,3-trimethyl -Benzene	10.42	C9H12	120.19	
2-Carene	18.92	C10H16	136.23	
Ethyl Acetate	3.87	C4H8O2	88.11	Esters
Formic acid butyl ester	9.26	C5H10O	102.13	
Acetic acid anhydride (Acetyl acetate)	6.44	C4H6O	102.09	
Propanedioic acid	12.70	C3H4O4	104.06	Acids
Butanoic acid	14.91	C4H8O2	88.11	
Hexanoic acid	17.42	C6H12O2	116.16	
Hexanal	7.10	C6H12O	100.16	Aldehydes
Nonanal	11.96	C9H18O	142.24	
2-Nonanone	11.88	C9H18O	142.24	Ketones
2-Heptanone	8.75	C7H14O	114.19	
Octane	2.60	C8H18	114.23	Alkanes
di-t-Butylacetylene	9.48	C10H18	138.25	Other

3.5. *Multiple factor comparison of ready-to-use ogi*

A full metabolic profile of *ogi* samples representing all technologies is provided in Fig. 9. This analysis combines all metabolites that were measured for characterization. The volatile organic compounds, free

Table 4
Non-volatile compounds in *ogi* samples at ready-to-use time †.

Technology	*Ogi* samples	Sucrose	Fructose	Glucose	Lactate	Pyruvate	Acetate	Citrate	Ethanol
G1	PoN1	0.17	0.67	0.34	5.91	0.03	0.46	0.28	1.07
	PoN2	0.10	0.33	0.33	5.94	0.07	0.70	0.29	1.45
	PoN3	0.17	0.56	1.38	8.00	0.01	0.64	0.22	1.40
	PoN4	0.12	0.05	1.13	16.88	0.09	1.46	0.25	1.05
	PoN5	0.05	0.97	1.40	12.64	0.00	0.94	0.31	1.68
	PoN6	0.30	0.42	0.23	5.59	0.04	0.60	0.15	1.44
G2	Boh3	0.09	1.52	1.57	11.95	0.02	0.66	0.39	1.26
F1	Boh1	0.02	0.08	0.93	12.24	0.00	0.43	0.12	2.00
	Boh2	0.02	0.03	1.10	13.54	0.01	0.66	0.24	1.04
	Abo2	0.02	0.08	0.25	12.79	0.12	0.80	0.50	1.65
	Abo3	0.04	0.05	0.65	16.54	0.05	1.52	0.41	0.79
	Cal	0.08	1.99	0.57	12.64	0.06	0.83	0.48	3.24
F2	Abo1	0.25	0.09	1.89	27.72	0.41	0.49	0.49	1.86
	Cot	0.03	0.12	0.77	13.98	0.03	0.50	0.39	1.78
Difference between technology		G1 ^{a}	G1 ^{a}	G1 ^{a}	G1 ^{b}	G1 ^{a}	G1 ^{a}	G1 ^{b}	G1 ^{a}
		F1 ^{b}	F1 ^{a}	F1 ^{a}	F1 ^{ab}	F1 ^{a}	F1 ^{a}	F1 ^{ab}	F1 ^{a}
		G2 ^{ab}	G2 ^{a}	G2 ^{a}	G2 ^{ab}	G2 ^{a}	G2 ^{a}	G2 ^{ab}	G2 ^{a}
		F2 ^{ab}	F2 ^{a}	F2 ^{a}	F2 ^{a}	F2 ^{a}	F2 ^{a}	F2 ^{a}	F2 ^{a}

†Compounds concentrations are expressed in g/L of dry *ogi*. Standard deviation for all values in the table is between 0.00 and 0.24. G1: Identified Goun technology, G2: Previously described Goun technology, F1: Identified Fon technology, F2: Previously described Fon technology. Technologies with the same letters (a, b) in column are not significantly different at $p > 0.05$.

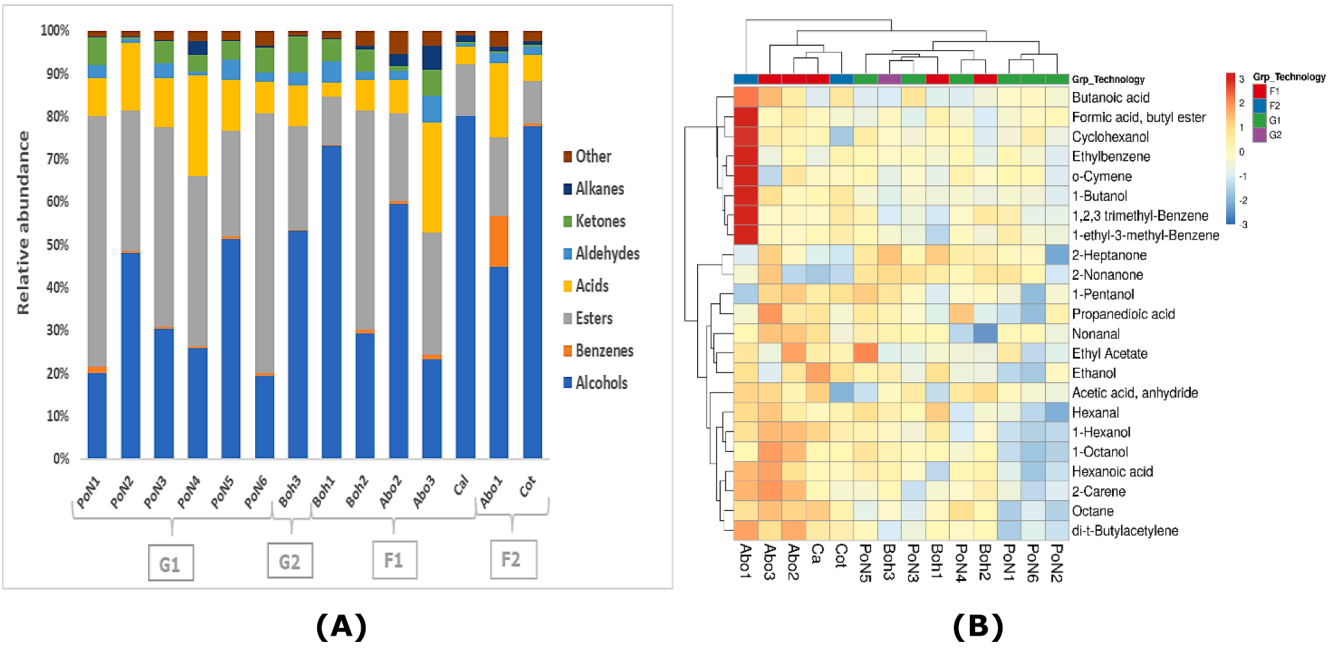


Fig. 7. Abundance of VOCs groups (%) in ready-to-use *ogi* (A) and aroma profile of each *ogi* sample (B). The heatmap is a hierarchical clustering of each VOC relative abundance (log_2) based on unit variant scaling, Euclidean distance and Ward's method. G1: Identified Goun technology, G2: Previously described Goun technology, F1: Identified Fon technology, F2: Previously described Fon technology.

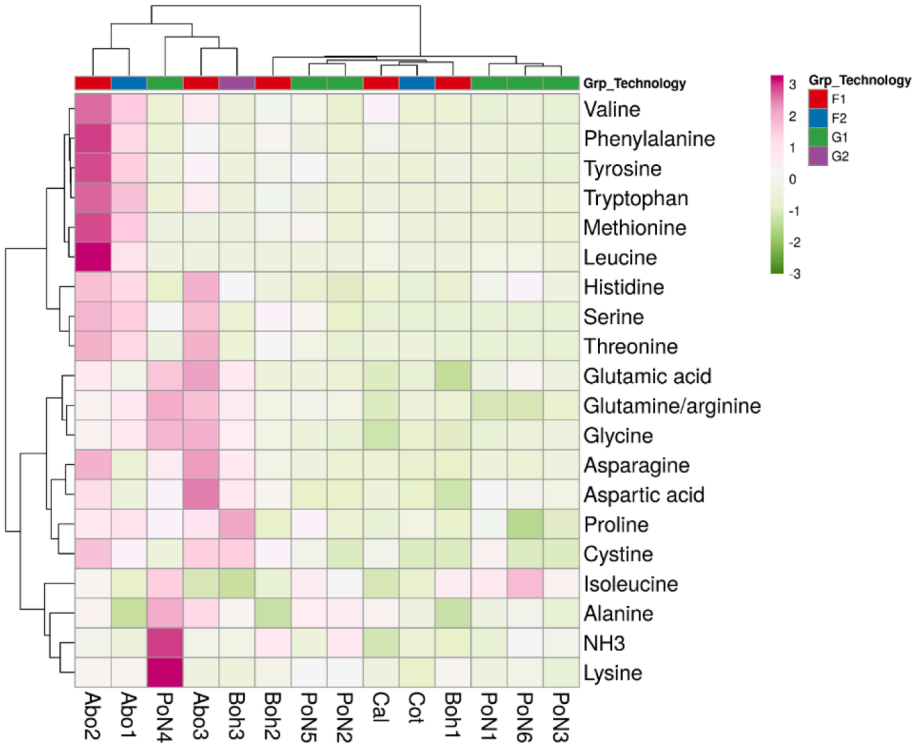


Fig. 8. Free amino acids profile of ready-to-use *ogi*. The heatmap is a hierarchical clustering of each FAA (log_2) based on unit variant scaling, Euclidean distance and Ward's method. G1: Identified Goun technology (Goun *ogi*: PoN1, PoN2, PoN3, PoN4, PoN5, PoN6), G2: Previously described Goun technology (Boh3), F1: Identified Fon technology (Boh1, Boh2, Abo2, Abo3, Cal), F2: Previously described Fon technology (Abo1, Cot).

amino acids except ammonia, organic acids, sugars and ethanol were merged into "VOCs", "FAAs", "OAs", "Sugars" and "Q_Ethanol" categorical variables, respectively. The first two dimensions (Dim1 and Dim2) explained 48.3% of the total variation among the Goun (G1, G2) and Fon (F1, F2) *ogi* processing technologies, which were distinctively positioned (Fig. 9A). However, some samples from Fon technologies were situated next to samples from Goun technologies, making a total of 7 clusters generated from the multifactorial analysis (Fig. 9B). The samples in cluster 1 had relatively low glucose and lactate contents. This cluster, consisting of Goun *ogi* "PoN1", "PoN2" and "PoN6" from G1 technology, was also characterised by low octane, di-t-butylacetylene, hexanal, alcohols (1-octanol, 1-hexanol, ethanol) and hexanoic acid

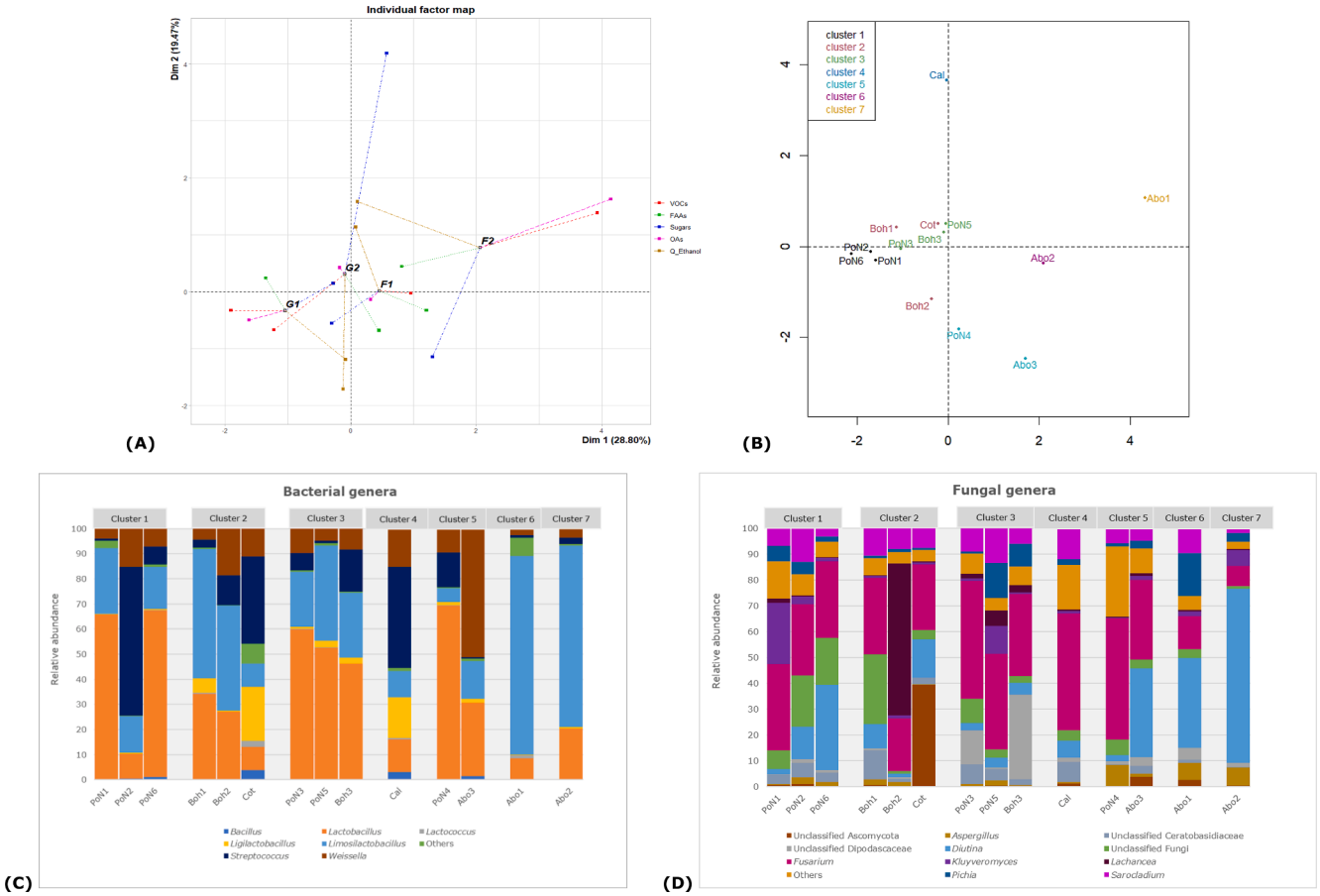


Fig. 9. Metabolic profile (A), hierarchical ascending classification with consolidation and Euclidean distance (B) followed by microbial profiles at genus level (C, D) of ready-to-use *ogi*. G1: Identified Goun technology (Goun *ogi*: PoN1, PoN2, PoN3, PoN4, PoN5, PoN6), G2: Previously described Goun technology (Boh3), F1: Identified Fon technology (Boh1, Boh2, Abo2, Abo3, Cal), F2: Previously described Fon technology (Abo1, Cot).

contents. Cluster 2 contained Fon samples "Boh1", "Boh2" and "Cot". The samples in this cluster had no specific characteristic. They only shared the categorical variables that did not significantly discriminate the 7 clusters at the threshold of 5% (i.e., sucrose, citrate, acetic acid, 2-heptanone, 1-pentanol, 2-nonanone, nonanal, 2-caren, serine, histidine, isoleucine, proline, and cystine). Goun samples "PoN3", "PoN5" and "Boh3" were mainly gathered as cluster 3 due to their high glucose content. Cluster 4 was a single sample ("Cal"), which contained high amounts of fructose and ethanol. The *ogi* samples "PoN4"and "Abo3" in cluster 5 were rich in acetate, propanedioic acid (malonic acid) and free non-essential amino acids such as glutamine/arginine, glutamic acid, glycine, alanine, lysine, aspartic acid and asparagine. Cluster 6 was a unique type of *ogi* ("Abo2"), particularly rich in free essential amino acids (leucine, phenylalanine, methionine, tyrosine, tryptophan, valine, threonine) and asparagine. Cluster 7 was a single complex *ogi* sample ("Abo1") high in aromatic hydrocarbons (ethylbenzene, benzene,1-ethyl-3-methyl, benzene,1,2,3-trimethyl, o-cymene), alcohols (cyclohexanol, 1-butanol), acids (butanoic acid, lactate and pyruvate) and formic acid butyl ester.

Similarities in metabolic characteristics of the ready-to-use *ogi* samples were crossed with their bacterial and fungal profiles, highlighting the top abundant genera (Fig. 9C,D). Interestingly, samples belonging to a same metabolic cluster reflected variability in the proportion of the microbial genera they share. There were significant and positive correlations ($p < 0.05$) between the relative abundances of the bacterial genera *Ligilactobacillus, Lactococcus* and *Bacillus*, and also between the fungal genera *Sarocladium* and unclassified members of Ceratobasidiaceae family.

4. Discussion

In this study, we analysed various aspects of maize *ogi* processing technology as a first step to contribute to research on the improvement and standardisation of *ogi* quality for upgraded production of *akpan*. We determined the state-of-the-art concerning traditional technologies currently used by *akpan* processors in south Benin to produce maize *ogi* and we reported the results for the *ogi* microbial composition and certain important characteristics of product quality, which are typical for this type of food (pH, titratable acidity, sugars and organic acids, free amino acids and volatile organic compounds) at ready-to-use time.

The literature on traditional technologies for *ogi* production in Benin is more than two decades old now, with reference to Nago et al. (1998a). We found that processing has changed, for instance, in terms of available resources, knowledge and practices, but also in how *akpan* processors operate to satisfy as many consumers as possible and to make a profit. This may explain the disappearance of the cold steeping technology described by Nago et al. (1998a), which is said to negatively impact the sensory quality of the *ogi*, and the current preference for medium to large and oval grains instead of small and floury kernels grains reported by Nago, Akissoë, Matencio, and Mestres (1997) and Sacca et al. (2012). Nowadays, only a short cold steeping treatment of the maize grains, with no steeping liquid renewal, is performed in combination with hot water steeping, see Fig. 1. However, the observed variations in *ogi* technologies could not significantly explain whether a processor would best make *akpan* after 6 h, 8 h, 12 h or 24 h of maize starch fermentation ($Chi^2 = 12.444$, $P = 0.189$), or make use of post-production practices such as replacing the supernatant right after 12 h of fermentation or not ($Chi^2 = 6.76$, $P = 0.344$). Similarly, Greppi et al. (2013) realized that the

production techniques of four fermented products, among which *ogi* and mawè in Benin, are specific to each traditional processor, who individually decides what is suitable for her and when to sell her product. In this context, the identified processing technologies and practices may have resulted over time from experiments, errors, remarks and adjustments that happen during production. Indeed, some processors said to have experienced that water absorption in the grains and starch yield increase when steeping maize grains in water at ambient temperature before hot water steeping. According to others, the same effect can be reached by prolonged steeping. Still others consider that kneading maize flour and filtering after a certain period of time is a way of increasing starch yield, improving the creamy and smooth texture of *akpan* and conferring a good aroma. For some producers of the latter category, kneading started from unforeseen situations where they could not complete the filtration as usual but had already added water to the wet flour. Gradual shifts in expectations of *akpan* consumers may also have played a role in the occurrence of new *ogi* technology variants. For example, certain consumers nowadays have specific preferences regarding acidity (i.e., not perceived, mild, moderate), or for aroma (i.e., natural fermented aroma, commercial/synthetic odorants) as reported by processors. In addition, most *akpan* processors are constantly searching for ways to benefit more from their activities and thus consequently experimenting with new variations in processing.

The composition of the microbial communities of the maize *ogi* samples at ready-to-use time shows, in terms of the bacterial species, a significant and large degree of variation, as shown by beta-diversity index (Fig. 3). This variation could be linked to differences in fermentation time, but also many other factors since *akpan* processors were asked to follow their usual *ogi* processing technologies and produce with their own materials. The type and source of maize grains, water for steeping and filtration, as well as storage containers, were all different. This also applies to the grinding machines, operators and production environment. All these factors may contribute to the diversity in microbiota in accordance with Chaves-López, Rossi, Maggio, Paparella, and Serio (2020) and Pswarayi and Gänzle (2022), who listed several intrinsic and extrinsic parameters that influence the microbiota of fermented maize products. Bacterial and fungal communities in ready-to-use *ogi* are diverse but two to eight species excluding pathogens (*Fusarium*, *Aspergillus*) seem to be the main players for they are the most abundant. This actually corroborates the fact that microbiota in traditional foods produced from a natural fermentation are more often diverse but not very complex (Alekseeva, Groenenboom, Smid, & Schoustra, 2021). The predominance of the four genera of lactic acid bacteria (LAB) in *ogi* samples was expected as lactate was found to be the most abundant organic acid (Table 4). However, some *Lactobacillus* species (including *Limosilactobacillus* formerly part of the genus *Lactobacillus*) and *Weissella* species are heterofermentative lactic acid bacteria known to produce a mixture of lactate, ethanol, carbon dioxide and/or acetic acid under different conditions (Schoustra, Kasase, Toarta, Kassen, & Poulain, 2013; Teixeira et al., 2021; Zheng et al., 2020). The organic acids produced during the fermentation are responsible for the low pH of *ogi* samples here varying between 3.3 and 4.2, which aligns with the values 3.4 $\pm$ 0.2 and 3.76 $\pm$ 0.1, respectively, found by Nago et al. (1998a) and Greppi et al. (2013) for *ogi* collected in Benin.

The bacterial genera associated with ready-to-use *ogi* are the same as found by Diaz et al. (2019) in spontaneously fermented *ogi* and other cereal and dairy products from eight different African countries, namely Ghana, Nigeria, Benin, Burkina Faso, Uganda, Kenya, Ethiopia and South Africa. Among the most abundant yeasts in ready-to-use *ogi*, only *Pichia* and *Kluyveromyces* were previously mentioned in *ogi* and mawè, respectively by Greppi et al. (2013) and Houngbédji et al. (2018), who specifically reported *Pichia kudriavzevii*, also referred to as *Candida krusei* or *Issatchenkia orientalis* (Douglass et al., 2018), and *Kluyveromyces marxianus*, as dominant in the two products. The other abundant yeast genera found in the samples were not reported before in Beninese *ogi*.

The high relative abundance of *Fusarium* species in maize *ogi* at ready-to-use time, irrespective of steeping procedure, other processing steps or fermentation duration, would have raised concern about *ogi* food safety if the microbial composition of the samples was not analysed through total DNA amplicon sequencing. Indeed, *Fusarium* is ubiquitous and one of the critical fungi besides *Aspergillus* and other filamentous fungi in cereals and cereal-based products, highly fought because of their mycotoxins and their negative impact on the crops and the health of consumers (Carbas et al., 2021; Chilaka, De Boevre, Atanda, & De Saeger, 2016). The initial quality of the maize grains used, depending on cultivation and storage conditions, as well as the uncontrolled traditional processing conditions in this study, might have contributed to the presence of DNA from those undesirable fungal species in *ogi* samples. However, DNA-based sequencing methods have the weakness of not discriminating between DNA from living and dead cells, as well as culturable and non-culturable microbial communities (Schneider, 2021; Ucak et al., 2022). These methods can actually reveal DNA months after organisms died, e.g. DNA of organisms died in the first hours of fermentation (Nielsen, Johnsen, Bensasson, & Daffonchio, 2007; Wuyts, Van Beeck, Oerlemans Eline, Wittouck, Claes Ingmar, De Boeck, Weckx, Lievens, & De Vuyst, 2018). Moreover, potential inhibitory effects on *Fusarium* growth and detoxification mechanisms against mycotoxins have been reported for lactic acid bacteria (Chilaka et al., 2016; Smaoui, Agriopoulou, D'Amore, Tavares, & Mousavi Khaneghah, 2022; Tsafrakidou, Michaelidou, & Biliaderis, 2020; Wafula, Muhonja, Kuja, Owaga, Makonde, Mathara, & Kimani, 2022). In addition, species of the genus *Sarocladium* (e.g., *Sarocladium zeae),* endophyte to cereals, have antagonistic effects against certain *Fusarium* species as well as other mycotoxigenic fungi for example, *Aspergillus* spp., *Nigrospora* and *Stenocarpella* (Błaszczyk, Waśkiewicz, Gromadzka, Mikołajczak, & Chełkowski, 2021; Kemp, Vaughan, McCormick, Brown, & Bakker, 2020; Liu et al., 2022).

Traditional ready-to-use maize *ogi* samples produced by *akpan* processors not only have shown variability in their microbial profiles but also in their quality characteristics. Significant differences were observed between Fon and Goun samples in terms of pH, residual sucrose, lactate, and citrate concentrations, as well as the total content of free essential amino acids. These could be linked to variations in processing technologies, but also to the nutritional properties of the different types of maize grains used by processors. Furthermore, the metabolic profile of *ogi* samples would be a result of both the activity of endogenous enzymes in maize grains, and bacteria and fungi present during the maize steeping and starch fermentation steps, in line with findings by Chaves-López et al. (2020). The 7 clusters generated from all metabolites except ammonia, show similarities mainly between Goun *ogi* samples. One Goun sample clustered with a Fon sample, and there is a cluster of Fon *ogi* samples and some clusters of single Fon *ogi* (Fig. 9B). The latter clusters seem generally related to differences in processing practices between previously reported and identified Fon *ogi* technologies (F2 and F1, respectively). These practices are, for example, the use of cold steeping, hot water steeping and a time gap after kneading maize flour before filtration in cluster 4 ("Cal"), and the daily replacement of steeping liquid in cluster 6 ("Abo2"). However, no specific bacterial or fungal associations were observed in the samples that could explain the metabolic clusters.

5. Conclusion

The Goun and Fon technologies investigated in this study for maize *ogi* production are not used in the same way by all *akpan* processors. The newly identified technological variants reveal the dynamic nature of traditional processing know-how and the importance of knowledge updating on food processing research. The traditional practices within either technology affect the microbial composition, acidity and metabolic profile of Goun and Fon maize *ogi* at ready-to-use time. Meanwhile, *akpan* processors are conscious of the differences between the *ogi* processing technologies they apply, and believe that those differences make

their products unique and competitive. The current findings do not support such uniqueness for every sample as there were similarities between samples obtained from different technologies. In summary, our findings contribute to a better understanding of maize *ogi* production in Benin and knowledge of how traditional fermented foods of the same designation could still be different. This knowledge provides insights into the underlying technological processes and some important characteristics of the traditional product, which are needed for the optimisation of product quality, ideally with a starter culture that minimizes variations due to spontaneous fermentation.

CRediT authorship contribution statement

A.K. Carole Sanya: Conceptualization, Investigation, Methodology, Formal analysis, Data curation, Writing – original draft. **Yann E. Madode:** Supervision, Project administration, Conceptualization, Methodology, Writing – review & editing. **Sijmen E. Schoustra:** Funding acquisition, Project administration, Supervision, Conceptualization, Writing – review & editing. **Eddy J. Smid:** Supervision, Conceptualization, Methodology, Writing – review & editing. **Anita R. Linnemann:** Supervision, Conceptualization, Methodology, Visualization, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw data are available from corresponding author.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113038.

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