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The impact of processing technology on microbial community composition and functional properties of Beninese maize *ogi*

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ABSTRACT

Traditionally fermented maize starch, called *ogi*, is produced to prepare *akpan*, a yoghurt-like street food widely consumed in Benin. Current maize *ogi* production practices were compared to assess the impact of different processing technologies on the characteristics of the fermented product as a basis to determine best practices. Maize starch slurry samples were collected from processors in five municipalities in southern Benin and analysed before fermentation (starch samples) and after spontaneous fermentation (*ogi* samples). Four technological pathways for maize starch production were distinguished based on variations in the duration of steeping the grains, which ranged from 6 to 72 h, and whether or not kneading of the wet flour before filtration was practised. Six categories of maize *ogi* were derived from the four technology groups based on the duration of the fermentation, which lasted from 6 to 24 h. The average pH of maize starch varied from 3.2 to 5.3, with the lowest values for the two technology groups that also had the highest lactate concentrations (9–11.8 g/L). The six maize *ogi* categories had a pH ranging from 3.1 to 4.0. Viable plate counts of lactic acid bacteria were similar for maize starch samples and for *ogi* samples, whereas yeast counts showed clear differences. Members of the genera *Limosilactobacillus*, *Lactobacillus*, *Weissella, Streptococcus* and *Ligilactobacillus*, dominated the bacterial community in maize starch, and were also dominant in maize *ogi*. The members of the genera dominating the fungal community in maize starch were also dominant in maize *ogi*, except for *Aspergillus* and *Stenocarpella* spp., which decreased in relative abundance by fermentation. The highest total free essential amino acid concentration was 61.6 mg/L in maize starch and 98.7 mg/L in *ogi*. The main volatile organic compounds in maize starch samples were alcohols, esters, and carboxylic acids, which also prevailed in maize *ogi* samples. The results indicate that the characteristics of traditional maize *ogi* depend on the processing technologies used to produce the maize starch before the intentional fermentation into *ogi*, with no clear-cut connection with the production practices due to high variations between samples from the same technology groups. This revealed the importance of a standardized maize starch production process, which would benefit controlling the starch fermentation and the characteristics of maize *ogi*. Further research is needed to understand the hidden fermentation during maize starch production for determination of the best practices that support the production of quality maize *ogi*.

1. Introduction

Traditional fermented foods are spread all over the world and very diverse for reasons such as the available raw materials (Tamang et al., 2016), environmental conditions, culture and history (Plessas, 2022). Traditional fermented foods and beverages are generally nutritious, have a long shelf life, desirable sensorial characteristics, and provide potential or proven health benefits (Cuamatzin-García et al., 2022; Shah et al., 2023). These foods are relatively cheap and accessible in developing countries, for example in Africa, where they also contribute to income generation as a small-scale economic activity, mainly performed by women (Materia et al., 2021; Zannou et al., 2022).

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Many traditional fermented foods and beverages in Africa are from cereals, usually maize, sorghum, and millets (Sulieman, 2022; Achi and Asamudo, 2019). The cereals themselves are important sources of carbohydrates, especially the non-digestible carbohydrates which are considered prebiotics (Kirmizigul and Sengun, 2023). The fermented products have additional benefits due to a range of bioactive and improved nutritional compounds (Achi and Asamudo, 2019; Hlangwani et al., 2023). Cereal-based fermented products are also rich in viable lactic acid bacteria acting in fermentation processes, and thereby contribute to healthy diets and the well-being of consumers (Kirmizigul and Sengun, 2023; Rawat et al., 2023). In addition, a large number of these products in Africa are non- or low-alcoholic foods and beverages obtained by lactic acid fermentation, which represent a significant proportion of the diets of people of all ages to whom they serve as complementary foods, thirst-quenching foods, snacks, or proper meals (Pswarayi and Gänzle, 2022; Apaliya et al., 2022; Misihairabgwi and Cheikhyoussef, 2017; Sulieman, 2022). All these explain the current trend of increased interest on traditional lactic acid fermented cereal products from Africa, and the research for understanding the production processes, the complex microbial populations associated, and their mechanisms of action during fermentation.

Akpan, for instance, a popular yoghurt-like food from Benin, West Africa, produced with fermented maize starch, called *ogi*, has recently attracted the attention of researchers. Primarily, *akpan* is a thirst-quenching food, which can be consumed plain, with added sugar, condensed milk, ice chunks, or a mix of these (Akissoé et al., 2015; Sacca et al., 2012). Nowadays, *akpan* is also seen as a potential health-promoting fermented food with prebiotic properties (Gullón et al., 2015). Yet, the quality of the fermented maize starch used as starting material is not standardized. Indeed, spontaneously fermented foods often suffer from inconsistencies resulting from the uncontrolled fermentation driven by a consortium of random microorganisms from the raw material, production environment, processor, utensils, and equipment (Chaves-López et al., 2020; Pswarayi and Gänzle, 2022). Moreover, the diversity in processing practices applied by the processors may differently impact the quality of these products (Phiri et al., 2020).

The traditional production of maize *ogi* for *akpan* preparation is done by different processing technologies (Sanya et al., 2023). These are consecutive processing steps by which the processor intends to extract maize starch from the maize grains to let it further ferment naturally. The variations in processing technologies are attributed to the traditions and food related cultural richness of Africa. These processing variants are transferred from generation to generation, whereby households have developed their specific practices (Ekpa et al., 2019; Pswarayi and Gänzle, 2022). Moreover, unforeseen situations during production may contribute to modifications of processing practices, along with changes in consumer preferences nowadays (Sanya et al., 2023).

As an attempt to find out how these processing differences affect maize *ogi* characteristics, our previous study evaluated the fermented product based on a comparison between newly identified and previously reported processing technologies currently used in southern Benin (Sanya et al., 2023). Special attention was given to the potential influence of maize grain steeping procedures on the microbial community present in *ogi* as mentioned by Nago et al. (1998). Significant dissimilarities were revealed in terms of the characteristics and microbial composition in maize *ogi* samples, but no clear relation was established with the different processing technologies, showing that the steeping procedure, as defined in the study, was not critical for the quality of the fermented maize starch samples (Sanya et al., 2023). Hence, here we look further into maize *ogi* characteristics by screening every point of divergence between the processing technologies used by *akpan* producers based on an inventory of current practices.

This study sought to highlight the stages of processing where variations in practices are crucial for the characteristics of maize *ogi*. In short, we first reported the differences between processing stages for maize *ogi* production. Secondly, we studied their impact on defined quality characteristics as well as the microbial community composition in maize starch before fermentation, and also after fermentation. Finally, the most influencing stages were used to categorise maize starch and maize *ogi*, then we investigated the development of functional properties of the fermented product.

2. Material and methods

2.1. Collection of processing information and maize starch samples

Fourteen traditional processors with at least 5 years of experience in *akpan* production were selected in five municipalities in southern Benin, namely Porto-Novo, Bohicon, Abomey, Abomey-Calavi, and Cotonou. The processors were all females, living in different districts of the rural and urban areas of the municipalities, with access to local markets and retailers for the supply of the maize grains they use.

Maize starch slurry was collected from every processor on the day of production. All the samples were obtained over the period January to March 2021 when the ambient temperature was 30 ± 3 °C (recorded with an iButton device temperature data logger, 0–125 °C; MAXIM, Philippines). The production of the starch slurries was carried out by processors in their own environment, using their own materials. Information regarding the maize grains used were recorded along with the processing steps and practices.

Samples were taken from the maize starch slurries immediately after extraction and kept on ice for the starch to settle down. The starch samples were collected after decantation of the supernatant and labelled 0 h samples. Other parts of produced maize starch slurries were transferred to the laboratory and kept at ambient temperature, for spontaneous fermentation. Samples of fermented maize starch (*ogi*) were subsequently taken at the time indicated by each processor as the end point of fermentation (i.e., 6 h, 8 h, 12 h or 24 h). The samples taken before and after fermentation were subsequently analysed or kept frozen at −20 °C until further analysis.

2.2. Physicochemical analysis

The samples of maize starch and maize *ogi* were tested for pH and titratable acidity (TTA) according to ISO 1842:1991 (ISO, 1991) and ISO 750:1998 (ISO, 1998), respectively. Briefly, 10 g of sample at room temperature diluted with 20 mL distilled water, were stirred and the pH was measured using a pH meter (Cybernscan pH 510, Eutech Instruments, Malaysia). Next, this suspension was mixed with an additional 70 mL of distilled water to determine the TTA by titration with a solution of 0.1 NaOH until a pH of 8.1 ± 0.2. The dry matter content of the samples was calculated from moisture content determination following the AACC 44-15A method (AACC, 2000), and used to express results on a dry weight basis.

Sugars (glucose, fructose, sucrose), organic acids (lactate, pyruvate, citrate, acetate) and ethanol concentrations of the samples were determined by High-Pressure Liquid Chromatography (HPLC), following the method described by van Mastrigt et al. (2018) and expressed in g/L dry weight basis. Free amino acids were quantified via Ultraperformance Liquid Chromatography (UPLC) using the AccQ•Tag method (Waters) as described by Lanzl et al. (2022) and expressed in mg/L dry weight basis. All samples were treated before HPLC and UPLC quantification according to our previous study (Sanya et al., 2023). The sum of sugars, organic acids and free essential amino acids (FEAAs) was calculated, respectively, to express the total sugar, total organic acid and total free essential amino acid concentrations of the samples.

Headspace solid-phase microextraction gas chromatography (HS – SPME - GC) coupled with mass spectrometry (MS) was performed to determine and quantify the volatile organic compounds (VOCs) in 2 g of maize starch and maize *ogi* samples, as described by Moonga et al. (2021). The peak areas of identified compounds were used to estimate the amount of the compounds in the samples. The relative abundance

expressed as a percentage of VOC groups in each *ogi* sample was calculated.

2.3. Microbial enumeration

Presumptive total viable plate counts (TC), lactic acid bacteria counts (LAB), yeast counts (YE) and Enterobacteriaceae counts (Entero) were determined by serial dilution and cultivation techniques. For this, the samples of maize starch and maize *ogi* were analysed immediately after sampling. A dilution of 1/10 was obtained by transferring 5 g of the sample in 45 mL sterile buffered peptone water (Thermo Scientific™ CM0509B) from which serial dilution was performed. Plate Count Agar (PCA) (Thermo Scientific™ CM0325B) was used for total viable counts enumeration after incubation at 30 °C for three days. The LAB counts were enumerated on double-layered plates of De_Man, Rogosa and Sharpe (MRS) agar (Thermo Scientific Oxoid CM1153B) after incubation at 30 °C for two days. Yeast Malt Agar (Sigma-Aldrich, Y3127) with added chloramphenicol and chlortetracycline at a ratio of 50 mg/L and 25 mg/L, respectively, was used for yeast enumeration after incubation at 25 °C for three days. Violet Red Bile Glucose (VRBG) Agar (Thermo Scientific Oxoid CM1082B) was used for Enterobacteriaceae enumeration on double-layered plates after incubation at 37 °C for 24 h. From the plates of each medium, colony-forming units (CFU) were counted and cell counts were expressed as "log CFU/g".

2.4. Total DNA extraction, amplicon sequencing and bioinformatic analysis

The microbial community composition in maize starch and maize *ogi* samples was determined from total DNA extracted directly from the samples using Qiagen DNeasy Blood & Tissue Kit (Qiagen, Venlo, The Netherlands) as described in our previous study (Sanya et al., 2023). Amplicon sequencing of 16S rRNA and ITS genes, with a focus on the regions V3-V4 and ITS1, respectively, was performed by Novogene Company Ltd. (Cambridge, United kingdom). The raw sequencing data of microbial community was deposited at 4TU.ResearchData, The Netherlands (URL: doi:10.4121/ab7bc46e-e163-4cec-8a5d-6d987025dd3a). Data were processed and further analysed in Microbiome Analyst web 2.0 (www.microbiomeanalyst.ca/) following the description in supplementary method.

2.5. Statistical analysis

For each sample, mean and standard deviation were calculated from duplicates. For groups of technology and *ogi* categories, mean, standard deviation and coefficient of variations were calculated among the samples belonging to a same group. Microsoft Excel 16 (Office 365) was used to arrange data, run calculations and plot histograms. The impact of factor of variation between technologies was tested in R v4.2.2 with a One-way analysis of variance followed by the Tukey HSD posthoc test, or Kruskal Wallis non-parametric ANOVA followed by Conover's all-pairs test. A p-value < 0.05 was considered significant. Statistics were performed on log-transformed values for the plate counts. Principal components analysis (PCA) was performed by FactoMineR and Factoshiny R packages. A correlation value of 0.3 was set as threshold for the description of the qualitative and quantitative variable on the PCA dimensions.

3. Results and discussion

3.1. Specificities of traditional processing technologies for maize ogi production

The process to obtain *ogi* from maize grains can be divided into three main stages according to the intermediate products (Fig. S1; Table S1). The first stage is completed by the end of the steeping. The second stage is completed when the starch slurry is obtained. The third stage entails fermentation of the starch slurry to obtain *ogi* after 6 to 24 h of incubation at ambient temperature, depending on the preferences of the processor.

Variations in processing technology are summarized in Table S1 and grouped by processing stages. Six potential influencing factors were identified, including cold steeping, heat treatment, change of steeping liquid and steeping time (Table S1; Fig. S1). In our study, the "Fon" and "Goun" heat treatments characterize the heating procedures described in previous research work (Nago et al., 1998; Sanya et al., 2023). Some of the processors applying similar procedures used charcoal, and others used wood, as source of energy for the heat treatment. The quantity of maize processed was also variable and the durations of the heat treatment (not reported) varied accordingly.

3.2. Effect of specificities in processing technologies on maize starch

To evaluate how variations in processing practices affect maize starch characteristics, the physicochemical properties (pH, TTA, sugars, organic acids and ethanol), microbial counts (total plate, LAB and Enterobacteriaceae) and microbial community composition of the maize starch samples, obtained from the starch slurries collected from processors before fermentation, were analysed as a function of the factors of variation identified in the processing stages 1 and 2 (Table S1; Fig. S1). Statistical tests comparing the starch samples for each parameter are provided by factors of variation in Tables S2.A and S2.B.

3.2.1. Physicochemical characteristics of maize starch

The physicochemical characteristics of maize starch samples are shown in Table 1. The pH of maize starch after extraction varied from 3.2 to 6.2. Significant impacts ($p < 0.05$) were found for heat treatment ("Goun" or "Fon"), steeping time ("common" or "long") and kneading ("no kneading" or "kneading") on the pH (Table S2.A). Maize starch samples had low pH values when water was boiled and poured on the maize grains (Fon samples), when long steeping of the maize grains (44 to 72 h) was applied, and when wet maize flour, after grinding, was kneaded and left for at least 8 h before filtration. The kneading operation also significantly increased the titratable acidity of maize starch ($p = 0.03$) compared to samples obtained without kneading. The simultaneous occurrence of a low pH and high titratable acidity (TTA) obtained as an effect of kneading was expected with regard to the negative and significant correlation between pH and TTA (cor $= -0.858$, $p = 5.16e\text{-}09$).

Total sugars, i.e. the sum of glucose, fructose and sucrose, in maize starch samples were significantly affected ($p < 0.05$) by the application of cold steeping for 1 or 6 h, and by the change of steeping liquid in the production process. Specifically, the glucose concentration was significantly affected ($p = 0.02$) by the heat treatment applied to the grains. Maize grains at maturity have low levels of free sugars (Chaves-López et al., 2020), which can explain the low sugar concentration in maize starch, as the samples were produced from mature and dried maize grains. However, the concentration of glucose in some starch samples was high, placing glucose as the most abundant sugar.

The steeping time, change of steeping liquid, as well as kneading before filtration, all significantly ($p < 0.05$) affected the total acid and ethanol concentrations of maize starch. These could be associated with biochemical and texture modifications that maize grains undergo during steeping, variously occurring depending on the steeping conditions and initial composition of the maize grains as a result of the activity of endogenous enzymes, and microbial colonisation, along with excretion of metabolites in the steeping liquid (Chaves-López et al., 2020; Gänzle and Salovaara, 2019; Oduro-Yeboah et al., 2016). In addition, the kneading step implemented by certain processors is followed by a rest period of 8 to 12 h during which the ground wet maize flour probably underwent microbial fermentation as reported by Gänzle and Salovaara (2019). The organic acids in the samples mainly consisted of lactic acid.

Table 1
Physicochemical characteristics of maize starch.

Starch samples	pH	TTA (g/100 g)	Sucrose	Fructose	Glucose	Lactate	Acetate	Citrate	Ethanol
P1_0h	4.7	0.5	1.3	0.8	0.9	2.5	0.2	0.7	1.1
P2_0h	5.8	0.4	1.2	1.1	0.4	0.6	0.2	0.8	1.0
P3_0h	5.8	0.2	0.4	0.2	1.1	3.8	0.4	0.3	0.8
P4_0h	4.6	0.6	0.3	1.7	0.4	3.0	0.5	0.5	1.0
P5_0h	4.5	0.5	0.9	2.1	3.2	2.1	0.4	0.7	0.9
P6_0h	6.2	0.2	2.2	0.2	0.7	0.2	0.0	1.2	1.0
B1_0h	4.2	0.5	0.3	0.2	4.5	1.6	0.2	0.2	1.0
B2_0h	3.2	1.2	0.1	0.0	1.4	9.0	0.3	0.3	1.4
B3_0h	6.0	0.2	2.2	0.6	1.7	0.1	0.0	0.8	0.9
A1_0h	5.2	0.4	1.9	1.3	12.7	0.9	0.1	0.8	1.2
A2_0h	3.5	0.7	0.0	0.8	0.3	11.8	0.8	0.5	1.8
A3_0h	3.7	0.9	0.4	0.1	1.4	11.7	1.4	0.6	1.1
Ca_0h	4.3	0.3	0.8	1.9	10.2	1.6	0.4	0.4	1.4
Co_0h	5.9	0.2	2.1	0.8	2.6	0.1	0.0	1.0	0.9

Compound concentrations are expressed in g/L dry basis of maize starch samples.
Most pyruvate concentrations were below 0.1 g/L, which are not shown in the table. TTA: titratable acidity. The standard deviation for all values in the table is between 0.00 and 0.08. The abbreviations in the first column are the starch samples represented by the processors who supplied the starch slurries (P1 - P6: Porto Novo, B1 - B3: Bohicon, A1 - A3: Abomey, Ca: Abomey-Calavi, Co: Cotonou), followed by "0 h" standing for the sampling time (here, before fermentation).

3.2.2. Microbial enumeration in maize starch

The total viable plate counts and viable counts of Enterobacteriaceae in maize starch samples were assessed (Fig. S2) in order to compare the production conditions adopted by processors. In general, high total viable counts (6.4 to 8.8 log CFU/g) were observed in the samples, dominated by lactic acid bacteria (LAB). Cereal grains themselves are important sources of microorganisms, up to 6 log CFU/g (Gänzle and Salovaara, 2019), coming from diverse origins such as the environment, cultivation practices, handling and transport operations. In addition, the traditional processing of maize grains was performed in uncontrolled conditions where contamination by ubiquitous living bacteria is inevitable. The dominance of LAB in the maize starch samples could be due to a combination of factors such as their natural presence in cereals (Yalmanci et al., 2022), the richness of maize grains in carbohydrates (Florou-Paneri et al., 2013), the steeping of whole maize grains and the steeping duration (Akinrele, 1970; Pswarayi and Gänzle, 2022), as well as environment and processing materials. The practice of steeping entire maize grains rather than their milled form may play a selective role in directing the growth of desired microorganisms and the predominance of lactic acid bacteria (Akinrele, 1970). Cereal grains generally start to ferment after 12 h of steeping, and LAB take the lead and remain prevalent after 24 h of steeping (Pswarayi and Gänzle, 2022). High initial total plate counts and LAB counts were also reported in the production process of mawè, a traditional lactic acid fermented dough produced in Benin (Hounhouigan et al., 1994).

Steeping duration ($p = 0.01$) significantly impacted the total plate counts as well as the LAB counts. Samples obtained from maize grains that were steeped for a long time (44–72 h) showed relatively higher counts than the samples obtained from grains that were steeped for 12 to 24 h. In addition, the type of heat treatment in the production process significantly influenced the total plate counts, LAB counts, as well as Enterobacteriaceae counts in maize starch samples. Enterobacteriaceae counts were generally 3.3 to 4.7 log CFU/g in samples from the Goun practice, whereas a great variability was observed in samples from the Fon practice with counts ranging from 1.5 to 5.9 log CFU/g.

3.2.3. Microbial composition and diversity in maize starch

Relative abundances of taxa in the bacterial and fungal communities in maize starch samples were assessed by 16S rRNA and ITS genes amplicon sequencing, respectively. These data were subsequently used to calculate the corresponding β-diversity values (Table S2.B). The bacterial community in maize starch was dominated by representatives of the phylum Bacillota (previously referred to as Firmicutes) (89.0 to 99.8 %), with species belonging to the class of Bacilli representing 85 to 99.8 % of the total community. A significant difference at the genus level ([ANOSIM] R: 0.46708; $p = 0.02$) in the bacterial communities of maize starch (Fig. S3.A) was observed as a function of processing technology. Significantly fewer *Lactobacillus* species were found in maize starch samples when a technology with a long steeping time (44–72 h) of the maize grains was used as compared to samples of maize starch that were obtained by applying a technology with a shorter steeping duration (12–24 h) (Fig. S3·B). None of the other processing practices significantly impacted ($p > 0.05$) on the bacterial community composition in the starch samples.

The diversity of the fungal community was found to be independent of the variations in processing technology ($p > 0.05$). The fungal community was dominated by the phylum Ascomycota (74.6–96.1 %), followed by members of Basidiomycota (2–20.7 %).

3.2.4. Discriminative factors in maize starch production

The most critical differential factor in the first stage of the maize starch production process, based on the above results with significant *p*-values ($p < 0.05$), was the steeping time (Table 2). Indeed, steeping of maize grains for 12 to 24 h or 44 to 72 h, simultaneously affected the physicochemical and microbial characteristics of maize starch samples, with significant effects mainly on lactic acid, sugars, microbial plate counts and community diversity. Subsequently, steeping time (stage 1) and kneading (stage 2) were used to subdivide the maize processing technologies for starch production into four groups, namely: "common steeping time plus kneading" (Cm_K), "long steeping time plus kneading" (Lg_K), "common steeping time without kneading" (Cm_NK), "long steeping time without kneading" (Lg_NK).

3.3. Maize starch characteristics shaped by the four technologies

To assess how these four processing technologies impacted maize starch, principal components analysis (Fig. 1) was performed with pH, TTA, total organic acids, total sugars and ethanol as important indicators. The between-samples (or within-groups) coefficients of variation (%CV) were estimated for each indicator to check the degree of variation between the samples belonging to similar technology group. The first dimension (Dim1) of the principal component analysis, explaining almost 76 % of the total variation among the groups of maize starch samples, was enough to describe all samples, in combination with the characteristics of the samples (Table S4). Long steeping of maize grains (≥ 44 h) plus kneading of the wet maize flour (Lg_K) resulted in starch samples with the highest sugar concentration (7.4 g/L, CV = 105.2 %) and glucose as the most abundant sugar (5.8 g/L, CV = 132.1 %). When no kneading was applied after a common steeping duration of 12 to 24 h (Cm_NK), maize starch after extraction had a sugar

Table 2
Summary-significant factors of variation ($p < 0.05$) in stage 1 of maize starch production.

	Cold steeping	Heat treatment	Steeping time	Steeping liquid change
pH	-	+	+	-
TTA	+	-	-	-
Glucose	+	+	-	+
Total sugars	+	-	-	+
Lactate	-	-	+	+
Total acids	-	-	+	+
Ethanol	-	+	+	+
TC	-	+	+	-
LAB	-	+	+	-
Entero	-	+	-	-
Microbial diversity	-	-	+	-

A significant effect of a factor of variation (in columns) on maize starch characteristics (in rows) is marked by a plus (+), while the absence of effect is marked by a minus (−). TTA: titratable acidity, TC: Total viable count, LAB: lactic acid bacteria count, Entero: Enterobacteriaceae count.

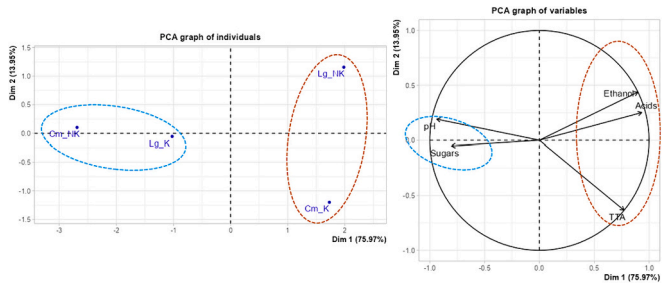


Fig. 1. Principal components analysis of maize starch characteristics as a function of the four technology groups. Cm_NK: common steeping time without kneading. Cm_K: common steeping time plus kneading. Lg_NK: long steeping time without kneading. Lg_K: long steeping time plus kneading. Common steeping time = 12–24 h, long steeping time = 44–72 h.

concentration of 5.0 g/L (CV = 81.8 %) and pH of 5.3 (CV = 14.2 %). In the cases of a long steeping of maize grains without kneading (Lg_NK) and a common steeping duration plus kneading (Cm_K), maize starch samples contained significantly more acids, mainly lactate (9–11.8 g/L), and ethanol (1.4–1.8 g/L), than the starch samples from the other two technologies.

Remarkably, some starch samples had very low pH values around 3.0–4.0 already right after extraction, for example in the technology groups Cm_K (3.2), Lg_NK (3.5) and Lg_K (4, CV = 9.7 %). On a first hand, this could be due to uncontrolled fermentation of the maize grains during the long steeping period of 72 h for the Lg_NK sample, even though the steeping liquid was changed every day (Fig. S1). On the other hand, for Cm_K and Lg_K samples, fermentation of wet maize flour during the waiting time of 8 to 12 h after kneading seems to be the cause, favoured by the presence of water at the kneading step and access by microorganisms to the compounds released during the previous processing steps (Horlacher et al., 2023).

The between-samples coefficient of variation exhibited for the technology groups Lg_K and Cm_NK were very high. This could be due to cofounding factors such as differences in the maize grains used by the processors (i.e., type, genotype, proximal composition, microbiological quality), in addition to variability in the steeping duration within each technology group (12 to 24 h for common steeping "Cm", 44 to 72 h for long steeping "Lg"). Moreover, in some cases where fermentation probably occurred, the microbial composition of the starch samples combined with the metabolic activity of the microorganisms that were present, may have induced variability in compounds availability in the extracted maize starch.

There was no significant difference in LAB counts between the four technology groups ($p > 0.05$), but significant differences in yeast counts ($p = 0.018$) were found: higher counts were found in starch samples from the Cm_K technology group (6.0 log CFU/g, CV = 0.4 %), than for the Cm_NK technology group (4.2 log CFU/g, CV = 23.1 %) that involved no kneading (Table S4). The introduction and mixing of oxygen during the kneading of wet maize flour in presence of air and water have probably boosted the yeasts to thrive and grow better leading to higher cell counts in starch samples from Cm_K group. Such effect of kneading corroborates yeast growth as reported by De Vuyst et al. (2023) for sourdough production.

The microbial community composition in maize starch was also studied as a function of the technology groups. The most abundant bacterial genera were *Limosilactobacillus* (18.6–78.4 %), *Lactobacillus* (15.5–55.9 %), *Weissella* (4–41.4 %), *Streptococcus* (≤ 12.7 %) and *Ligilactobacillus* (≤ 1.6 %), see Fig. 2A. Furthermore, a significant difference at genus level ([ANOSIM] R: 0.35266; $p = 0.042$) was observed between the technology groups, showing more *Limosilactobacillus* species ($p = 0.013$) in samples from maize grains that had been subjected to long steeping without kneading (Lg_NK). The observed five LAB genera were reported as the main bacterial genera in fermented ready-to-use maize *ogi* in our previous study (Sanya et al., 2023). Their abundance in maize starch (before the actual fermentation into *ogi*) bears witness to the occurrence of fermentation during the processing of maize grains to obtain the starch, which could be due to the steeping phase, the waiting time observed after kneading, as well as to microbial transfer from previous batches of fermented product retained in the walls of containers and materials used during production (Chaves-López et al., 2020; Pswarayi and Gänzle, 2019, 2022; Schoustra et al., 2013).

The fungal community composition at genus level of maize starch samples was not significantly different between the technology groups ([ANOSIM] R: 0.32077; $p = 0.068$). Generally, *Fusarium* was abundant in all groups (22.2–39.4 %), sometimes dominating in Cm_NK and Lg_K samples (Fig. 2B). This was not surprising since members of the genus *Fusarium* are worldwide recognized as plant pathogens living as a parasite and endophyte on maize grains, and commonly occurring during cultivation in the field, particularly under the warm climatic

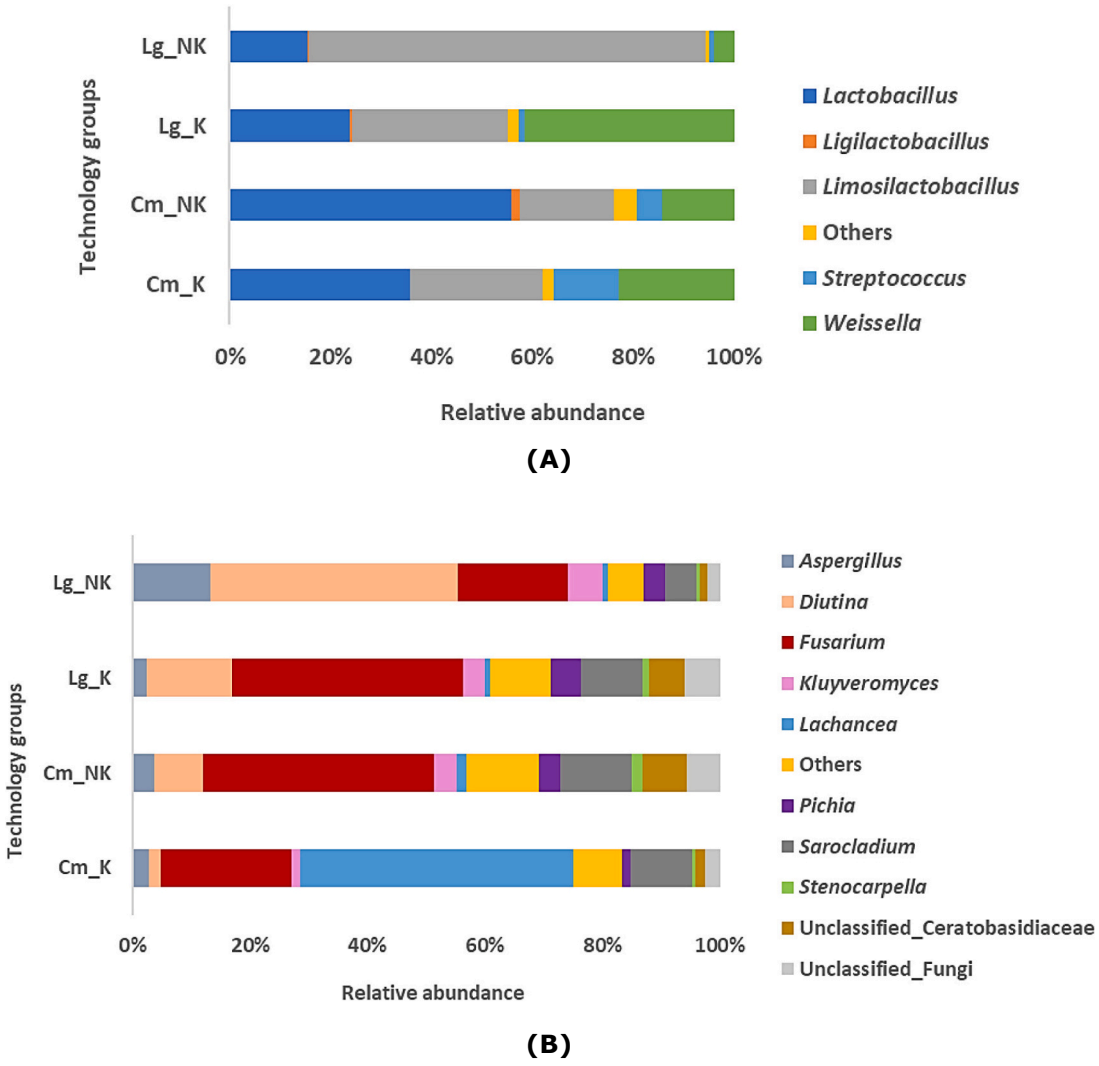


Fig. 2. Relative abundance of bacteria (A) and fungi (B) at the genus level in maize starch samples of four technology groups, namely Cm_NK: common steeping time without kneading. Cm_K: common steeping time plus kneading. Lg_NK: long steeping time without kneading. Lg_K: long steeping time plus kneading. Common steeping time = 12–24 h, long steeping time = 44–72 h.

conditions (Ademola et al., 2021; Bryła et al., 2022; Katati et al., 2023) that characterize the weather in southern Benin. The yeast genus *Lachancea* dominated in samples from the group Cm_K (46.4 %) and members of the yeast belonging to the genus *Diutina* dominated in the group Lg_NK (42.1 %). The genus *Sarocladium* was present in all groups in varying amounts (5.2–12.2 %), while a few members of the genera *Pichia* (1.5–5.2 %), *Kluyveromyces* (1.4–6.0 %), and *Aspergillus* (2.4–13.3 %), unclassified members of the Ceratobasidiaceae family (1.4–7.5 %), as well as unclassified fungal members (2.1–6.0 %) were randomly present in the different maize starch samples.

3.4. Effect of specificities in processing technologies on maize ogi

Fermentation is the last step (stage 3, Fig. S1) of the traditional maize *ogi* production scheme, where maize starch is transformed by spontaneous fermentation into the final fermented product called "*ogi*". This step is intentionally performed by processors and generally consists of storing the obtained maize starch slurry in a plastic container with a lid and leaving this at ambient temperature. The starch in the slurry will settle down in the plastic container, leaving water with soluble matter at the top. After the fermentation period defined by processors as their preference for the maize starch to be transformed enough, the resulting spontaneously fermented maize starch, referred to as *ogi*, is ready for use to prepare *akpan*. The processors in our study applied a fermentation time between 6 and 24 h of the maize starch slurry, following their usual practices. Samples fermented for 6 and 8 h were grouped together in a category of samples with the same duration of the fermentation period (6–8 h).

To evaluate how differences in processing technology affect the final fermented product, maize *ogi* samples were analysed for their physicochemical properties (pH, TTA, sugars, organic acids and ethanol), microbial counts (LAB, yeasts and Enterobacteriaceae) and microbial community composition, as a function of the factors of variation in the maize *ogi* production process (i.e., from stage 1 to 3). Statistical tests comparing the *ogi* samples for each parameter are provided by factors of variation in Tables S3.A and S3.B.

3.4.1. Physicochemical characteristics of maize ogi

The physicochemical characteristics of maize *ogi* samples are shown in Table 3. The pH of fermented maize starch (*ogi*) samples varied from 3.1 to 4.2. Significantly high pH values ($p < 0.05$) were observed for samples obtained from technologies in which maize grains were cold steeped for 1 to 6 h, heated together with water at the beginning of the steeping step (Goun samples), and from technologies in which samples were fermented for 6 to 8 h. Fermentation time also had a significant influence on the titratable acidity (TTA). As expected, there was a

Table 3
Physicochemical characteristics of maize *ogi*.

Ogi samples	pH	TTA (g/100 g)	Sucrose	Fructose	Glucose	Lactate	Acetate	Citrate	Ethanol
P1_6h	3.9	0.7	0.2	0.7	0.3	5.9	0.5	0.3	1.1
P2_12h	4.2	0.8	0.1	0.3	0.3	5.9	0.7	0.3	1.5
P3_12h	3.7	1.0	0.2	0.6	1.4	8.0	0.6	0.2	1.4
P4_24h	3.7	1.8	0.1	0.1	1.1	16.9	1.5	0.2	1.1
P5_12h	3.5	1.5	0.1	1.0	1.4	12.6	0.9	0.3	1.7
P6_8h	4.1	0.8	0.3	0.4	0.2	5.6	0.6	0.1	1.4
B1_12h	3.1	1.6	0.0	0.1	0.9	12.2	0.4	0.1	2.0
B2_12h	3.1	1.5	0.0	0.0	1.1	13.5	0.7	0.2	1.0
B3_12h	3.4	1.1	0.1	1.5	1.6	12.0	0.7	0.4	1.3
A1_24h	3.2	1.5	0.3	0.1	1.9	27.7	0.5	0.5	1.9
A2_12h	3.5	1.0	0.0	0.1	0.2	12.8	0.8	0.5	1.6
A3_12h	3.5	0.9	0.0	0.1	0.6	16.5	1.5	0.4	0.8
Ca_12h	3.3	0.8	0.1	2.0	0.6	12.6	0.8	0.5	3.2
Co_24h	3.4	0.7	0.0	0.1	0.8	14.0	0.5	0.4	1.8

Compound concentrations are expressed in g/L dry basis of maize *ogi* samples.
Most pyruvate concentrations were below 0.1 g/L, which are not shown in the table. The standard deviation for all values in the table is between 0.00 and 0.11. TTA: titratable acidity. The abbreviations in the first column are the *ogi* samples represented by the processors who supplied the starch slurries (P1 - P6: Porto Novo, B1 - B3: Bohicon, A1 - A3: Abomey, Ca: Abomey-Calavi, Co: Cotonou), followed by 6, 8, 12 or 24 h standing for the fermentation period in hours.

significant and negative correlation (cor = −0.509, $p = 0.0056$) between the pH and TTA of the *ogi* samples.

The total sugar concentration in maize *ogi* samples was significantly lower ($p < 0.05$) when a change of steeping liquid of the maize grains was applied in the production process compared to *ogi* for which the steeping liquid was not replaced. Same trend was observed for only the glucose concentration. Moreover, samples from a process applying long steeping of maize grains (44–72 h), and from a process where maize starch slurry was fermented for 6 to 8 h, generally had lower glucose concentrations than the samples from common steeping time (12 to 24 h) or 12 to 24 h of fermentation. Indeed, sugars are released by maize grains into steeping liquid as the consequence of amylolytic and enzymatic activities from both the grains and the microorganisms present (Chaves-López et al., 2020; Oduro-Yeboah et al., 2016). Therefore, changing the steeping liquid every day, or long steeping periods with continuous degradation of maize starch into sugars and consumption of these sugars by the microorganisms in the steeping liquid, can cause losses of sugars in the steeped grains. In contrast, very short fermentation times probably led to less sugar release because of insufficient exposure of the maize starch to the starch-degrading enzymes. Either glucose or fructose was the most abundant sugar in maize *ogi* samples. Low concentrations (0.1 g/L) of fructose in maize *ogi* samples were obtained when water was first boiled and then poured onto the maize grains during the heat treatment at the start of the steeping step (Fon samples), except for sample Ca12 of which the fructose concentration (2.0 g/L) was the highest among all *ogi* samples.

Lactate was the main organic acid in maize *ogi* with significant variations ($p < 0.05$) as a function of heat treatment as well as fermentation time, which also similarly affected the total organic acid concentration, significantly. The ethanol concentration of maize *ogi* samples was significantly higher ($p < 0.05$) when a cold steeping of maize grains was applied compared to samples produced without cold steeping.

3.4.2. Microbial enumeration in maize ogi

Lactic acid bacteria, the dominant microorganisms in maize *ogi* samples, had high counts ranging from 8.5 to 9.2 log CFU/g (Fig. S4). LAB counts were not significantly impacted ($p > 0.05$) by any of the differentiators of the processing technologies (i.e., the factors of variation). However, changing the steeping liquid in the maize *ogi* production process induced significantly high yeast counts (5.7 log CFU/g, $p = 0.02$). The LAB counts in maize *ogi* samples were similar to the mean counts of 9 log CFU/g found in commercial maize *ogi* samples as reported by Nago et al. (1998). The average yeast counts found in our study (4.8 log CFU/g) were relatively higher than the value of 3.7 ± 1.1 log CFU/g reported by Greppi et al. (2013) but lower than the 7 log CFU/g reported by Nago et al. (1998).

Most *ogi* samples contained <1 log CFU/g Enterobacteriaceae, which corroborates the findings of Nago et al. (1998) and indicates the effect of acidification and organic acids production during fermentation. However, there were a few samples of *ogi* that contained up to 4.4 log CFU/g Enterobacteriaceae, suggesting either an incomplete fermentation or a potential fermentation failure (Dinardo et al., 2019; Pswarayi and Gänzle, 2019). No significant impact of any of the differentiators of the processing technologies was observed in the comparison of the *ogi* samples containing Enterobacteriaceae.

3.4.3. Microbial composition and diversity in maize ogi

The relative abundance and β-diversity index of the microbial taxa present in maize *ogi* samples were analysed as a function of the differentiators of the processing technologies. Thus, the bacterial community in maize *ogi* was found to be dominated by representatives of the phylum Bacillota (95.8–99.9 %), being mainly species belonging to the class of Bacilli, which represented 92.2–99.9 % of the total bacterial community. Similar dominance of Bacillota (i.e., Firmicutes) was reported in millet-based *ogi* produced in Nigeria (Chibuzor-Onyema et al., 2021), as well as in sorghum and millet slurries from Zimbabwe (Gabaza et al., 2019). Only the heat treatment applied to maize grains (Table S3·B) had a significant impact (p-value = 0.019) at the genus level on the bacterial community composition in maize *ogi* (Fig. S5.A), showing more *Lactobacillus* counts in Goun samples as compared to Fon samples (Fig. S5·B).

The fungal community in maize *ogi* was dominated by members of the phylum Ascomycota (61.7–98.7 %), with most species belonging to the class Saccharomycetes (5.8–80.4 %) and Sordariomycetes (10.4–76.6 %). None of the differentiators of the processing technologies had a significant impact on the composition of the fungal community ($p > 0.05$).

3.4.4. Discriminative factors in the maize ogi production process

To define the discriminative factors in the maize *ogi* production process, the results with significant p-values ($p < 0.05$) for the differentiators of the processing technologies (cf. Sections 3.4.1 to 3.4.3) were evaluated. As outcome, none of the factors had a significant and simultaneous impact on the physicochemical characteristics, microbial plate counts and community diversity of the samples (Table 4). Hence, no critical differentiating factor was found in the first stage of the maize *ogi* production process. Moreover, the kneading in stage 2 of the production process (Table S1; Fig. S1) had no significant impact on any of the evaluated traits. Therefore, it was not possible to discriminate maize *ogi* samples directly using the variations between processing

Table 4
Summary-significant factors of variation ($p < 0.05$) in stage 1 of maize *ogi* production.

	Cold steeping	Heat treatment	Steeping time	Steeping liquid change
pH	+	+	-	-
TTA	-	-	-	-
Sugars	-	-	-	+
Lactate	-	+	-	-
Acids	-	+	-	-
Ethanol	+	-	-	-
LAB	-	-	-	-
Yeast	-	-	-	+
Entero	-	-	-	-
Microbial diversity	-	+	-	-

A significant effect of a factor of variation (in columns) on maize *ogi* characteristics (in rows) is marked by a plus (+), while the absence of effect is marked by a minus (−). TTA: titratable acidity, LAB: lactic acid bacteria count, Entero: Enterobacteriaceae count.

technologies.

3.5. Technology groups for starch production shape maize ogi characteristics

The maize *ogi* samples could not be categorised directly according to the differentiators of the processing technologies as inferred from our findings (Section 3.4.4). However, maize starch samples could be grouped according to variations in steeping time and kneading between processing technologies as shown in earlier results (Section 3.3). Hence, the four groups of maize processing technologies for starch production (i.e., Cm_NK, Cm_K, Lg_NK, Lg_K) were combined with the subsequent fermentation step to categorise maize *ogi* samples (Table S5). The variations in fermentation time (6–8 h, 12 h, 24 h) within the group Cm_NK (common steeping time without kneading) led to three categories of *ogi* samples: "Cm_NKt68" (fermented for 6 to 8 h), "Cm_NKt12" (fermented for 12 h), "Cm_NKt24" (fermented for 24 h). The samples from the other technology groups were fermented for 12 h ("Cm_Kt12", "Lg_NKt12", "Lg_Kt12"). This resulted in six categories of maize *ogi* samples to compare.

3.5.1. pH, titratable acidity, organic acids and ethanol

Principal components analysis (Fig. 3) was performed with pH, TTA, total organic acids, total sugars and ethanol as important indicators to assess how the processing technologies for maize starch production affect the characteristics of maize *ogi*. The first two dimensions (Dim1 and Dim2) explained 76.7 % of the total variation among the *ogi* samples, but Dim3 (not plotted) was needed to describe all the samples and explain 94.9 % of the total variation between samples. This was done in combination with the samples characteristics (Table S6). Maize *ogi* samples with high pH values (4.0, CV = 14.2 %), low TTA (0.7 %, CV = 13.0 %) and low total acid concentration (6.5 g/L, CV = 2.9 %) were obtained when maize grains were steeped for the common duration of 12 to 24 h, when the wet maize flour was not kneaded, and the resulting starch fermented for 6 to 8 h (Cm_NKt68). After 12 h of fermentation, the resulting *ogi* samples (Cm_NKt12) showed a high residual sugar concentration (1.9 g/L, CV = 49.7 %). Then, after 24 h of fermentation, maize *ogi* samples (Cm_NKt24) had the highest total acid concentration (20.9 g/L, 31.5 %), a high TTA (1.4 %, CV = 38.9 %), but low pH values (3.4, CV = 6.1 %). High total acid concentrations and TTA values also characterised the *ogi* samples obtained from the process combining steeping of the maize grains for 12 to 24 h with kneading of the maize flour and fermentation of the maize starch for 12 h (Cm_Kt12). A long

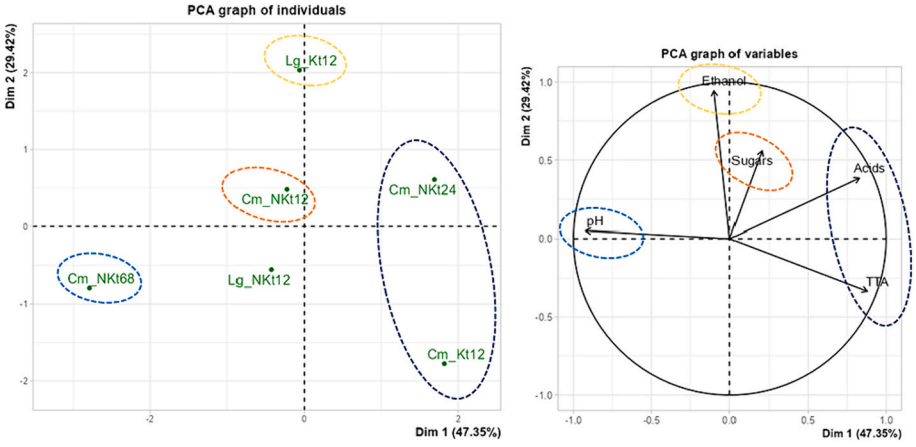


Fig. 3. Principal components analysis of maize *ogi* characteristics applied to the technology groups for maize starch production combined with fermentation time. Cm_NKt68, Cm_NKt12, Cm_NKt24: common steeping time without kneading, fermented for 6–8 h, 12 h, 24 h, respectively. Cm_Kt12: common steeping time plus kneading, fermented for 12 h. Lg_NKt12: long steeping time without kneading, fermented for 12 h. Lg_Kt12: long steeping time plus kneading, fermented for 12 h. Common steeping time = 12–24 h, long steeping time = 44–72 h.

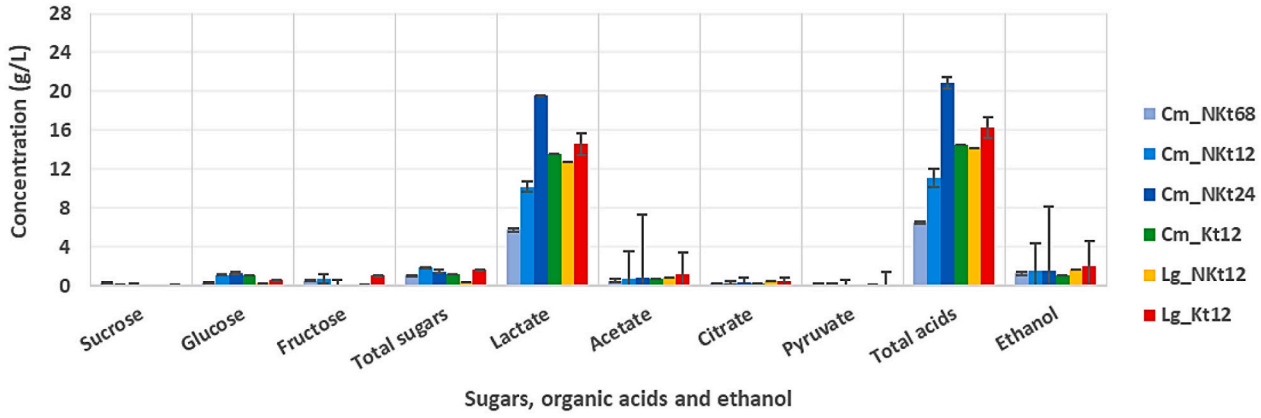
steeping of maize grains (44–72 h) associated with kneading and 12 h fermentation of maize starch gave *ogi* samples (Lg_Kt12) with a high ethanol concentration (2.0 g/L, CV = 70.1 %), whereas no kneading during processing led to samples with a high residual total sugar concentration (Lg_NKt12).

Generally, the sugar and ethanol concentrations in the maize *ogi* categories were low (Fig. 4A). Lactate was the main organic acid present, ranging from 5.8 g/L (CV = 3.4 %) to 19.5 g/L (CV = 33.2 %). A significant increase (p = 3.12e-4) in lactate was observed for the *ogi* samples within the Cm_NK technology group (Cm_NKt68, Cm_NKt12, Cm_NKt24) as a function of the increase in fermentation duration. There was no significant difference ($p > 0.05$) between the *ogi* categories for the ethanol concentration, which ranged from 1.0 to 2.0 g/L.

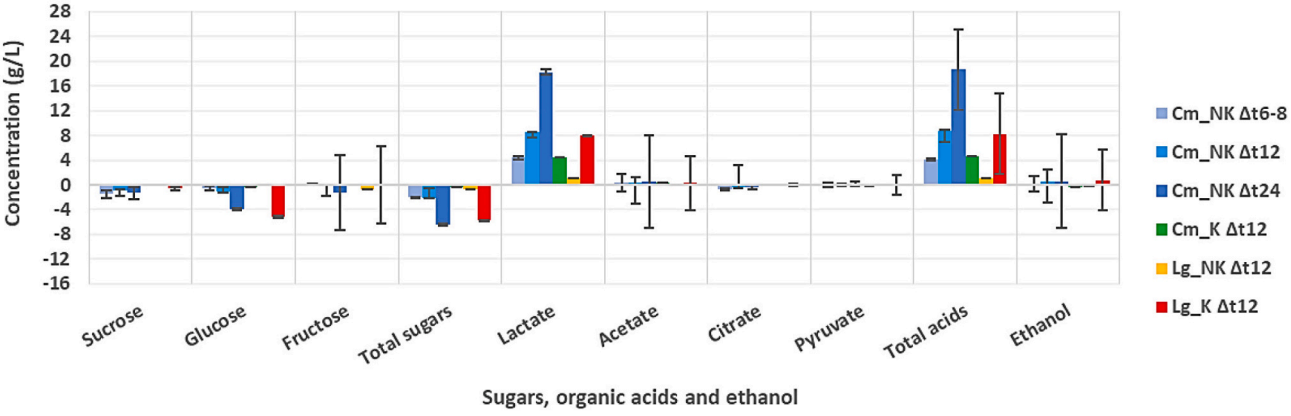
Trends in the presence of organic compounds were comparable ($p > 0.05$) for the different maize *ogi* categories during the fermentation step, but the changes observed (both positive and negative) were variable, sometimes slightly lower or higher, yet not significant (Fig. 4B). This could be due to the high coefficient of variation (%CV) within maize *ogi* categories expressing an important variability between *ogi* samples from the same technology group and fermentation duration. Nonetheless, a general observation was that sugars were consumed, acids were produced and the pH decreased (Fig. 4B). The above characteristics of maize *ogi* categories, and observed trends, align with findings on several other cereal-based fermented foods showing concomitant lower pH values, higher TTA amounts, more organic acids, mainly lactate, ethanol production, as well as production of simple sugars and their subsequent consumption, all depending on the duration of the steeping of the grains and fermentation times, but also the composition of the microbial communities present (Chaves-López et al., 2020; Chibuzor-Onyema et al., 2021; Gabaza et al., 2019; Oduro-Yeboah et al., 2016).

3.5.2. *Free essential amino acids*

Total free essential amino acids (FEAAs) in maize starch and maize *ogi* samples were calculated (Table 5) and the difference between the initial and end points of fermentation were plotted, see Fig. 5. The four technology groups yielded maize starch with similar total FEAAs concentration (p = 0.196), ranging from 29.3 mg/L to 61.6 mg/L. The variability between samples of the groups Cm_NK and Lg_K, expressed by high values of the within-group coefficients of variation CV (32.5 % and 37.5 %, respectively), might explain the absence of significant



(A)



(B)

Fig. 4. Sugars, organic acids and ethanol (g/L dry basis) in fermented *ogi* samples from the four technology groups for maize starch production combined with fermentation time (A) and the difference Δ between end and initial fermentation points according to the fermentation duration indicated by the processor (B). Cm_NKt68, Cm_NKt12, Cm_NKt24: common steeping time without kneading, fermented for 6–8 h, 12 h, 24 h, respectively. Cm_Kt12: common steeping time plus kneading, fermented for 12 h. Lg_NKt12: long steeping time without kneading, fermented for 12 h. Lg_Kt12: long steeping time plus kneading, fermented for 12 h. Cm_NKΔt6–8, Cm_NKΔt12, Cm_NKΔt24: changes for common steeping time without kneading, after fermentation of 6–8 h, 12 h, 24 h, respectively. Cm_KΔt12: change for common steeping time plus kneading, after fermentation of 12 h. Lg_NKΔt12: change in long steeping time without kneading, after fermentation for 12 h. Lg_KΔt12: change in long steeping time plus kneading, after fermentation for 12 h. Common steeping time = 12–24 h, long steeping time = 44–72 h. Error bars are the standard deviation values of the corresponding compounds.

Table 5
Total free amino acids in maize starch and *ogi* samples.

Samples	Type	Mean (mg/L)	SD	%CV
Cm_NK	Starch	29.3	9.53	32.5
Cm_NKt68	*Ogi*	25.7	2.47	9.6
Cm_NKt12	*Ogi*	24.3	4.12	16.9
Cm_NKt24	*Ogi*	43.5	25.19	57.9
Cm_K	Starch	31.7	0.00	0.0
Cm_Kt12	*Ogi*	34.8	0.00	0.0
Lg_NK	Starch	61.6	0.00	0.0
Lg_NKt12	*Ogi*	98.7	0.00	0.0
Lg_K	Starch	39.6	14.87	37.5
Lg_Kt12	*Ogi*	41.4	15.16	36.6

Cm_NK and Cm_NKt68, Cm_NKt12, Cm_NKt24: common steeping time without kneading, not fermented and fermented for 6–8 h, 12 h, 24 h, respectively. Cm_K and Cm_Kt12: common steeping time plus kneading, not fermented and fermented for 12 h. Lg_NK and Lg_NKt12: long steeping time without kneading, not fermented and fermented for 12 h. Lg_K and Lg_Kt12: long steeping time plus kneading, not fermented and fermented for 12 h. Common steeping time = 12–24 h, long steeping time = 44–72 h.

differences. Conversely, there was a significant difference ($p = 0.021$) between maize *ogi* categories, shown as an increase in total FEAAs in samples from Lg_NKt12 category (long steeping time without kneading, 12 h of fermentation), compared to *ogi* obtained from Cm_NK group (common steeping time without kneading) after 6–8, 12 and 24 h of fermentation. The observed trend can be linked to the significant increase in methionine, isoleucine, leucine and tryptophan in the samples during fermentation (Fig. 5). The change in FEAAs concentration would result from variability in the proteolytic capacity of fermenting microorganisms. Some LAB species release amino acids from the proteins contained in the food under fermentation, and so contribute to their increase in fermented products, while others use amino acids for their activity, leading to a decrease (Horlacher et al., 2023; Sharma et al., 2020).

3.5.3. Volatile organic compounds

The volatile organic compounds (VOCs) in maize starch and maize *ogi* samples belong to eight compound groups from which "alcohols", "esters" and "carboxylic acids" were the most abundant (Fig. 6). No significant differences in relative abundance in these groups of compounds were found in maize starches from the four technology groups except for alcohols ($p = 3.93e\text{-}05$) and the group of miscellaneous (i.e., "other") compounds ($p = 0.027$). Maize starch from the Lg_NK group (long steeping time without kneading) had more alcohols than maize starch from the Cm_NK technology (common steeping time without kneading). For esters, observations revealed an average higher abundance, yet not significant, in maize starch samples from common steeping times of the maize grains (i.e., Cm_NK and Cm_K groups) as

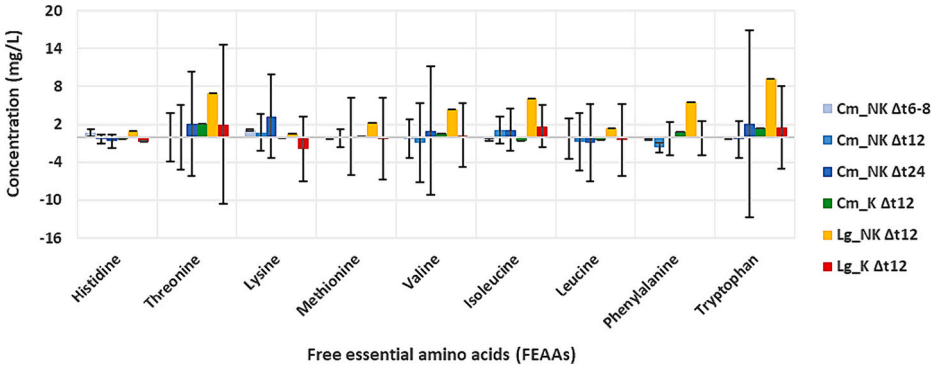


Fig. 5. Changes (Δ) in free amino acids (mg/L dry basis) in fermented *ogi* samples from the four technology groups for maize starch production combined with fermentation time. Cm_NKΔt6–8, Cm_NKΔt12, Cm_NKΔt24: changes for common steeping time without kneading, after fermentation of 6–8 h, 12 h, 24 h, respectively. Cm_KΔt12: change for common steeping time plus kneading, after fermentation of 12 h. Lg_NKΔt12: change in long steeping time without kneading, after fermentation for 12 h. Lg_KΔt12: change in long steeping time plus kneading, after fermentation for 12 h. Common steeping time = 12–24 h, long steeping time = 44–72 h. Error bars are the standard deviation values of the corresponding compounds.

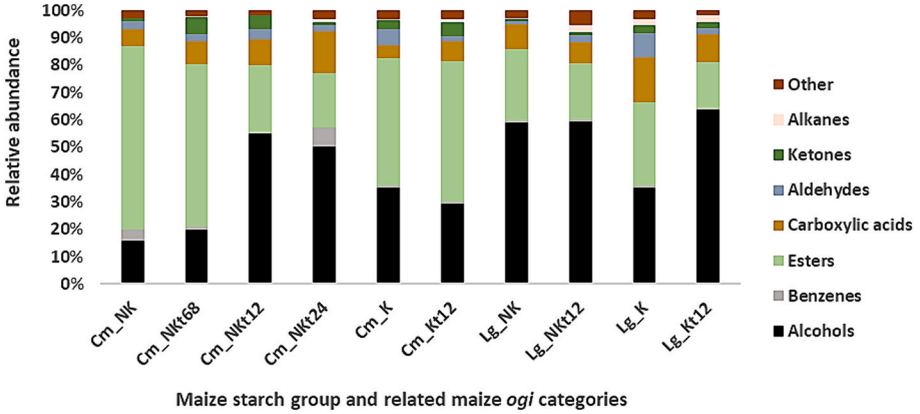


Fig. 6. Volatile organic compounds (%) in maize starch samples and subsequent fermented *ogi* per technology groups for maize starch production. Cm_NK: common steeping time without kneading, not fermented. Cm_NKt68, Cm_NKt12, Cm_NKt24: common steeping time without kneading, fermented for 6–8 h, 12 h, 24 h, respectively. Cm_K: common steeping time plus kneading, not fermented. Cm_Kt12: common steeping time plus kneading, fermented for 12 h. Lg_NK: long steeping time without kneading, not fermented. Lg_NKt12: long steeping time without kneading, fermented for 12 h. Lg_K: long steeping time plus kneading, not fermented. Lg_Kt12: long steeping time plus kneading, fermented for 12 h. Common steeping time = 12–24 h, long steeping time = 44–72 h.

compared to samples from long steeping times (i.e., Lg_NK and Lg_K groups). The large variations between the relative abundance of esters within the groups Cm_NK (CV = 23.4 %) and Lg_K (CV = 28.0 %) could explain this absence of significant difference. However, considering the unintended fermentation occurring during maize starch production, esters abundance was not in line with the yeast counts (Table S4) when comparing maize starch samples from Cm_NK group to Cm_K, Lg_NK and Lg_K groups. In fact, the production of volatile esters is mainly attributed to the important contribution of yeasts in lactic acid fermentation of cereal foods (Chaves-López et al., 2020; Johansen et al., 2019). Volatile alcohols, esters and acids were also reported for the first hours of fermentation for sorghum-based Gowé production (Vieira-Dalodé et al., 2016), and potentially originate from the metabolic activities of LAB and yeasts during the steeping of the grains, and also during the waiting time of 8–12 h after kneading wet maize flour, before filtration.

The VOCs in *ogi* were further compared between the six categories of samples obtained after fermentation (Fig. 6, Table S7). A general increase in volatile alcohols, yet not significant ($p > 0.05$), was observed in all *ogi* categories except in samples Lg_NK (long steeping time without kneading) after 12 h of fermentation. This trend also occurred with the increase in fermentation time from 6 to 12 h within the group Cm_NK (common steeping time without kneading). The relative abundance of alcohols in Cm_NK group after 12 h of fermentation (Cm_NKt12) and after 24 h (Cm_NKt24) were similar. The relative abundance of volatile esters decreased in all *ogi* categories after fermentation, while the content of volatile acids increased in samples from the technology groups with a common steeping time (Cm_NKt68, Cm_NKt12, Cm_NKt24, Cm_Kt12) but decreased in samples from the groups with a long steeping time (Lg_NKt12 and Lg_Kt12). In general, esters are produced during lactic acid fermentation of cereals, by the metabolism of the available substrates through a combination of biochemical degradation and enzymatic activities specific to the yeasts and LAB co-existing in the fermenting food and supporting each other by mutualistic interactions (Chaves-López et al., 2020; Johansen et al., 2019; Kayitesi et al., 2023; Sieuwerts et al., 2018). The decrease in volatile esters as observed in our study appears counter-intuitive. However, other authors also reported differing effects of cereal lactic acid fermentation in terms of volatile esters (Masha et al., 1998; Vieira-Dalodé et al., 2016). For example, the presence of esters and their increase were reported in the production of sorghum-based Gowé, namely during the primary fermentation (0−12 h) and the first 12 h of secondary fermentation, while they were no longer detectable after 24 h during the secondary fermentation (Vieira-Dalodé et al., 2016).

3.5.4. Bacterial and fungal genera in maize ogi by technology groups

Finally, the microbial community composition in maize *ogi* from the technology groups for maize starch production combined with variations in fermentation time, were analysed for diversity and species abundance at genus level, after enumeration of LAB and yeast counts.

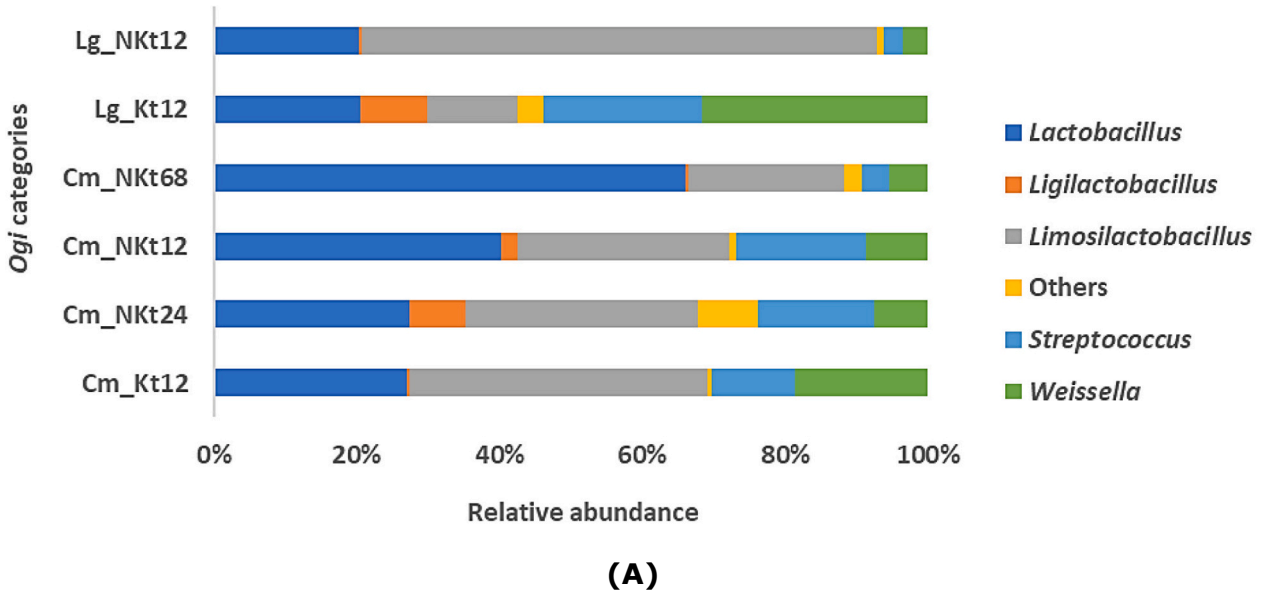
The maize *ogi* categories contained 8.6 to 9.1 log CFU/g LAB and 4.3 to 5.7 log CFU/g yeast (Table S6). The differences between the technology groups did not affect the LAB counts after fermentation, while significant shifts were observed for the yeast counts. Specifically, the maize starch samples from the technology group Cm_K (common steeping time plus kneading) contained more yeast than samples from Cm_NK group (common steeping time plus kneading) before fermentation, but similar counts were found in the resulting maize *ogi* after fermentation. Several factors could have influenced the yeast growth during fermentation such as the static nature of the starch fermentation happening in submerged water and close containers, or the composition of the bacterial community present. The samples of the Cm_NK group fermented for 24 h (Cm_NKt24) had higher yeast counts (5.3 log CFU/g, CV = 3.2 %) than the samples in the same group fermented for 12 h (Cm_NKt12) containing 4.3 log CFU/g (CV = 19.9 %). This shows continued fermentation between 12 and 24 h of incubation during the fermentation stage. Nonetheless, LAB and yeast counts generally did not vary considerably after fermentation of maize starch into maize *ogi*.

The bacterial community composition in maize *ogi* was quite similar among the six categories of samples ([ANOSIM] R: 0.057895; $p = 0.356$), and was dominated by the same five genera that dominated in maize starch after extraction (Fig. 7A). The relative abundance of *Limosilactobacillus* increased after fermentation in samples from the common steeping duration of maize grains (from 18.6–26.2 % to 21.8–41.8 %), but decreased in samples from the long steeping duration (from 30.8–78.4 % to 12.6–72.2 %). In general, the proportion of *Lactobacillus* species decreased in all groups after 12 h of fermentation, except in samples from Lg_NKt12 group (long steeping time without kneading). However, there was a slight increase in *Lactobacillus* species after 6–8 h of fermentation in samples Cm_NKt68 (common steeping time without kneading). The genus *Ligilactobacillus* increased generally in all groups, while *Weissella* generally decreased. The decrease in *Weissella* species during fermentation seems in line with the succession dynamics of microbiota in spontaneous lactic acid fermentation of cereals established in three subsequent phases: (i) the most abundant facultative anaerobes in cereal grains (Enterobacteriaceae) initiate the fermentation, (ii) *Weissella* species increase their abundance in the next phase, and finally, (iii) acid-resistant lactobacilli take over and remain dominant in the fermented products (Gänzle and Salovaara, 2019; Pswarayi and Gänzle, 2022).

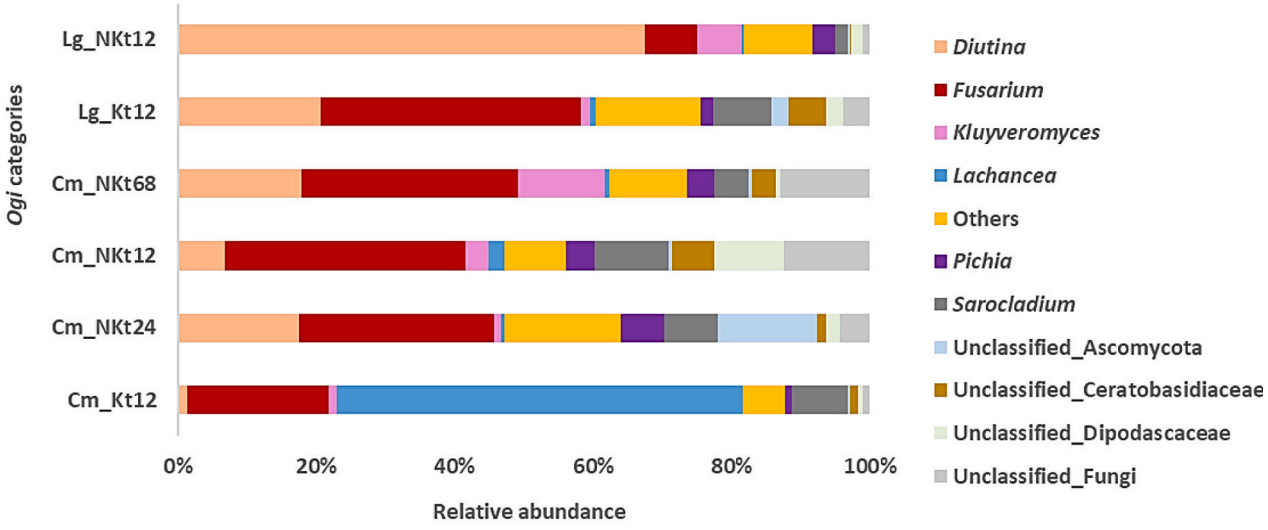
The fungal community composition in maize *ogi* was slightly different between *ogi* categories ([ANOSIM] R: 0.34737; $p = 0.041$), see Fig. 7B. *Fusarium* remained one of the leading genera in the different samples (20.5–37.5 %) except in Lg_NKt12 where *Diutina* was highly abundant (67.5 %). Overall, *Diutina*, *Kluyveromyces*, *Pichia*, *Lachanceae*, *Sarocladium*, as well as unclassified fungi and unclassified members of the family Ceratobasidiaceae remained the most dominant taxa (Fig. 7B). Species of these taxa are probably acid tolerant, as they are abundant in the fermented products of which the pH ranges from 3.1 to 4.0. Interestingly, the genera *Aspergillus* and *Stenocarpella*, which were abundant in maize starch after extraction, were no longer abundant in the fermented starch, being replaced by unclassified members of the family Dipodascaceae and unclassified Ascomycota. This could be attributed to the pronounced acidification of the fermented products combined with the production of metabolites such as lactic acid, acetic acid and potentially other substances that possess antifungal properties (Chaves-López et al., 2020; Guan et al., 2023). Members of the genus *Diutina* showed a significantly different abundance ($p = 0.006$) between the six categories of maize *ogi*. *Diutina* spp. were highly present in the samples Lg_NKt12 (long steeping time without kneading, 12 h of fermentation) but hardly so (1.3 %) in samples Cm_Kt12 (common steeping time plus kneading, 12 h of fermentation).

4. Conclusion

Different processing technologies are currently used for the traditional production of maize *ogi* by *akpan* producers in Benin. A direct connection between the technological variations and the characteristics of *ogi* samples was not found in our study. However, variations in steeping duration of maize grains and either or not kneading followed by a waiting time before filtration was applied, significantly differentiated maize starch samples before fermentation. Subsequent shifts in the composition of the bacterial and fungal communities of maize starch were found in maize *ogi*, and the functional properties of each *ogi* category provided insights into the impact of fermentation in relation to differences in processing technologies. In addition, we found that the traditional maize *ogi* production process actually also comprises unintentional fermentation occurring during the processing of maize grains into maize starch. This unintentional fermentation seems to play a critical role in (i) the quality of the extracted maize starch, (ii) the course of the intentional fermentation, and (iii) the final maize *ogi* characteristics. However, our findings were entirely based on an inventory of the characteristics of maize starch and maize *ogi* from the slurries produced



(A)



(B)

Fig. 7. Relative abundance of bacteria (A) and fungi (B) at the genus level in maize *ogi* samples merged into technology groups in combination with fermentation time. Cm_NKt68, Cm_NKt12, Cm_NKt24: common steeping time without kneading, fermented for 6–8 h, 12 h, 24 h, respectively. Cm_Kt12: common steeping time plus kneading, fermented for 12 h. Lg_NKt12: long steeping time without kneading, fermented for 12 h. Lg_Kt12: long steeping time plus kneading, fermented for 12 h. Common steeping time = 12–24 h, long steeping time = 44–72 h.

by different traditional processors to see the spectrum in terms of the impact of processing practices. Other factors such as the quality of the water, the variety of maize, and the processing environment, were not evaluated in this study but may also contribute as cofounding factors to the differentiation of the samples. Such information is needed, including the labour inputs required by each technology, to determine the best practices for maize *ogi* production.

CRediT authorship contribution statement

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

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

The raw sequencing data of microbial community was deposited at

4TU.ResearchData, The Netherlands (URL: https://doi.org/10.4121/ab7bc46e-e163-4cec-8a5d-6d987025dd3a).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2024.110683.

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