

Microbiological, Physicochemical and Sensory Characterization of Several Variants of *Tchakpalo*, a Traditional Beninese Beer

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Abstract

Tchakpalo is a traditional Beninese beer produced using artisanal methods from malted sorghum (*Sorghum bicolor* (L.) Moench) and/or maize (*Zea mays* L.). The beer is originally associated with sociocultural groups in central Benin and has spread throughout the country. Today, many variants of *Tchakpalo* are produced and commercialized, particularly in southern Benin. This study aimed to evaluate the impact of different production processes on the sensory attributes, microbiological and physicochemical characterization of *Tchakpalo*. Eight different varieties of *Tchakpalo* were produced and subjected to hedonic testing based on seven organoleptic characteristics, involving sixty panelists. The microbiological and physicochemical characteristics of the different variants of *Tchakpalo* were determined using standard laboratory methods. Panelist perceptions regarding the color, aroma, aftertaste, sweetness, acidity, and alcohol content of *Tchakpalo* varied significantly depending



on the production method used. The same applied to the microbiological and physicochemical parameters evaluated. Overall, the *Tchakpalo* varieties produced using the MFM1 (24-hour single-fermentation maize) and DFM1 (24-hour double-fermentation maize) methods were the most appreciated by tasters. They exhibited pleasant color, aroma, and aftertaste, as well as greater sweetness and high values for Brix, dry matter, and density. Conversely, they were less acidic, had lower alcohol content, and showed a less abundant microbial flora. The results of this study provide a wealth of important information to support ambitions for modernizing and promoting this traditional beer, which is highly popular among the Beninese population.

Keywords

Tchakpalo, Locally Produced Beer, Fermentation Technology, Food Quality, Microorganisms

1. Introduction

Beer is a fermented beverage produced by the alcoholic fermentation of a mash obtained mainly from malted grains to which water, hops, and yeast are generally added, through a manufacturing process that includes malting, brewing, fermentation, and maturation [1]. Produced in both developed and developing countries, the beers are today the most popular alcoholic beverages in the world because they satisfy physiological, psychological, and sociological needs. In the cultures and customs of many communities around the world, beer plays an essential role because it provides nutritional benefits. It is consumed to reduce tiredness and depression, providing a sense of escape and euphoria, and creating a festive mood [2].

In Benin, many traditional beers are produced and commercialized, including *Tchakpalo*, an iconic beer that represents Benin's brewing heritage. Indeed, *Tchakpalo* is a locally low-alcohol beer produced by a traditional process, containing mainly a step of malting, brewing, and spontaneous fermentation of a mash obtained from water and grits of germinated maize, germinated sorghum, or a mixture of the two [3]. It is originally associated with the Idaatcha and Nago sociocultural groups in central Benin [4]. It is spreading gradually across the country, especially in major cities, where its popularity is increasing.

Throughout this process of technological migration, the traditional *Tchakpalo* production method has been territorially adapted, influencing not only unit operations and their parameters, but also the raw materials, ingredients, and the equipment used for production and marketing. Thus, from a technological viewpoint, many *Tchakpalo* variants have been produced and commercialized, notably in southern Benin. Moreover, the spontaneous character of the fermentation, combined with technological diversity, leads to significant variation in the beverage's organoleptic characteristics between different production methods and different

producers. These factors constitute a serious obstacle to the standardization and label certification of this local beer. In addition, there is insufficient documentation on the sensory characteristics of *Tchakpalo* variant.

In a previous study, Dognon *et al.* [5] identified in southern Benin, particularly in the Grand Nokoué area, many *Tchakpalo* variants almost similar to the original *Tchakpalo*. The production processes for these different variants involve practically the same unit operations. Nevertheless, the operations chronology, the number of fermentation steps (one or two), the fermentation duration (24 hours or 48 hours), and the raw material (maize, sorghum, or a maize-sorghum mixture) differ among variants. Given its economic, cultural, and religious importance, traditional *Tchakpalo* beer which is highly prized by the people of Benin, needs to be better promoted. The achievement of this objective requires standardizing the production process based on the sensory attributes most valued by consumers today.

The present research aims to contribute to nutrition security and food safety through the evaluation of the sensory, microbiological and physicochemical characteristics of *Tchakpalo* variant. Ultimately, it identifies the processes most conducive to producing a high-quality *Tchakpalo* that is better accepted by consumers and consistent with the objectives of standardization, promotion, and improved food safety for this traditional Beninese beer.

2. Materials and Methods

2.1. Plant Materials

The plant materials consisted of the maize (*Zea mays* L.) varieties AK 94 DMR ESR, BENIN, and the sorghum (*Sorghum bicolor* (L.) Moench) variety Chorossoya (Figure 1). They were identified at the Southern Agricultural Research Center (CRA-SUD) of the National Institute for Agricultural Research of Benin (INRAB). These varieties were the most used for *Tchakpalo* production by the processors within the study area.



Figure 1. (a) Maize variety (AK 94 DMR ESR Y BENIN) and (b) Sorghum variety (Chorossoya) used for *Tchakpalo* production.

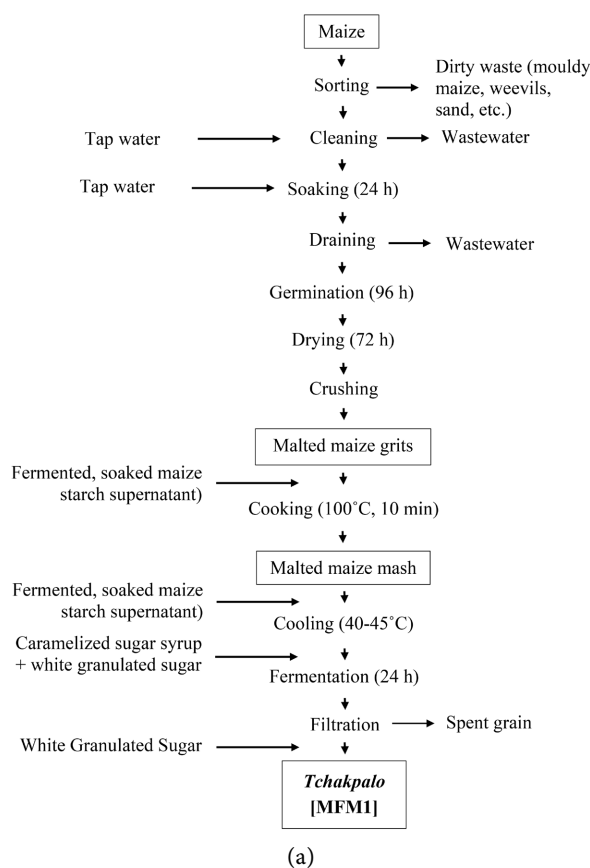
2.2. *Tchakpalo* Variants Evaluated

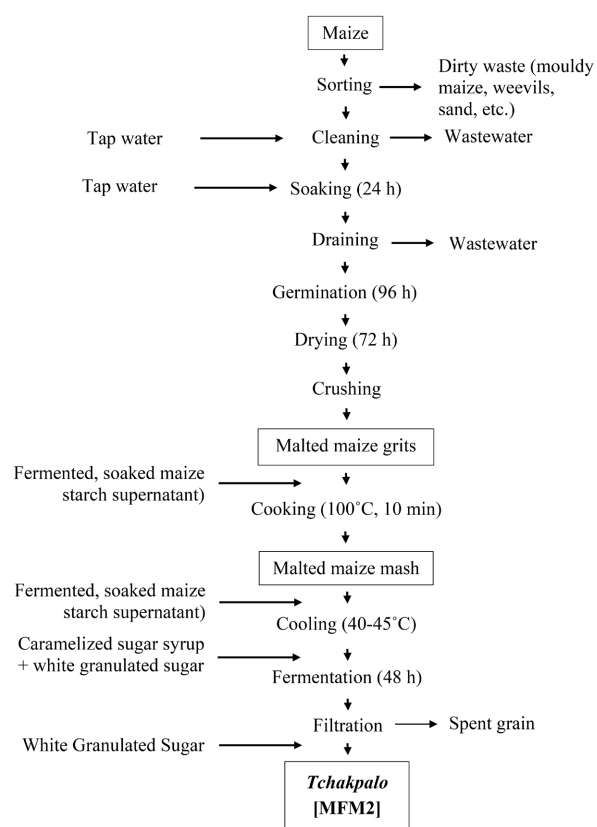
In a previous study, six (6) technological variants of *Tchakpalo* production were identified in the Grand Nokoué area (1°58'45,214"E and 2°43'8,072"E; 6°19'39,826"N and 6°42'5,043"N) by Dognon *et al.* [5]. These different variants are presented in **Table 1**. In addition to these identified *Tchakpalo* variants, two (2) others inspired by the original traditional *Tchakpalo* production process were added.

Table 1. Different variants of *Tchakpalo* studied.

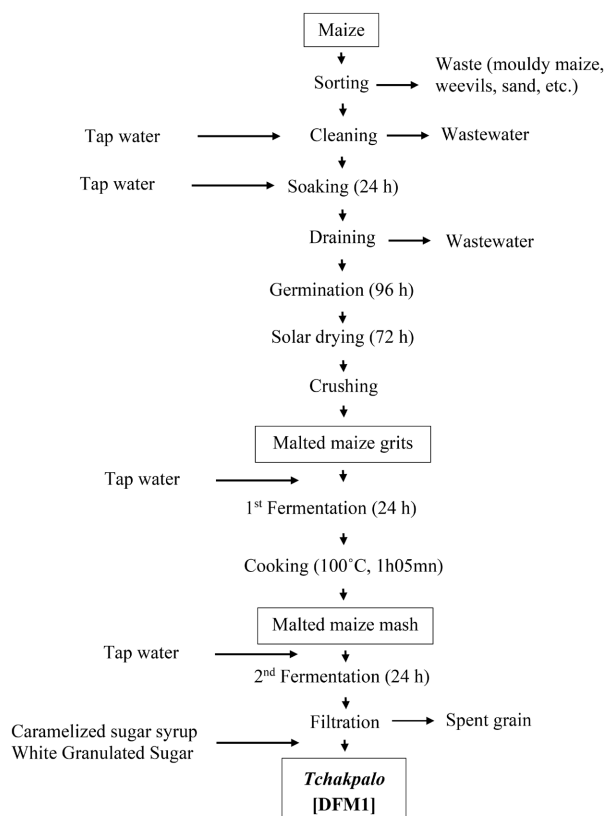
No	Variant code	Variant description
1	MFM1	24-hour Single-Fermented Maize
2	MFM2	48-hour Single-Fermented Maize
3	DFM1	24-hour Double-Fermented Maize
4	DFM2	48-hour Double-Fermented Maize
5	MFMS1	24-hour Single-Fermented Maize-Sorghum
6	MFMS2	48-hour Single-Fermented Maize-Sorghum
7	DFMR	24-hour Double-Fermented Maize Reference
8	DFSR	24-hour Double-Fermented Maize Reference

2.3. Production of *Tchakpalo* Variants

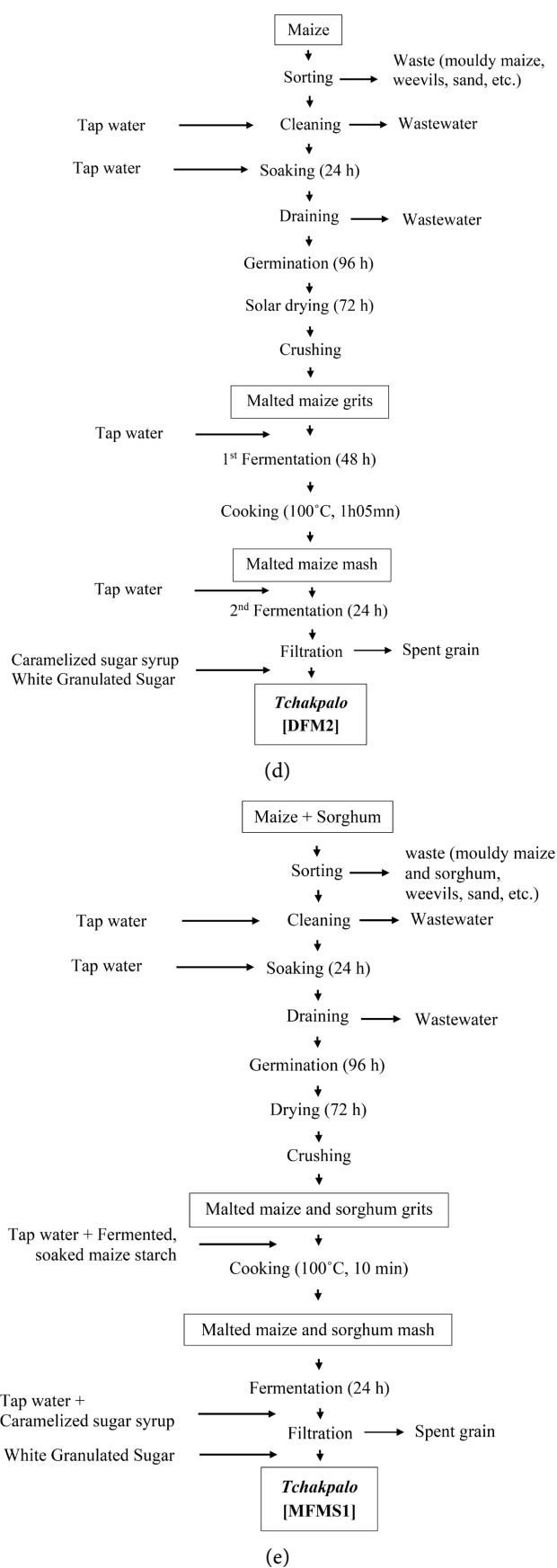


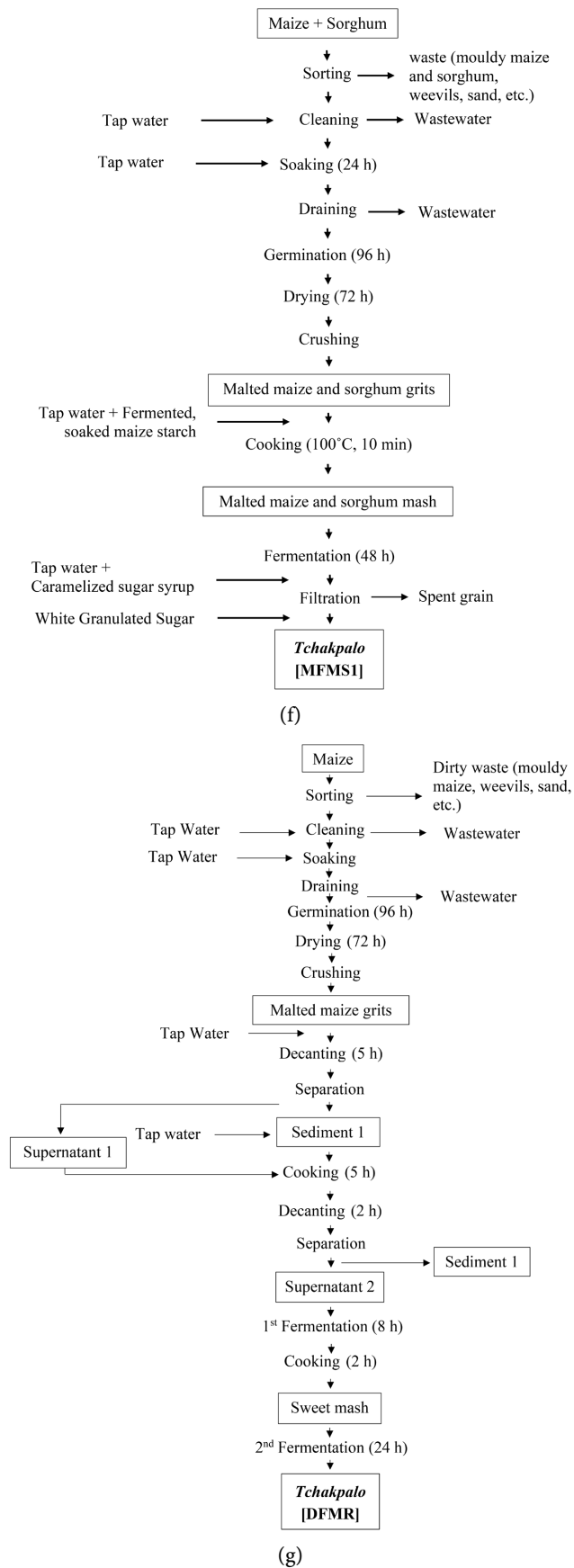


(b)



(c)





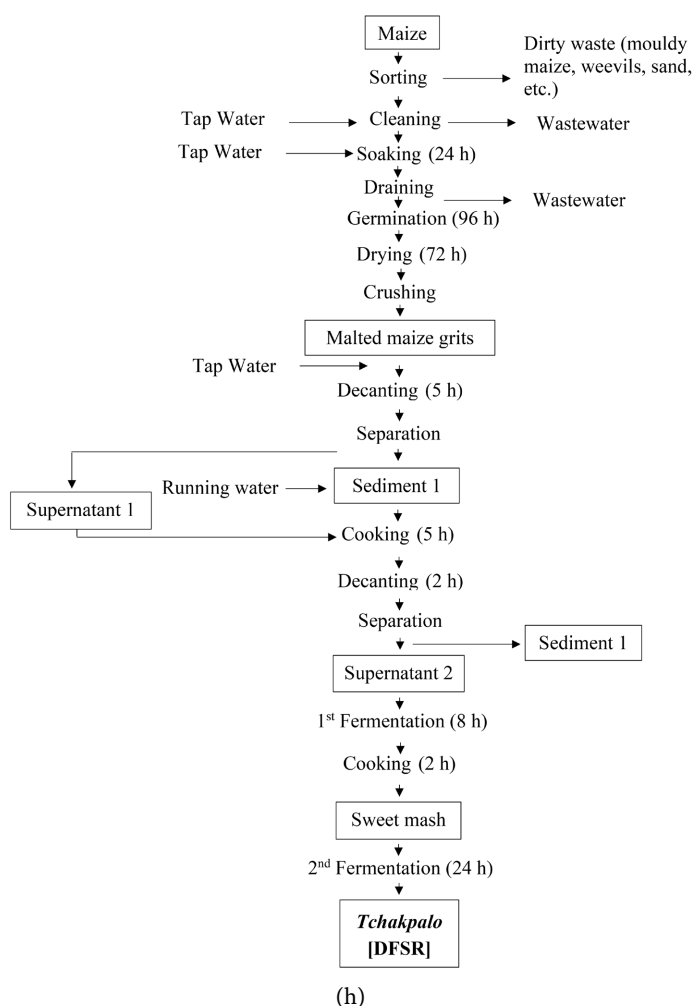


Figure 2. (a) Technological diagrams of production *Tchakpalo* variants MFM1; (b) Technological diagrams of production *Tchakpalo* variants MFM2; (c) Technological diagrams of production *Tchakpalo* variants DFM1; (d) Technological diagrams of production *Tchakpalo* variants DFM2; (e) Technological diagrams of production *Tchakpalo* variants MFMS1; (f) Technological diagrams of production *Tchakpalo* variants MFMS2; (g) Technological diagrams of production *Tchakpalo* variants DFMR; (h) Technological diagrams of production *Tchakpalo* variants DFSR.

The technological diagrams of the production of these different *Tchakpalo* variants are presented in **Figure 2**. The production was carried out in such a way that different *Tchakpalo* were obtained on the same day and made by the processors with substantial experience in the task. At the end of production, samples of each variant were collected aseptically in plastic containers (3L) and transported to laboratories for sensory, microbiological, and physicochemical analyses.

2.4. Sensory Evaluation of the *Tchakpalo* Products

The sensory evaluation of the eight (8) variants of *Tchakpalo* produced was carried out using a hedonic test. The test was conducted with 60 untrained panelists who were accustomed to consuming *Tchakpalo* and had provided their written

and signed consent to participate in the study. The test was conducted in a room set up for this purpose (with good ventilation, adequate lighting, and shielded from noise) at the University of Abomey-Calavi (Benin) (**Figure 3**). Each *Tchakpalo* variant was assessed on 7 specific sensory attributes (appearance, aroma, aftertaste, sweetness, acidity, alcohol content, and general appreciation) using a 5-point hedonics scale varying from 1 (very disagreeable) to 5 (very agreeable). The hedonic test was conducted in two (2) steps. The first step consisted of comparing *Tchakpalo* samples made from the same raw material and using the same production method, based on the duration of the main fermentation (24 hours and 48 hours). Thus, MFM1 was compared to MFM2, MFMS1 to MFMS2, and DFM1 to DMF2. At the end of this step, the three most preferred variants (MFM1, DFM1 and MFMS1) were selected and added to the two reference variants (DFMR and DFSR) for the second step. Consequently, only these five variants were subjected to microbiological and physicochemical analyses.



Figure 3. Sensory evaluation session.

The microbiological analysis of the *Tchakpalo* sample produced consisted of enumeration of the Total Mesophilic Aerobic Flora on Plate Count Agar at 30°C for 72 hours [6]; yeasts and molds on Sabouraud chloramphenicol agar at 25°C for 5 days [7]; and finally, lactic acid bacteria on MRS (de Man, Rogosa, and Sharpe) agar at 30°C for 72 hours [8].

2.5. Determination of the Physicochemical Characteristics of the *Tchakpalo* Products

The physicochemical parameters evaluated were: pH, titratable acidity, degrees Brix, alcohol content, dry matter, water content, and density.

2.5.1. Determination of pH and Titratable Acidity

The pH and titratable acidity were determined according to the method described by Nout *et al.* [9]. To 10 mL of *Tchakpalo*, 20 mL of sterile distilled water was

added. After homogenization, the pH was measured using a pH meter (Medidor pH Pometer, pH-009). Following the pH measurement, 70 mL of distilled water and 2 to 3 drops of phenolphthalein (color indicator, 1% by weight) were added to the solution. The titratable acidity was determined by titration with a sodium hydroxide (NaOH, 0.1 N) solution. The titratable acidity was expressed in grams of lactic acid per liter.

2.5.2. Determination of Moisture and Dry Matter Content

The moisture and volatile matter content of the *Tchakpalo* sample was determined in accordance with French standard NF V03-921:2021 of the French Standards Association (AFNOR) [10]. It corresponds to the loss in mass of the sample after drying in an oven at $103^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until a constant mass is reached. The moisture and volatile matter content were expressed as a percentage.

2.5.3. Determination of Soluble Dry Matter (Brix Degrees)

The soluble dry matter content of the *Tchakpalo* sample was determined using a portable refractometer (Sopelem 9596, France) calibrated from 0 to 30° Brix. After calibrating the instrument with distilled water, one to two drops of the *Tchakpalo* sample were placed on the refractometer's prism. The device was then closed, and the Brix reading was taken through the eyepiece on the device [11].

2.5.4. Determination of Alcohol Content

The alcohol content was determined using an ATC (Automatic Temperature Compensation) alcoholometer, according to the method described by Alborno [12]. The process involved measuring the alcohol content by placing one to two drops of the *Tchakpalo* sample on the prism of the alcoholometer after calibrating it with distilled water. The device was then closed, and the alcohol content was read through the lens on the display.

2.5.5. Density Determination

The relative density of *Tchakpalo* at 20°C was determined using the method described by Diakabana *et al.* [13]. This is the ratio of the mass of a given volume of *Tchakpalo* to the mass of the same volume of distilled water at the same temperature (20°C).

2.6. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and mean comparisons using the Student-Newman-Keuls test at the 5% threshold, performed using R software (version 4.3.0) [14] and the agricolae package [15]. All microbiological and physicochemical analyses were carried out in triplicate. The reported values correspond to analytical replicates performed on samples obtained from the same production batch and are expressed as mean $\pm$ standard deviation ($n = 3$). Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were performed using the mean physicochemical and microbiological characteristics of the five selected *Tchakpalo* variants. PCA was used as an exploratory tool to visu-

alize the relationships among variables and variants. Analyses were performed using the FactoMineR [16] and factoextra [17] packages.

3. Results

3.1. Comparative Sensory Characteristics of the 24-Hour and 48-Hour *Tchakpalo* Variants

The results of the first phase of the sensory analysis are shown in **Figure 4**, comparing *Tchakpalo* samples prepared with the same raw material and production process, but differing in the duration of the main fermentation (24 hours versus 48 hours).

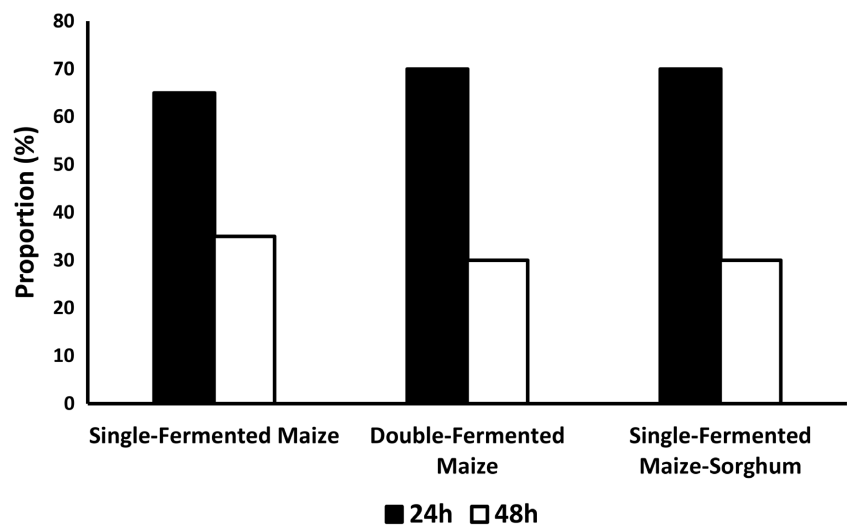


Figure 4. Hedonic test on *Tchakpalo* variants made from the same raw material.

For the two variants of single-fermented maize *Tchakpalo* (24 and 48 hours), the majority of panelists (65%) preferred the 24-hour fermentation variant to the 48-hour variant. Specifically, 70% of panelists reported that the color, odor and aftertaste of these two variants were agreeable or very agreeable. However, the panelists felt that the 24-hour single-fermented maize *Tchakpalo* was sweeter (70%), less acidic (70%), and had a less alcoholic taste (90%) than the 48-hour fermented variants. For the two variants of double-fermented maize *Tchakpalo* (24 and 48 hours), the majority of panelists (70) preferred the 24-hour variant to the 48-hour variant. As previously noted, 68.33% of the panelists found the color, aroma, and aftertaste of both variants to be agreeable or very agreeable. However, all panelists (100%) noticed that the 24-hour double-fermented maize *Tchakpalo* was less acidic and had a less alcoholic taste than the 48-hour variants while the majority of panelists (80%) recorded it was sweeter than the 48-hour double-fermented maize *Tchakpalo*.

Finally, the *Tchakpalo* resulting from the mixture of maize and sorghum (single-fermented) for 24-hour scored more agreeable for 70% of the panelists than the single fermented for 48-hour. Specifically, 70% of the panelists reported that

the color, odor, and aftertaste of both variants were agreeable or very agreeable. The 24-hour maize-sorghum *Tchakpalo* was less acidic and had a less alcoholic taste for all panelists (100%) than the 48-hour version. Conversely, the majority of the panelists (60%) recorded that it was sweeter than the 48-hour maize-sorghum *Tchakpalo*.

The key finding from this first phase of sensory evaluation is that, regardless of the raw material (maize, sorghum, or maize-sorghum) and the production process (single or double fermentation), the panelists prefer the *Tchakpalo* samples with 24-hour of primary fermentation over those with 48-hour. The results indicate that the 24 h *Tchakpalo* samples were sweeter, less acidic, and had a less alcoholic taste than the 48 h samples.

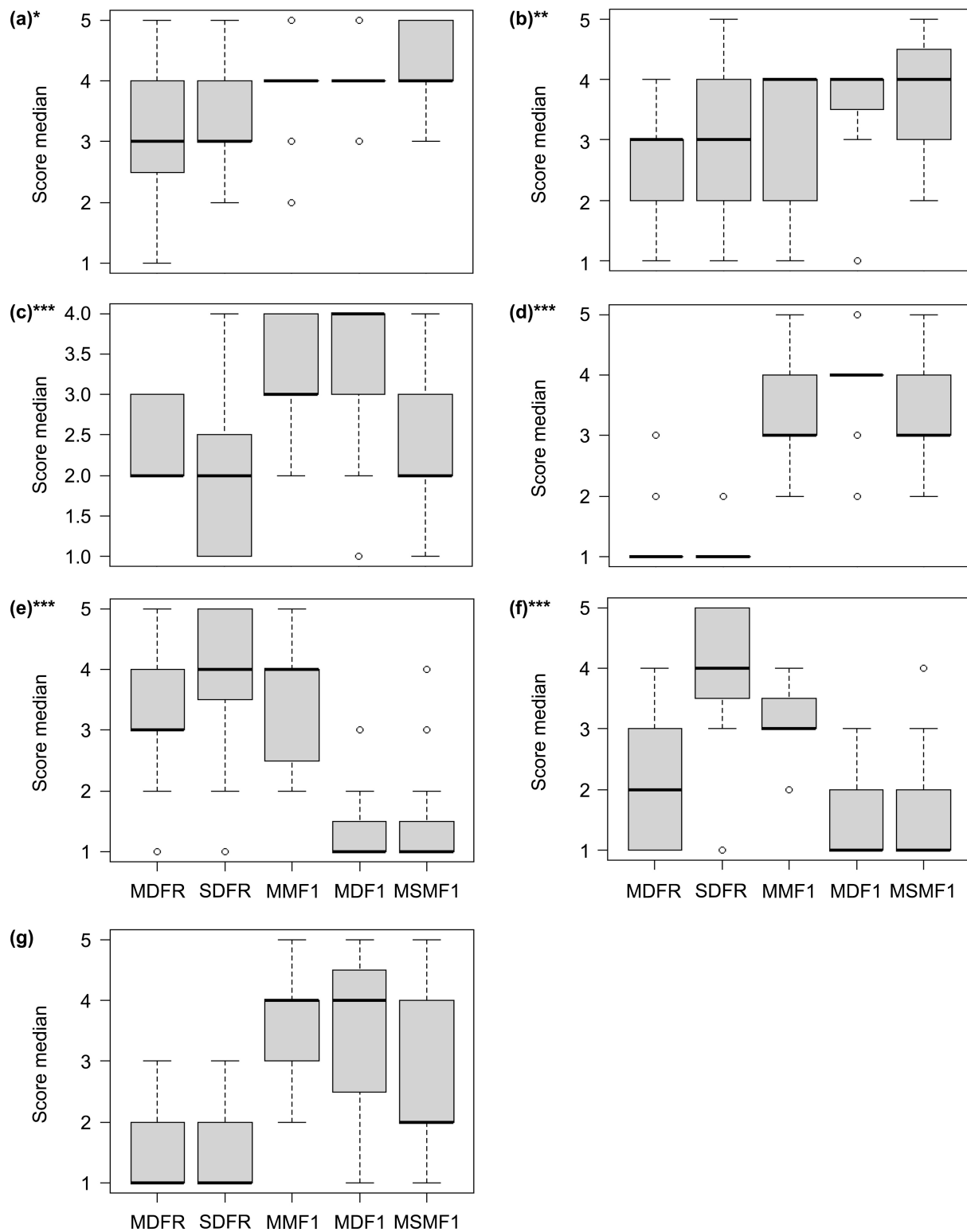
3.2. Comparative Sensory Characteristics of the 24-Hour Fermented *Tchakpalo* and the Reference Variants

The results of the second phase of the sensory analysis comparing the reference *Tchakpalo* samples with those fermented for 24 hours are presented in **Figure 5**.

The results revealed that the panelists' assessment of *Tchakpalo* color, odor, aftertaste, sweetness, acidity, and alcohol content varied significantly from one production method to another (**Figure 5**). Thus, the color of the *Tchakpalo* produced using the MFM1 scored (4 [2 - 5]), DFM1 (4 [3 - 5]), and MFMS1 (4 [3 - 5]) technologies and is agreeable, contrary to those produced using DFMR (3 [1 - 5]) and DFSR (3 [2 - 5]) technologies, toward which the tasters are indifferent. The same trend is observed for the aroma of *Tchakpalo*. Furthermore, sensory scores obtained for *Tchakpalo* are from MFM1 (4 [2 - 5]) to DFM1 (4 [3 - 5]) regarding color, and for DFM1 (4 [1 - 4]) regarding aroma.

In fact, during the MFM1 and DFM1 variant *Tchakpalo* production, caramelized sugar syrup was added. This is intended to make the color and aroma of the *Tchakpalo* more pleasant according to the processors. Regarding aftertaste, the *Tchakpalo* produced using the DFM1 technology is the most highly rated (pleasant) with a median score of 4 [1 - 4], while MFM1, MFMS1, DFMR, and DFSR *Tchakpalo* variants aftertaste seems nor agreeable nor disagreeable (3 [2 - 4]) and found to be unpleasant. The *Tchakpalo* produced using the reference technologies (DFMR and DFSR) are perceived as unsweet with median score of (1 [1 - 3] and 1 [1 - 2]) by the panelists, unlike the one produced using the DFM1 technology, which they find sweet (4 [2 - 5]).

Regarding to acidity and alcohol content, the DFM1 and MFMS1 *Tchakpalo* variant from were scored neither acidic nor alcoholic with median score. In contrast, the *Tchakpalo* derived from the DFSR and MFM1 technologies are the most acidic, whereas the one derived from the DFSR technology has the highest alcohol content. The *Tchakpalo* produced from the MFM1 and DFM1 technologies are globally more appreciated (4 [1 - 5] and 4 [2 - 5]) by the panellist, contrary to the one obtained from the MFMS1 technology, which is less appreciated (2 [1 - 5]), and those resulting from the DFMR and DFSR technologies, which are disliked (1 [1 - 3] and 1 [1 - 3]).



DFMR: 24-hour Double-Fermented Maize Reference; DFSR: 24-hour Double-Fermented Sorghum Reference; MFM1: 24-hour Single-Fermented Maize; DFM1: 24-hour Double-Fermented Maize; MFMS1: 24-hour Single-Fermented Maize-Sorghum; (a): Color; (b): Odor; (c): Aftertaste; (d): Sweetness; (e): acidic taste; (f): alcohol content; (g): overall acceptability; ° = $p > 0.05$ (not significant); * = $p < 0.05$ (significant); ** = $p < 0.01$ (highly significant); *** = $p < 0.001$ (very highly significant).

Figure 5. Box plot of the comparative sensory evaluation of the different *Tchakpalo* variants.

3.3. Microbiological Characteristics of the Different *Tchakpalo* Variants

The mean values for each microbiological parameter vary according to the production method (Table 2). Except the density of *Tchakpalo* which varies very significantly ($p < 0.01$) between technologies used, all other parameters show a p -value less than 0.001; these parameters are therefore highly influenced by the different production variants and methods involved in this study (raw materials and fermentation technologies).

Mean values for Total Aerobic Mesophilic Flora (TAMF), Lactic Acid Bacteria (LAB), and Yeasts and Molds (YM) are generally higher in *Tchakpalo* produced using reference technologies (DFSR and DFMR) than in those from their variants (DFM1, MFM1, and MFMS1). Specifically, the microbial load (TAMF and YM) in *Tchakpalo* from reference technologies is approximately twice that of the variants. The same trend applies to the LAB load, although the ratio is slightly less than twofold. *Tchakpalo* produced via the DFSR technology exhibits the highest levels of TAMF, LAB, and YM, whereas *Tchakpalo* from the MFMS1 technology shows low microbial abundance.

Table 2. Microbiological characteristics of the different variants of *Tchakpalo*.

		Total aerobic mesophilic flora ($\times 10^7$ UFC/ml)	Lactic acid bacteria ($\times 10^5$ UFC/ml)	Yeasts & molds ($\times 10^6$ UFC/ml)
DFMR	$\bar{x}^-$	4.64 ^b	2.40 ^b	3.16 ^b
	σ_x^-	0.72	0.33	0.8
DFSR	$\bar{x}^-$	5.63 ^a	2.83 ^a	3.5 ^a
	σ_x^-	0.67	0.11	0.3
MFM1	$\bar{x}^-$	1.38 ^d	1.5 ^{cd}	1.39 ^d
	σ_x^-	0.22	0.13	0.3
DFM1	$\bar{x}^-$	2.2 ^c	1.63 ^c	2.49 ^c
	σ_x^-	0.37	0.15	0.6
MFMS1	$\bar{x}^-$	1.23 ^d	1.1 ^d	1.07 ^e
	σ_x^-	0.41	0.22	0.4
p-value		***	***	***

DFMR: 24-hour Double-Fermented Maize Reference; DFSR: 24-hour Double-Fermented Sorghum Reference; MFM1: 24-hour Single-Fermented Maize; DFM1: 24-hour Double-Fermented Maize; MFMS1: 24-hour Single-Fermented Maize-Sorghum; $\bar{x}^-$: Mean; σ_x^- : Standard Error; In the same column, means marked with different letters are significantly different at the threshold of 5% according to the Student-Newman-Keuls test. ° = $p > 0.05$ (not significant); * = $p < 0.05$ (significant); ** = $p < 0.01$ (highly significant); *** = $p < 0.001$ (very highly significant).

3.4. Physicochemical Characteristics of the Different *Tchakpalo* Variants

With regard to the physico-chemical parameters, it can be seen that the average pH values are higher for *Tchakpalo* produced using the MFM1 and MFMS1 processes, intermediate for DFM1, and low for DFMR and DFSR (Table 3). As for titratable acidity, high average values were observed for the DFSR and DFMR technologies, lower values for DFM1, and low values for MFM1 and MFMS1.

The soluble dry matter content of *Tchakpalo* produced using the MFM1, MFMS1 and DFM1 processes is higher than that of *Tchakpalo* produced using the DFMR and DFSR processes. In terms of alcohol content, the *Tchakpalo* produced using the DFMR and DFSR technologies have the highest alcohol content, in contrast to those produced using the MFM1, DFM1 and MFMS1 technologies. Maize-based *Tchakpalo* have a slightly higher density than sorghum-based *Tchakpalo*. Finally, the assessment of dry matter content revealed that the *Tchakpalo* produced using the MFM1 and DFM1 technologies have the highest values, with intermediate values for DFMR and MFMS1, and low values for DFSR. *Tchakpalo* produced using the MFM1 technology has a distinctive feature: it has the highest values for dry matter, density and soluble dry extract, whilst, conversely, it has low values for acidity and alcohol content.

Table 3. Physicochemical characteristics of the different variants of *Tchakpalo*.

Variants		pH	Titrateable Acidity (g/l of Lactic acid)	Soluble solids (°Brix)	Alcohol content (% v/v)	Density	Dry matter (g/100ml)
DFMR	$\bar{x}$	4.17 ^c	0.21 ^b	5.1 ^c	6.67 ^a	0.93 ^a	17 ^c
	σ_x^-	0.01	0.00	0.22	0.33	0.01	0.45
DFSR	$\bar{x}$	4.16 ^c	0.24 ^a	4.0 ^d	7.67 ^a	0.84 ^b	4 ^e
	σ_x^-	0.01	0.00	0.19	0.23	0.01	0.44
MFM1	$\bar{x}$	4.25 ^b	0.11 ^d	9.0 ^a	3.67 ^b	0.98 ^a	35 ^a
	σ_x^-	0.01	0.00	0.24	0.11	0.01	0.38
DFM1	$\bar{x}$	4.21 ^{bc}	0.15 ^c	8.0 ^b	4.67 ^b	0.95 ^a	26 ^b
	σ_x^-	0.01	0.00	0.31	0.26	0.01	0.42
MFMS1	$\bar{x}$	4.37 ^a	0.11 ^d	8.3 ^{ab}	3.67 ^b	0.88 ^b	12 ^d
	σ_x^-	0.01	0.00	0.21	0.17	0.01	0.38
p-value		***	***	***	***	**	***

DFMR: 24-hour Double-Fermented Maize Reference; DFSR: 24-hour Double-Fermented Sorghum Reference; MFM1: 24-hour Single-Fermented Maize; DFM1: 24-hour Double-Fermented Maize; MFMS1: 24-hour Single-Fermented Maize-Sorghum; $\bar{x}$: Mean; σ_x^- : Standard Error; In the same column, means marked with different letters are significantly different at the threshold of 5% according to the Student-Newman-Keuls test. ° = $p > 0.05$ (not significant); * = $p < 0.05$ (significant); ** = $p < 0.01$ (highly significant); *** = $p < 0.001$ (very highly significant).

3.5. Correlation between the Technologies and the Parameters Evaluated

Figure 6 illustrates the results of the Principal Component Analysis (PCA), showing the correlation between sensory, microbiological, and physicochemical parameters along the first two principal axes, as well as the hierarchical classification showing the technologies that yield *Tchakpalo* with the most similar sensory, microbiological, and physicochemical characteristics.

The first two axes of the principal component analysis alone account for 86.66% of the total variability (70% for axis 1 and 16.66% for axis 2), thereby ensuring good data representativeness (**Figure 6(a)**).

The first axis (Dim 1) is strongly and positively correlated with color and odor ($r = 0.98$), aftertaste ($r = 0.59$), sweetness ($r = 0.92$), Brix value ($r = 0.99$), dry matter ($r = 0.71$), density ($r = 0.63$), and overall acceptability ($r = 0.83$). Thus, *Tchakpalo* samples produced using the MFM1 and DFM1 technologies which possess a pleasant color, also exhibit a pleasant odor and aftertaste, a sweet taste, and high values for degree Brix, dry matter, and density, while achieving high overall acceptability. This indicates that these attributes contribute to a positive perception of *Tchakpalo*, suggesting that a product with an attractive color, pleasant aroma, and sweet taste is likely to be better accepted by consumers. Conversely, this PC axe is strongly and negatively correlated with total flora ($r = -0.99$), lactic acid bacteria ($r = -0.94$), yeasts and molds ($r = -0.88$), alcohol content ($r = -0.98$), and titratable acidity ($r = -0.97$), indicating that *Tchakpalo* samples (produced using DFMR and DFSR technologies) exhibit high acidity, high alcohol content, and high titratable acidity.

The second axis (Dim 2), which accounts for 16.56% of the variance, is positively correlated with aftertaste ($r = 0.70$) and overall acceptability ($r = 0.54$) and negatively correlated with pH ($r = -0.79$). This suggests that aftertaste has a significant impact on the general perception of *Tchakpalo*. In contrast, the axis is negatively correlated with pH ($r = -0.79$), suggesting that a high pH could negatively affect the perception of the product.

This highlights the importance of optimizing pH during the fermentation process to improve product acceptability. Factorial analysis reveals that *Tchakpalo* variants are distributed according to production technology types, demonstrating that manufacturing processes influence the final characteristics of the *Tchakpalo*. Indeed, MFM1 and DFM1 show strong correlations with positive sensory attributes, such as good color and odor. The dendrogram illustrates the relationship between the various *Tchakpalo* manufacturing technologies (**Figure 6(b)**). Two groups can be distinguished: the first (G1), comprising the reference technologies (DFMR and DFSR), and the second (G2), including MFMS1, MFM1, and DFM1. Notably, within group G2, the MFM1 and DFM1 technologies exhibit greater similarity. Thus, *Tchakpalo* produced using MFM1 and DFM1 technologies share similarities; the same applies to those produced using DFMR and DFSR technologies.

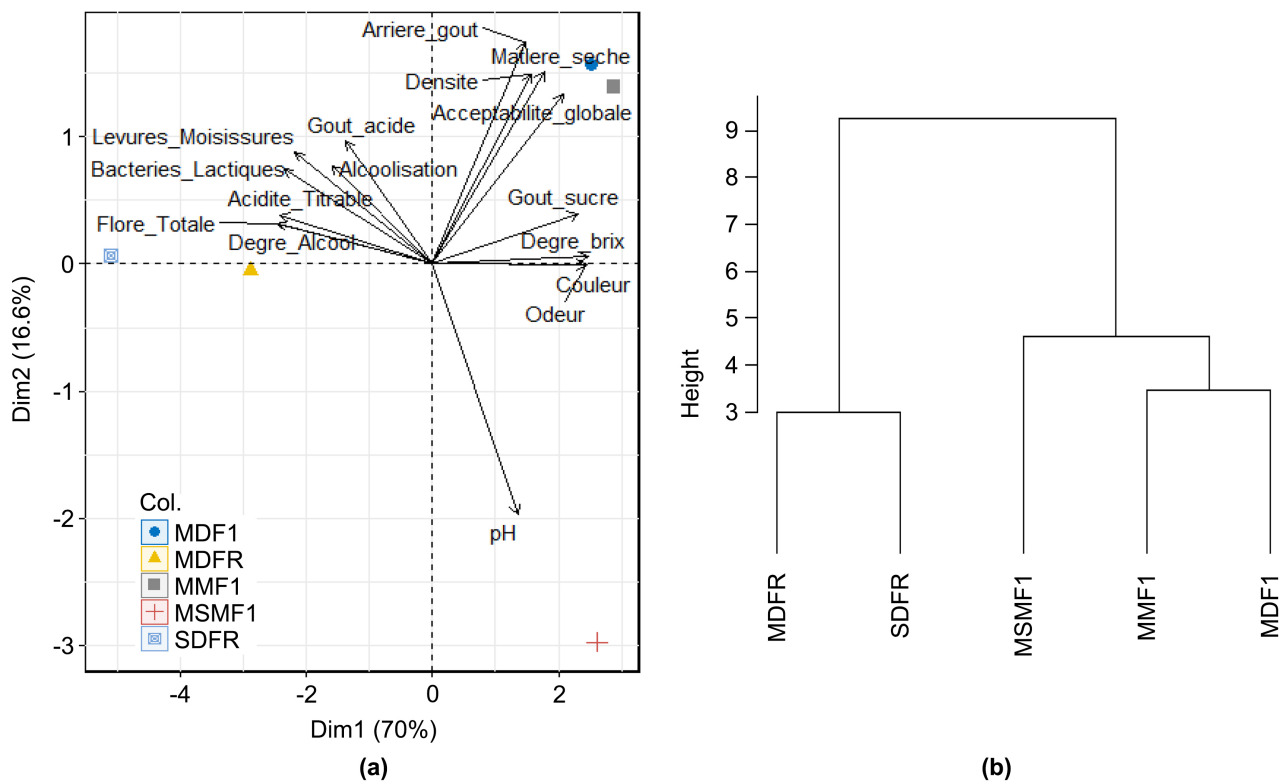


Figure 6. (a) Correlation between sensory, microbiological, and physicochemical parameters on the first two principal axes, and (b) Hierarchical classification showing the identical technologies used to produce the different *Tchakpalo* variants. DFMF: Maize Double-Fermented Reference; DJSR: Sorghum Double-Fermented Reference; MFM1: 24-hour Single-Fermented Maize; DFM1: 24-hour Double-Fermented Maize; MFMS1: 24-hour Single-Fermented Maize-Sorghum.

4. Discussion

The results of the first phase of the sensory evaluation revealed that, regardless of the raw material (maize, sorghum, or a maize-sorghum mixture) and the production process (single or double fermentation), the panelists preferred the *Tchakpalo* samples of 24 hours of fermentation as opposed to those of 48 hours of fermentation. The 24-hour fermented *Tchakpalo* samples were considered sweeter, less acidic, and had a lower alcohol content than those fermented for 48 hours. It can therefore be concluded from these results that sugar content, alcohol content, and acidity are the key parameters influencing consumers' preference for *Tchakpalo*. These findings are consistent with those obtained by Dossou *et al.* [2] in a study on the economic analysis of artisanal *Tchakpalo* production in Benin. The study, later identified Dassa-Zoumè and Glazoué (central Benin) as the places of origin of this local beer—revealing that the criteria for evaluating *Tchakpalo* beer, as described by consumers, are: taste, color, alcohol content, and acidity. Taste and alcohol content were identified by Kayodé *et al.* [18] and Pale *et al.* [19] as the main quality criteria consumers use to evaluate certain traditional African beers (e.g. *Tchoukoutou*, *Dolo*).

These results also corroborate the observations of Madilo *et al.* [20] on multiple fermented African cereal beverages, notably Ivorian *Tchakpalo*, Burkinabe *Dolo*,

and Ghanaian *Pito*, for which the reduction of fermentation time is associated with a better sensory appreciation due to the preservation of a mild flavor and moderate acidity. Furthermore, the increase in alcohol content observed after 48 hours of fermentation may also have negatively influenced acceptability. Regular consumers of traditional African beverages generally prefer moderate alcohol content, which is associated with better drinkability and a greater sensation of freshness [21]. Thus, the decrease in acceptability observed after 48 hours could result from the combined effect of increased acidity and a higher alcohol content.

Comparative analysis between the variants fermented for 24 hours and the reference processes showed that samples MFM1 and DFM1 received the highest overall acceptability scores. Consumers particularly appreciated their color, aroma, sweetness, and low acidity. These results may be attributed to the addition of powdered white sugar and caramelized sugar syrup at the end of production for the *Tchakpalo* samples produced using the MSM1, DFM1, and MFM1 methods, unlike the DFMR and DFSR reference samples. This addition not only increases the soluble solids content but also improves the product's color and aromatic characteristics. Furthermore, color is a key criterion in the sensory evaluation of fermented beverages. According to Kayitesi *et al.* [22], consumers generally associate a uniform amber color with superior technological quality. The caramelized sugar added during the process promotes the formation of brown pigments resulting from Maillard and caramelization reactions, thereby enhancing the product's visual appeal. These results confirm findings from the previous sensory test, which identified sweetness, acidity, and alcohol content as the primary parameters influencing consumer appreciation of *Tchakpalo*.

These results align with those obtained by Egounlety *et al.* [3] in a study regarding the production process and the microbiological, physicochemical, and sensory characteristics of *Tchakpalo*. Indeed, in that study, most of the panelists preferred the sweeter and slightly acidic taste of pasteurized *Tchakpalo* over the less sweet and highly acidic taste of unpasteurized *Tchakpalo*. Similar findings were reported in a study by Konfo *et al.* [23] on the changes in physicochemical, microbiological, and sensory parameters during the production of *Tchakpalo* supplemented with *Hemizygia bracteosa* powder.

That study showed that the control *Tchakpalo* (produced using the standard/reference method) was the least appreciated, as it was the most acidic and least sweet of the samples. Furthermore, in a study on the consumption of thirst-quenching beverages in schools in the city of Abomey-Calavi, Kayodé *et al.* [24] stated that sweetness and aroma were the primary reasons cited by students for consuming such beverages specifically *Tchakpalo*, Adoyo, and Bissap (hibiscus) juice. It is worth highlighting, however, that *Tchakpalo* produced using these standard technologies (DFMR and DFSR) is not widely available in southern Benin. Such products are typically found at special events—such as christenings, weddings, dowry ceremonies, brunches, and official celebrations and are therefore not accessible to the general public.

Microbiological analyses revealed significant differences among the technologies studied. *Tchakpalo* samples produced using the reference technologies exhibited the highest counts of total aerobic mesophilic flora, lactic acid bacteria, and yeasts/molds. These results may be attributed to technological differences between the production processes for the reference *Tchakpalo* and their variants. Double cooking and double fermentation promote the hydrolysis of starch into fermentable sugars, thereby increasing the availability of energy substrates required for the growth of fermentative microorganisms [25]. Indeed, unlike other *Tchakpalo* types (produced via DFM1, MFM1, and MFMS1 technologies) which require only a single cooking step, the production of reference *Tchakpalo* (via DFSR and DFMR technologies) involves two cooking stages: the first cooks the sediment obtained after the settling of the diluted malted maize or sorghum grits (to which the supernatant is gradually added), while the second cooks the fermented supernatant (from the first fermentation) obtained after the settling of the product from the first cooking stage. The settling step facilitates the separation of the sediment composed of undissolved matter from the liquid supernatant, which contains soluble sugars and enzymes. Extracting these enzymes is essential for the subsequent hydrolysis of the starch.

In a study on optimizing the *Tchakpalo* production process in Ivory Coast, Amane *et al.* [25] stated that the pre-cooking stage facilitates enzymatic action on the starch. Indeed, the starch paste obtained after pre-cooking is readily hydrolyzed into dextrins, maltotriose, maltose, and glucose through enzymatic activity. Consequently, the enzyme's diastatic action is more effective on pregelatinized starch than on raw starch. Obtaining starch paste is thus a crucial step in *Tchakpalo* production, as it facilitates the conversion of starch into sugar. The cooking stage, meanwhile, concentrates the diluted mash into a sugary mash through water evaporation, yielding the sugary mash required for fermentation. In our study, the pre-cooking and cooking stages could be equivalent to a first and second cooking phase, respectively; this benefited the fermentation process, explaining the higher levels of yeasts and lactic acid bacteria found in *Tchakpalo* produced using DFSR and DFMR technologies. It should be noted that lactic acid bacteria and yeasts are the dominant microorganisms in most cereal-based fermented products [26].

Lactic acid bacteria play a role during the souring and acidification stage of the mash, thereby creating conditions favorable for yeast growth (lactic fermentation). Yeasts, for their part, carry out alcoholic fermentation, the final step in the production process of this traditional beer. The yeast, hence, plays a decisive role in shaping the characteristics of the finished product. This is the result of a symbiotic association between yeasts and lactic acid bacteria: the bacteria create an acidic environment conducive to yeast proliferation, while the yeasts produce vitamins and other compounds essential for bacterial growth. Consequently, the high populations of lactic acid bacteria observed in the reference *Tchakpalo* samples (produced using DFSR and DFMR methods) indicate more active lactic fermentation. Species belonging to the genera *Lactiplantibacillus*, *Levilactobacillus*,

and *Pediococcus* are frequently found in cereal-based fermented beverages and contribute to the production of organic acids responsible for the decrease in pH [20]. The simultaneous increase in yeast populations reflects more intense alcoholic fermentation. Yeasts primarily *Saccharomyces cerevisiae* convert fermentable sugars into ethanol and carbon dioxide, thereby contributing to the development of the beverage's final organoleptic characteristics [27]. The microbiological results obtained in this study should be interpreted only with respect to the microbial groups enumerated (total aerobic mesophilic flora, lactic acid bacteria, and yeasts and molds). Therefore, lower microbial counts cannot be directly considered as evidence of improved food safety.

The physicochemical results obtained confirm this microbiological dynamic. The reference technologies exhibit the lowest pH values and the highest titratable acidities. This inverse relationship between pH and titratable acidity is characteristic of fermented cereal beverages and reflects the gradual accumulation of organic acids produced during fermentation [28].

This difference could be explained by the fact that the *Tchakpalo* produced using the MFM1 and MFMS1 technologies under a single fermentation, unlike the *Tchakpalo* produced using the DFMR, DFM1, and DFSR technologies, which undergo a double fermentation process. These results corroborate the tasters' perceptions during the sensory evaluation, except for the *Tchakpalo* produced using the DFM1 technology, which, according to the tasters, is less acidic; this could be explained by the sweet taste of this *Tchakpalo*, which may have influenced its perceived acidity.

As for titratable acidity, high average values were observed for the DFSR and DFMR technologies, lower values for DFM1, and low values for MFM1 and MFMS1. These high levels of titratable acidity in the reference *Tchakpalo* samples may be linked to the accumulation of organic acids, which tend to make the medium more acidic [29]. Indeed, titratable acidity corresponds to the total amount of mineral and organic acids contained in a food product [30]. Egounlety *et al.* [3] also noted a decrease in pH during the *Tchakpalo* fermentation process. They attributed this decrease to the production of organic acids resulting from the activity of lactic acid bacteria, which break down dry matter and soluble solids to produce other metabolites, the most important of which are lactic acid, acetic acid, citric acid, and oxalic acid.

The soluble dry matter of *Tchakpalo* produced from MFM1, MFMS1, and DFM1 technologies is higher than that produced from DFMR and DFSR technologies, to which no white sugar or caramelized sugar was added. In terms of alcohol content, the *Tchakpalo* produced using the DFMR and DFSR technologies have the highest alcohol content, unlike those produced using the MFM1, DFM1, and MFMS1 technologies. Maize based *Tchakpalo* have a slightly higher density than sorghum-based *Tchakpalo*. Finally, the evaluation of dry matter content revealed that the *Tchakpalo* produced from MFM1 and DFM1 technologies have the highest values, while intermediate values are recorded by DFMR and MFMS1, and low

values by DFSR.

Tchakpalo produced from MFM1 technology has a distinctive feature: it has higher values for dry matter, density, and soluble dry extract, and, conversely, lower values for acidity and alcohol content. These results are comparable to those obtained by Amané *et al.* [31], whose finding revealed that some samples had a dry matter content 4.5 times higher than others. This difference is likely due to the clarification method or to an inadequacy in that method. It was observed that, while some brewers use very fine-mesh filters, others use only jute bags for filtration. Furthermore, the high alcohol content observed in the reference *Tchakpalo* samples supports the microbiological results, which revealed an abundance of lactic acid bacteria (LAB) and yeasts and molds (YM). Indeed, lactic fermentation is carried out by lactic acid bacteria (production of acids), and alcoholic fermentation by yeasts (production of alcohol and carbon dioxide) [32].

From our results, it can therefore be deduced that the high alcohol contents observed in the DFMR and DFSR technologies corroborate the high yeast loads recorded in these samples. More extensive fermentation leads to greater conversion of sugars into ethanol, thus explaining the higher alcohol content values observed. Conversely, the MFM1, DFM1, and MFMS1 variants exhibited the highest values for dry matter, density, and soluble dry extract. These results suggest that a significant proportion of the carbohydrates remains unmetabolized due to less advanced fermentation. This accumulation of residual sugars directly contributes to the perception of sweetness and partly explains their higher sensory acceptability.

Principal component analysis revealed the link between the various parameters studied. The first two axes account for 86.66% of the total variability, indicating an excellent interpretation of the variation observed. The first axis clearly contrasts two distinct technological profiles. On the one hand, the MFM1 and DFM1 technologies are associated with high values for Brix, dry matter, density, color, odor, sweetness, and overall acceptability. On the other hand, the DFMR and DFSR technologies are correlated with high levels of total flora, lactic acid bacteria, yeast, titratable acidity, and alcohol content. This underscores the importance of optimizing pH during the fermentation process to improve product acceptability. Extensive fermentation enhances microbiological activity and promotes the production of alcohol and organic acids, but it simultaneously reduces the amount of residual sugars and may decrease consumer acceptance.

From a factorial perspective, *Tchakpalo* variants are categorized according to production technology types, indicating that manufacturing processes influence the final characteristics of *Tchakpalo*. Indeed, MFM1 and DFM1 show strong correlations with positive sensory attributes, such as good color and aroma. The negative correlation observed between overall acceptability and parameters related to fermentation intensity confirms that consumers prefer moderately fermented beverages, characterized by a balance between sweetness, acidity, and alcohol content. Similar trends have been reported in several recent studies on traditional fer-

mented cereal beverages from West Africa [22] [27] [28].

The dendrogram confirms this structure by distinguishing two main groups corresponding to the traditional reference processes and the adapted variants. This classification shows that the technological modifications introduced in the studied variants simultaneously influence the microbiological, physicochemical, and sensory properties of *Tchakpalo*.

Overall, the results demonstrate that the MFM1 and DFM1 variants represent the best balance between sensory acceptability and technological characteristics. They produce beverages with good organoleptic quality while maintaining microbiological and physicochemical parameters compatible with the requirements of artisanal *Tchakpalo* production.

5. Conclusion

This study enabled us to compare the sensory, microbiological, and physicochemical characteristics of different *Tchakpalo* variants. The findings indicate that consumers' perceptions regarding the color, aroma, aftertaste, sweetness, acidity, and alcohol content of *Tchakpalo* vary significantly depending on the production method used. The same applies to the microbiological and physicochemical parameters evaluated. Overall, *Tchakpalo* samples produced using the MFM1 (24-hour Single-Fermented Maize) and DFM1 (Maize double Fermented of 24 hours) methods were the most preferred by the panellists. Principal Component Analysis (PCA) highlighting the link between sensory, microbiological, and physicochemical parameters reveals that *Tchakpalo* from these two methods possesses a pleasant color, aroma, and aftertaste, as well as greater sweetness and higher Brix, dry matter, and density values. Conversely, these samples are less acidic and have lower alcohol content and a less abundant microbial flora (total flora, lactic acid bacteria, and yeasts/molds). The results of this study provide valuable information to support efforts to modernize and promote this traditional beer, which is highly popular among the Beninese population.

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All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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