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Thèse en cotutelle

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Qualité microbiologique du lait caillé et du *Wagashi Gassirè* produits au Bénin: état des lieux et pistes d'amélioration

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Résumé

Le fromage *Wagashi Gassirè* (WG) et le lait caillé (*Fanirè* ou lait fermenté) (LC) figurent parmi les produits laitiers les plus populaires au Bénin et au-delà, grâce à leur valeur nutritionnelle. Cependant, leur production traditionnelle soulève des préoccupations quant à leur sécurité sanitaire. Cette étude vise à évaluer la qualité microbiologique de ces produits disponibles sur le marché et dans les campements ainsi que ceux issus de procédés améliorés à travers plusieurs approches.

Dans un premier temps, une étude préliminaire a été réalisée sur les fromages WG (colorés et non colorés) des marchés de Cotonou et Abomey-Calavi. Les résultats ont révélé que les WG non colorés étaient dominés par les *Lactobacillaceae* (55%, principalement *Lactobacillus delbrueckii*) et les *Streptococcaceae* (32%), tandis que les WG colorés étaient majoritairement composés de *Streptococcaceae* (73%) avec une faible présence de *Lactobacillaceae* (2%). Bien que la diversité bactérienne globale des deux types de fromage n'ait pas présenté de différence significative, la faible concentration en *Lactobacillus* des WG colorés pourrait être liée à leur procédé de coloration.

Une enquête menée auprès des éleveurs, producteurs, commerçants et consommateurs a permis de recueillir des informations sur les pratiques de transformation du lait en WG et LC, ainsi que sur leur conservation. Suite à cette enquête, la qualité microbiologique des laits crus, WG et LC issus des suivis de production et ceux collectés sur les marchés a été évaluée. Sur 70 échantillons analysés, *Salmonella enterica* et *Bacillus cereus* ont été isolés dans respectivement 10 et 14% des échantillons

Les niveaux d'*Escherichia coli* ont dépassé les normes du règlement CE 2073/2005 dans le lait cru, le lait caillé et le WG coloré, tandis que les niveaux de *Staphylococcus aureus* étaient acceptables dans la majorité des échantillons.

Pour approfondir cette analyse, le séquençage à haut débit des amplicons du gène de l'ARNr 16S bactérien a été utilisé sur 114 échantillons de produits laitiers. Les phylums bactériens dominants étaient les Firmicutes/Bacillota (64%) et les Proteobacteria/ Pseudomonadota (36%). Les produits laitiers des camps et marchés de Nikki et Dassa-Zoumé partageaient principalement des bactéries des familles *Streptococcaceae* (44%), *Enterobacteriaceae* (23%), *Moraxellaceae* (9%), *Lactobacillaceae* (7%) et *Bacillaceae* (5%). Les espèces dominantes étaient *Lactococcus lactis* (21%), *Streptococcus* sp. (18%), *Lactobacillus delbrueckii* (6%), *Streptococcus thermophilus* (3%), *Weissella confusa* (2%), *Lactobacillus fermentum* (1%) et *Lactobacillus helveticus* (1%). La dynamique des communautés bactériennes des fromages WG rouges et blancs a montré une différence significative pendant la durée de conservation. L'analyse a également détecté plusieurs pathogènes potentiels, tels que *B. cereus*, *Clostridium perfringens*, *Klebsiella pneumoniae* et *Streptococcus infantarius*. Ces résultats soulignent l'importance de la diversité et de la dynamique des microbiotes dans ces produits laitiers traditionnels et devraient aider à résoudre les problèmes de sécurité et de détérioration associés.

Pour améliorer leur qualité sanitaire, des propositions d’améliorations ont ensuite été formulées et testées en laboratoire, et les produits ainsi améliorés ont fait l'objet des mêmes analyses microbiologiques. Les

échantillons de WG ont été soumis à un traitement thermique à 100°C pendant 20 min, puis emballés de deux manières: en emballage simple et sous vide.

Par ailleurs, les échantillons de *Fanirè* ont été soumis à une fermentation avec diverses cultures de départ, notamment *Lactococcus lactis* seul, ainsi qu'en combinaison avec *Leuconostoc mesenteroides*, *Lactobacillus plantarum* et *L. fermentum*. La fermentation contrôlée a duré 24 h à 30°C, suivie d'une évaluation 48 h après production. Les échantillons de WG ont été surveillés sur trois jours, avec des analyses effectuées le premier jour et après trois jours de stockage. Les résultats ont montré que le lait fermenté produit avec *L. lactis* seul avait une qualité microbiologique adéquate, quelle que soit la période d'analyse. Les produits obtenus par backslopping et combinaison de bactéries lactiques se sont améliorés avec le stockage. La double cuisson des échantillons de WG, avec ou sans emballage sous vide, a préservé la qualité sanitaire des fromages.

Afin d'améliorer la sécurité des produits laitiers traditionnels, il est essentiel de mettre en place des protocoles stricts de contrôle de la qualité microbiologique et de sensibiliser les acteurs de la chaîne de production aux bonnes pratiques d'hygiène. Des campagnes de sensibilisation auprès des consommateurs sur les risques et les bonnes pratiques de consommation sont également nécessaires. L'exploration d'autres bactéries bénéfiques présentes dans ces produits, ainsi que leur rôle potentiel dans l'inhibition des pathogènes et des microorganismes d'altération, contribuera à préserver et améliorer la valeur nutritionnelle de ces produits étudiés.

Mots clés: *Wagashi Gassirè, Fanirè*, qualité sanitaire, NGS, Bénin**.**

Abstract

Wagashi Gassirè cheese (WG) and spontaneously curdled milk (*Fanirè* or fermented milk) (LC) are among the most popular dairy products in Benin and beyond due to their nutritional value. However, their traditional production raises concerns about their sanitary safety. This study aims to evaluate the microbiological quality of these products available on the market and in camps, as well as those derived from improved processes, through various approaches.

Initially, a preliminary study was conducted on WG cheeses (stained and unstained) from the markets of Cotonou and Abomey-Calavi. The results revealed that unstained WG were dominated by *Lactobacillaceae* (55%), mainly *Lactobacillus delbrueckii*, and *Streptococcaceae* (32%), while stained WG were primarily composed of *Streptococcaceae* (73%) with a low presence of *Lactobacillaceae* (2%). Although the overall bacterial diversity of both types of cheese did not show a significant difference, the low concentration of *Lactobacillus* in stained WG could be linked to their colouring process.

A survey conducted among breeders, producers, traders, and consumers gathered information on the practices of transforming milk into WG and LC, as well as their preservation. Following this survey, the microbiological quality of raw milk, WG, and LC from production monitoring and those collected from the markets was evaluated. Out of 70 samples analyzed, *Salmonella enterica* and *Bacillus cereus* were isolated in 10 and 14% of the samples, respectively. *Escherichia coli* levels exceeded the standards of EC Regulation 2073/2005 in raw milk,

curdled milk, and stained WG, while *Staphylococcus aureus* levels were acceptable in the majority of samples.

To further this analysis, high-throughput sequencing of bacterial 16S rRNA gene amplicons was used on 114 dairy product samples. The dominant bacterial phyla were Firmicutes/Bacillota (64%) and Proteobacteria/Pseudomonadota (36%). Dairy products from the camps and markets of Nikki and Dassa-Zoumé mainly shared bacteria from the families *Streptococcaceae* (44%), *Enterobacteriaceae* (23%), *Moraxellaceae* (9%), *Lactobacillaceae* (7%), and *Bacillaceae* (5%). The dominant species were *Lactococcus lactis* (21%), *Streptococcus sp*. (18%), *Lactobacillus delbrueckii* (6%), *Streptococcus thermophilus* (3%), *Weissella confusa* (2%), *Lactobacillus fermentum* (1%), and *Lactobacillus helveticus* (1%). The dynamics of bacterial communities in red and white WG cheeses showed a significant difference during storage. The analysis also detected several potential pathogens, such as *B. cereus*, *Clostridium perfringens*, *Klebsiella pneumoniae*, and *Streptococcus infantarius*.

These results highlight the importance of the diversity and dynamics of microbiomes in these traditional dairy products and help address associated safety and spoilage issues.

To improve their sanitary quality, improvement proposals were formulated and tested in the laboratory, and the improved products were subjected to the same microbiological analyses. The *Wagashi Gassirè* samples were subjected to heat treatment at 100°C for 20 min, then packaged in two ways: simple packaging and vacuum packaging.

Abstract

Fanirè samples were subjected to fermentation with various starter cultures, including *Lactococcus lactis* alone and in combination with *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, and *L. fermentum*. The controlled fermentation lasted 24 h at 30°C, followed by an evaluation 48 h after production. *Wagashi Gassirè* samples were monitored over three days, with analyses conducted on the first day and after three days of storage. The results showed that fermented milk produced with *L. lactis* alone had adequate microbiological quality, regardless of the analysis period. Products obtained through backslopping and combinations of lactic bacteria improved with storage. Double cooking of *Wagashi Gassirè* samples, with or without vacuum packaging, preserved the sanitary quality of the cheeses.

To improve the safety of traditional dairy products, it is essential to implement strict protocols for microbiological quality control and to raise awareness among production chain actors about good hygiene practices. Awareness campaigns for consumers on risks and best consumption practices are also necessary. Exploring other beneficial bacteria present in these products and their potential role in inhibiting spoilage microorganisms and pathogens will help preserve and enhance the nutritional value of these studied products.

Keywords: *Wagashi Gassirè, Fanirè,* sanitary quality, NGS, Benin.

Préambule

Cette thèse a été réalisée en cotutelle entre le Laboratoire de Microbiologie de l'Alimentation (MAIE) du *Earth and Life Institute* (ELI-ELIm) à l'Université catholique de Louvain (UCLouvain, Belgique) et l'Unité de Recherche sur les Maladies Transmissibles à l'Université d'Abomey-Calavi au Bénin. Le travail a bénéficié du soutien financier de l'Académie de Recherche et d'Enseignement Supérieur, Commission de la Coopération au Développement (ARES-CCD) par le biais du projet WALAC intitulé: « Amélioration des procédés de production et de conservation du lait caillé (LC) et du *Wagashi Gassirè* (WG) par la recherche action en partenariat avec les acteurs de la filière lait au Bénin » et d'une bourse d'achèvement de thèse ARES-CCD - UCLouvain.

Le projet WALAC visait à améliorer les procédés de production et de conservation du LC et du WG au Bénin, au profit des femmes rurales productrices de ces denrées, des entreprises laitières locales et des consommateurs. Le projet est intervenu dans les communes de Dassa-Zoumé et de Nikki, situées respectivement au centre et au nord du Bénin et connues comme faisant partie des principales zones d'élevage du pays. Les deux aliments indexés par le projet WALAC sont très appréciés au Bénin du fait de leurs valeurs nutritionnelles, de leurs accessibilités et du fait qu'ils représentent une importante source de revenus pour les différents acteurs impliqués dans leurs productions et commercialisations. Cependant, le non-respect des normes hygiéniques et les difficultés de conservation de ces denrées périssables limitent la

mise à disposition aux consommateurs de produits de qualité irréprochable.

Le présent travail s'inscrit dans les objectifs dudit projet et comprend plusieurs chapitres dont le premier présente une revue de littérature générale (Chapitre 1) sur la production laitière au Bénin, suivi des objectifs du travail (Chapitre 2). La section résultats contient cinq chapitres chacun faisant l'objet d'un manuscrit. Le premier a pris en compte une étude préliminaire qui porte sur la diversité bactérienne des WG échantillonnés à Abomey-Calavi et Cotonou. Les résultats de cette étude ont été associés à ceux d'un travail similaire réalisé sur des aliments fermentés du Bénin, Niger et de l'Inde, et ont fait l'objet d'une publication (Chapitre 3). Le deuxième manuscrit prend en compte une enquête menée auprès des acteurs sur les pratiques d'élevage, de la traite et de la transformation pouvant favoriser la contamination du lait cru, du LC et du WG au Bénin (Chapitre 4). Les caractéristiques microbiologiques des laits crus, LC et WG échantillonnés dans les deux zones d'intervention du projet ont été évaluées à trois temps, espacés de 24h, chacun dans le but d'apprécier les changements microbiologiques que subissent les produits lorsqu'ils sont conservés sans aucun traitement, à température ambiante (28-32°C). Les résultats de cette étude (Chapitre 5) ont également été présentés sous forme d'un manuscrit. Pour mieux comprendre la diversité bactérienne des produits, une autre étude présentée dans le Chapitre 6 a été menée à travers une approche métagénomique utilisant la technique NGS Illumina Miseq. Suite à ces différentes études, une méthode de production améliorée a été proposée pour les LC et WG. La qualité

sanitaire de ces produits a également été évaluée (Chapitre 7). Une discussion générale est présentée au Chapitre 8, tandis que le Chapitre 9 reprend les conclusions et perspectives de cette thèse.

Une liste d'articles publiés supplémentaires est présentée à l'Annexe A. Ils portent sur les aspects technologiques, chimiques et socio-économiques de la chaine WG et LC, et ont aussi fait l'objet de thèse de doctorat dans le cadre du même projet. Enfin, une liste de communications scientifiques (orales et posters) est rapportée à la fin de la thèse (Annexe B).

Articles inclus

Komagbe G.S., Dossou A., Seko Orou B.M.T., Sessou P., Azokpota P., Youssao I., Hounhouigan J., Scippo M.-L., Clinquart A., Mahillon J., Farougou S. **(2023)** State of the art of breeding, milking, and milk processing for the production of curdled milk and *Wagashi Gassirè* in Benin: Practices favoring the contamination of its dairy products. *Front. Sustain. Food Syst.* 6, 1050592.

Sessou P., Keisam S., Gagara M., **Komagbe G.S.**, Farougou S., Mahillon, J., Jeyaram K. **(2023)** Comparative analyses of the bacterial communities present in the spontaneously fermented milk products of Northeast India and West Africa. *Front. Microbiol*. 14: 1166518.

Komagbe G.S., Sessou P., Dossou A., Seko Orou B.M.T., Hinson C., Azokpota P., Youssao I., Hounhouigan J., Clinquart A., Scippo M.-L., Bragard C., Farougou S., Mahillon J. - Microbiological quality assessment of dairy products from Benin and techno-functional properties of dominant lactic acid bacteria isolated from *Fanirè*, a naturally fermented milk. *In preparation.*

Komagbe G.S., Sessou P., Keisam S., Clinquart A., Farougou S., Bragard C., Jeyaram K., Mahillon J. - Bacterial diversity and dynamics in *Fanirè* spontaneously fermented milk and *Wagashi Gassirè* cheese during shelf-life in Benin. *In preparation.*

Komagbe G.S., Sessou P., Dossou A., Azokpota P., Scippo M.-L., Clinquart A., Farougou S., Bragard C., Mahillon J. - Effect of heat treatment and packaging on *Wagashi Gassirè* and controlled fermentation on *Fanirè* safety. *In preparation.*

Liste des sigles et abréviations

ADN	:	Acide désoxyribonucléique
ADRI	:	Administration des relations internationales
ARES	:	Académie de Recherche et d'Enseignement Supérieur
ARN	:	Acide ribonucléique
°C	:	Degré Celsius
CAC	:	*Codex Alimentarius Commission*
CCD	:	Commission de la Coopération au Développement
CE	:	Commission Européenne
CFU	:	*Colony Forming Unit*
CI	:	*Confidence Interval*
CO_2	:	Dioxyde de carbone
EFSA	:	*European Food Safety Authority*
EC	:	*European Commission*
ELI	:	*Earth and Life Institute*
EPAC	:	Ecole Polytechnique d'Abomey-Calavi
EPEC		*Enteropathogen Escherichia coli*
ETEC	:	*Enterotoxinogen Escherichia coli*
EUCAST	:	*European Committee on Antimicrobial Susceptibility Testing*
FAO	:	*Food Agriculture Organization*
FAOSTAT	:	*Food and Agriculture Organization Statistics*
FDA	:	*Food and Drug Administration*
GBAD	:	Groupe de la Banque Africaine de Développement

GDP	:	*Gross Domestic Product*
GRAS	:	*Generally Regarded as Safe*
H_2O_2		Peroxyde d'hydrogène
ICFMH	:	*International Committee on Food Microbiology and Hygiene*
INSAE	:	Institut National de Statistique et de l'Analyse Economique
ISO	:	*International Organization for Standardization*
ITS	:	*Internal Transcribed Spacer*
LAB	:	*Lactic Acid Bacteria*
LC	:	Lait Caillé
MAEP	:	Ministère de l'Agriculture, de l'Elevage et de la Pêche
mg	:	Milligramme
MIAE	:	Microbiologie Alimentaire et Environnementale
mL	:	Millilitre
MPD	:	Ministère du Plan et du Développement
N_2	:	Diazote
NCBI	:	*National Center for Biotechnology Information*
ND	:	Non déterminé
NGS	:	*Next-Generation Sequencing*
O_2	:	Dioxygène
OMS	:	Organisation mondiale de la Santé
OT	:	Oxytétracycline
OTU	:	*Operational Taxonomic Unit*
PAFILAV	:	Projet d'Appui aux Filières LAit et Viande
PacBio	:	*Pacific Biosciences*
p.ex.	:	Par exemple

PCR	:	*Polymerase Chain Reaction*
PEAR	:	*Paired-End reAd mergeR*
PGM	:	*Personal Genome Machine*
PNIASAN	:	Plan National d'Investissements Agricoles et de Sécurité Alimentaire et Nutritionnelle
PPCB		Péripneumonie Contagieuse Bovine
PRD	:	Projet de Recherche et de Développement
PSDSA	:	Plan Stratégique de Développement du Secteur Agricole
RC	:	*Red Cheese*
RDP	:	*Ribosomal Database Project*
STEC	:	*Shiga Toxin-producing Escherichia coli*
SxT	:	Sulfaméthoxazole triméthoprime
Ty	:	Tylosine
UAC	:	Université d'Abomey-Calavi
UCL	:	Université catholique de Louvain
UE	:	Union Européenne
ufc	:	Unités Formant Colonies
µg	:	Microgramme
UHT	:	Ultra Haute Température
UI	:	Unité internationale
WALAC	:	*WAgashi Gassirè* et LAit Caillé
WC	:	*White Cheese*
WG	:	*Wagashi Gassirè*
WGB	:	*Wagashi Gassirè* blanc (non coloré)
WGR	:	*Wagashi Gassirè* rouge (coloré)
WGS	:	*Whole Genome Sequencing*

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I. INTRODUCTION ET OBJECTIFS

Chapitre 1

1.1. Les produits laitiers et leur importance dans le régime alimentaire

1.1.1. Composition biochimique et valeur nutritionnelle du lait

L'histoire du lait commence à l'ère de la révolution néolithique, époque à laquelle l'homme a substitué la chasse et la cueillette à un mode de vie plus sédentaire (Greenfield, 2010). Cela a permis de nouvelles possibilités d'adaptation des ressources pour acquérir de la nourriture. La plus importante, avec le développement de l'agriculture, a été la domestication des animaux, qui signifiait un accès régulier à leur fourrure, à leur viande et, bien sûr, à leur lait (Barłowska et al., 2011). Le lait a été défini par le congrès international de la répression des fraudes tenu à Genève en 1908 comme étant « le produit intégral de la traite totale et ininterrompue d'une femelle laitière bien portante, bien nourrie et non surmenée. Il doit être recueilli proprement et ne pas contenir de colostrum ». Selon Lambrini et al. (2021), « le lait est un liquide blanc, opaque, de saveur légèrement sucrée, constituant un aliment complet et équilibré, sécrété par les glandes mammaires de la femme et par celles des mammifères femelles pour la nutrition des jeunes ». Le lait de vache est généralement considéré comme un aliment équilibré et nutritif en raison de sa richesse en nutriments essentiels tels que l'eau (87%), les glucides (4,7%), les protéines (3,4%), les graisses (3,4%) et de cendres (0,7%) (Chandan, 2015; Foroutan et al., 2019).

En plus de ses nutriments principaux, il contient d'autres constituants en concentrations plus faibles (p. ex. vitamines, minéraux, enzymes et gaz dissous) qui participent également à ses propriétés biologiques et technologiques (Lambrini et al., 2021). L'utilisation du lait mature de divers mammifères dans l'alimentation humaine s'est accrue ces dernières années à cause de sa richesse en nutriments, de sa biodisponibilité et de ses propriétés thérapeutiques (Barreto et al., 2019). De plus, la matière grasse du lait est l'une des rares sources alimentaires d'acide butyrique, considéré comme étant un inhibiteur de la prolifération des cellules cancéreuses (Pietrzak-Fiećko et Kamelska-Sadowska, 2020). Bien que les brebis et les chèvres aient été les premiers animaux laitiers domestiqués parce qu'elles sont plus faciles à gérer, les bovins sont aujourd'hui les animaux laitiers dominants (Fox, 2003). Le lait de vache est donc de nos jours le plus produit et le plus consommé (81% de tous les laits) à travers le monde (FAO, 2018). Sa valeur nutritionnelle, sa composition chimique ainsi que ses teneurs en vitamines varient en fonction de la race, de l'âge, du régime alimentaire, du stade de lactation et des conditions environnementales (Mehra et al., 2021). Le Tableau 1.1 présente la composition moyenne en acides aminés, matières grasses, vitamines et minéraux du lait de vache.

Tableau 1.1: Composition moyenne en acides aminés, matières grasses, vitamines et minéraux du lait de vache (Landi et al., 2021; Antunes et al., 2022; Martini et al., 2023).

Acides aminés (mg)		**Acide gras (mg)**		**Vitamines (mg)**		**Minéraux (mg)**	
Histidine	170	Arachidique	80	A	155,3 UI	Calcium	121,2
Isoleucine	190	Butyrique	2230	B1	0,04	Cuivre	0,01
Leucine	410	Caproïque	1970	B2	0,18	Fer	0,03
Lysine	370	Caprylique	1400	B3	0,09	Magnésium	11,1
Méthionine	140	Caprique	3500	B6	0,04	Manganèse	0,002
Phénylalanine	220	Laurique	4060	B9	5,03 µg	Phosphore	84,4
Proline	310	Margarique	590	B12	0,47 µg	Potassium	144,2
Sérine	280	Myristique	12190	C	0,87	Sélénium	2,33
Thréonine	230	Pentadécylique	1350	D	22,8 UI	Sodium	44,1
Tyrosine	230	Oléique	20640	E	0,04	Sulfure	32
Valine	230	Stéarique	8020	K	0,26 µg	Zinc	0,42

Les valeurs sont exprimées pour 100 g de lait pour les acides aminés, vitamines et minéraux et pour 100 g de matière grasse pour les acides gras. UI: unité internationale.

1.1.2. Productions laitières au Bénin

L'économie de la plupart des pays africains au sud du Sahara, y compris le Bénin, est principalement basée sur l'agriculture et l'élevage (Benin et al., 2016). Parmi les produits d'élevage, le lait de vache joue un rôle majeur aussi bien dans l'alimentation que dans l'économie des communautés pastorales et autres, puisqu'il favorise la création d'emploi et de revenus pour la population (Dossou et al., 2006). Les Peuls (Peulhs) sont des acteurs majeurs de l'élevage bovin du pays et donc de la production laitière. Le Bénin possède un important cheptel bovin diversifié par les races locales et adaptées aux différentes conditions climatiques. Les principales races élevées sont les Somba, Lagunaire, Borgou, Zébu, M'Bororo et le Goudali. Il existe aussi la race exotique Girolando, importée du Brésil pour améliorer la production laitière des races locales (Abdoukarim et al., 2013).

Au Bénin, la production du lait de vache est passée de 118.903 tonnes en 2017, à 128.415 tonnes en 2020 (FAOSTAT, 2022). Malgré cet accroissement, les besoins de la population en lait et produits laitiers ne sont pas encore satisfaits, aussi bien qualitativement que quantitativement et le déficit est comblé par les importations de produits laitiers européens.

Du fait de sa forte périssabilité, la conservation du lait au Bénin est un véritable défi pour les acteurs, surtout compte tenu des températures ambiantes élevées que connait le pays. En outre, il existe un tabou Peulh qui interdit le retour d'un lait invendu du marché vers le campement (Dossou et al., 2006). Cela entraîne du gaspillage, car le lait non vendu est jeté. Pour pallier cette situation et réduire les pertes économiques

qui en découlent, les acteurs ont mis au point différents procédés traditionnels adaptés à leur contexte socio-économique et environnemental pour conserver le lait (Sessou et al., 2019). Ainsi, le lait est transformé en divers produits tels que le beurre, le yaourt, le lait caillé (LC) et le fromage traditionnel "*Wagashi Gassirè* " (WG). Ces deux derniers sont les plus répandus et les plus consommés par la population béninoise (Sessou et al., 2013).

1.1.3. Production du lait caillé (LC)

Le lait caillé encore appelé *Fanirè* en langue locale *Fulfuldé* (langue parlée par les Peulhs) est un lait fermenté spontanément par les microorganismes naturellement présents dans le lait entier de vache, non pasteurisé et laissé au moins un jour à température ambiante (28-32°C). C'est l'un des produits laitiers les plus populaires et les plus traditionnels des aliments fermentés, consommés dans de nombreux pays comme rafraîchissement ou dessert (Boko et al., 2016; Sessou et al., 2019). Sa production dans les zones rurales et urbaines joue un rôle important dans la sécurité nutritionnelle et économique des ménages. Toutefois, la production du LC reste domestique et artisanale, laissant agir de manière aléatoire les microorganismes intrinsèques au lait cru et ceux extrinsèques qui peuvent provenir des contaminations croisées (Katinan et al., 2012). Cette manière de faire favorise une diversité microbienne précieuse et riche, dominée par les bactéries lactiques et les levures, mais constitue aussi un facteur de risque lié à la croissance potentielle d'agents pathogènes (Agyei et al., 2020). Par ailleurs, le LC est considéré, après le lait cru, comme l'une des principales causes d'intoxications alimentaires (Ahmed et al., 2010; Gran et al., 2002).

1.1.4. Production du *Wagashi Gassirè* (WG)

Connu sous l'appellation courante de *Wagashi* au Bénin, et plus spécifiquement de *Gassirè* en langue locale *Fulfuldé*, ce fromage Peulh à pâte molle est de haute valeur nutritionnelle (Sessou et al., 2023). Il est obtenu par coagulation à chaud du lait frais entier de vache, sous l'action de la calotropaïne, une enzyme végétale de *Calotropis procera*, aussi appelé le pommier de Sodome (Dossou et al., 2006). Pour la préparation du WG, le lait filtré est soumis à un préchauffage à 60°C environ, pendant 5 minutes, avant l'ajout du coagulant, extrait par trituration des feuilles et/ou tige de *C. procera* dans du lait cru. Le mélange subit ensuite une cuisson à 100°C environ jusqu'à la formation du caillé surnagé par le lactosérum (ou petit lait). L'ensemble reste sur le feu pendant encore 3 à 5 minutes avant d'être égoutté dans des passoires. La cuisson est arrêtée lorsque le petit lait devient jaunâtre et transparent et que le caillé qui se trouvait au fond de la marmite monte à la surface où il est brisé en morceaux. Le coagulum ainsi obtenu, cuit, égoutté et moulé peut être coloré à l'extrait des panicules de sorgho (*Sorghum vulgaris*) avant d'être présenté et vendu sur le marché sous des formes et tailles différentes en fonction du prix de vente (Aïssi, 2009). La Fig. 1.1 présente le diagramme technologique de fabrication du *Wagashi Gassirè*.

Pour colorer le *Wagashi Gassirè*, les productrices utilisent les panicules de sorgho, soit à chaud (Fig. 1.2), soit à froid, selon leurs préférences. La coloration à froid se réalise en triturant les panicules de sorgho dans de l'eau, avec ou sans ajout de sel ou de potasse. Le *Wagashi Gassirè* blanc est ensuite immergé dans cette eau colorée, permettant ainsi sa

teinture (rouge). Cette méthode améliore non seulement l'attrait visuel du produit, mais contribue également à sa bonne conservation (Dossou, 2006).

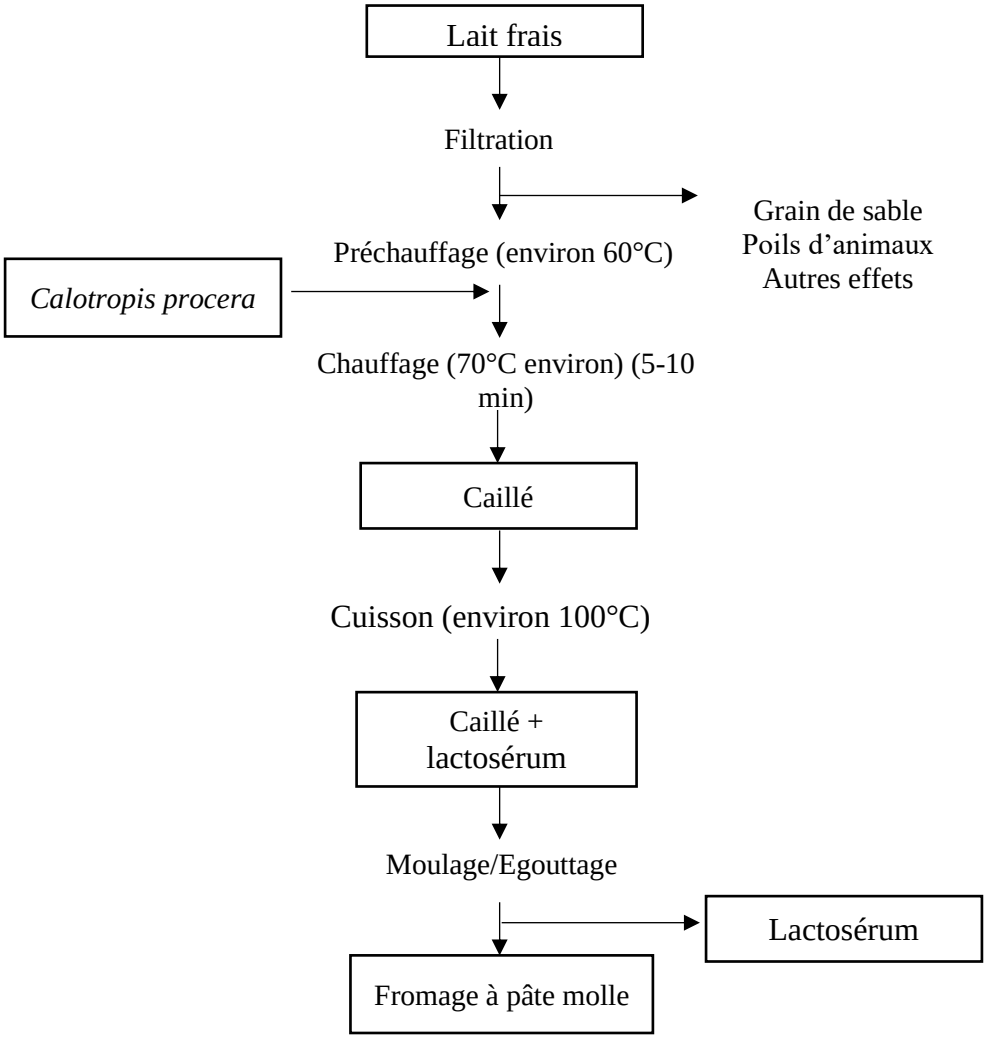

Figure 1.1: Diagramme technologique de fabrication du *Wagashi Gassirè* (Dossou et al., 2006).

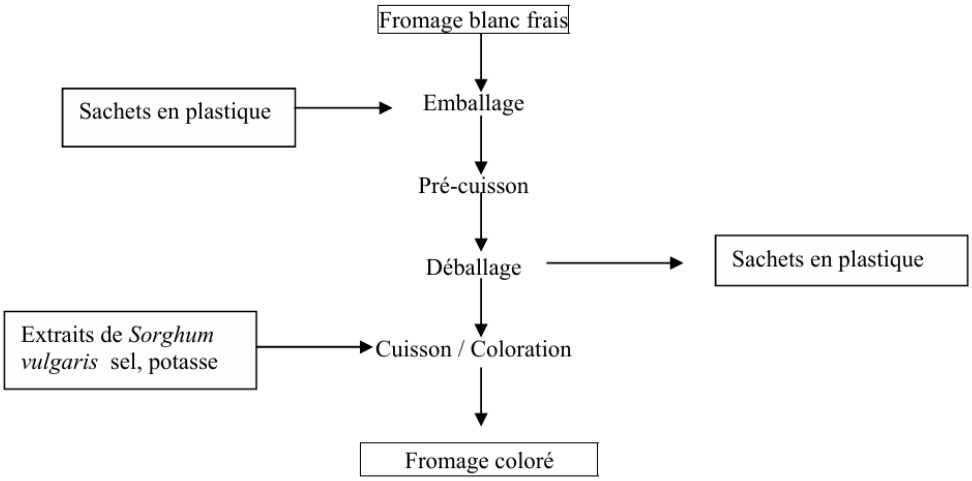

Figure 1.2: Diagramme technologique de coloration du *Wagashi Gassirè* à l'extrait de panicule de *Sorghum vulgaris*.

1.2. Microbiote du lait et des produits laitiers

1.2.1. Diversité et taxonomie des bactéries lactiques

Les bactéries lactiques (LAB) font partie des groupes importants de bactéries qui ont des effets bénéfiques sur la santé humaine, animale et végétale. Ce sont des bactéries Gram-positives présentes sous forme de bâtonnets ou de coques, catalase négative, immobiles et non sporulées, anaérobies et aérotolérantes, avec une grande tolérance aux faibles pH (Saeed et Salam, 2013). Les bactéries lactiques sont généralement associées à des habitats riches en nutriments, tels que divers produits alimentaires (lait, viande, légumes, vins ou produits céréaliers), et certaines font également partie des muqueuses humaines et animales (Khalid, 2011). Elles sont classées en deux groupes sur la base des

produits issus du glucose qu'elles fermentent: les bactéries lactiques dites « homofermentaires » donnent lieu à une fermentation homolactique tandis que les « hétérofermentaires » produisent une fermentation hétérolactique. Si les premières produisent uniquement de l'acide lactique à partir de sucres, les secondes produisent de l'acide lactique, du dioxyde de carbone, de l'alcool et parfois d'autres acides (Zuniga et al., 1993). Suivant la taxonomie actuelle, les bactéries lactiques appartiennent à l'embranchement des *Bacillota*, à la classe des *Bacilli,* à l'ordre des *Lactobacillales* et comprennent six familles: *Aerococcaceae*, *Carnobacteriacea*, *Enterococcaceae*, *Lactobacillaceae*, *Leuconostocaceae* et *Streptococcaceae* (Bintsis, 2018; Mathur et al., 2020).

1.2.2. Caractères phénotypiques et biochimiques des bactéries lactiques

Les bactéries lactiques sont classées en fonction de la morphologie cellulaire, du mode de fermentation du glucose et de la plage de température de croissance (Quinto et al., 2014). Le tableau 1.2 reprend la classification actuelle des familles et genres de bactéries lactiques, ainsi que certaines de leurs caractéristiques.

Tableau 1.2: Caractéristiques des bactéries lactiques (Vinderola et al, 2019).

Famille	Genre	Coque (C) ou bâtonnet (B)	Fermentation lactique	Croissance à 10°C	Croissance à 45°C	Croissance à 6,5% de NaCl	Croissance à 18% de NaCl	Croissance à pH 4,4	Croissance à pH 9,6	Isomère d' acide lactique
Aerococcaceae	*Aerococcus*	C	Homo	+	-	+	-	-	+	L
Carnobacteriacea	*Carnobacterium*	B	Homo et hétéro	+	-	ND	-	ND	-	L
Enterococcaceae	*Enterococcus*	C	Homo	+	+	+	-	+	+	L
	Tetragenococcus	C	Homo	+	-	+	+	±	+	L
	Vagococcus	C	Homo	+	-	-	-	ND	-	L
Lactobacillaceae	*Lactobacillus*	B	Homo et hétéro	±	±	±	-	±	-	D, L, DL
	Pediococcus	C	Homo	±	±	±	-	+	-	L, DL
Leuconostocaceae	*Leuconostoc*	C	Hétéro	+	-	±	-	±	-	D
	Fructobacillus	B	Hétéro	ND	ND	+	-	ND	ND	D
	Oenococcus	C	Hétéro	+	-	±	-	±	-	D
	Weissella	C (B)	Hétéro	+	-	±	-	±	-	D, DL
Streptococcaceae	*Lactococcus*	C	Homo	+	-	-	-	±	-	L
	Streptococcus	C	Homo	-	±	-	-	-	-	L

+: Croissance; -: pas de croissance; ND: non déterminé; ±: réponse variable selon les espèces.

1.2.3. Rôles des bactéries lactiques dans les produits laitiers

L'une des plus anciennes formes de conservation des aliments, dont les produits laitiers, est la fermentation lactique qui est liée à une baisse du pH consécutive à la production d'acide lactique (Gemechu, 2015; Liu et al., 2011). Le but de cette conservation est d'empêcher le développement des agents nuisibles, non seulement pour les produits (modifications des caractéristiques organoleptiques), mais aussi pour la santé humaine. Ainsi de nos jours, les bactéries lactiques sont largement utilisées dans le monde en raison de leur statut de microorganismes généralement reconnus comme sûrs (GRAS, *Generally Recognised As Save*) et de leurs bienfaits pour la santé. Toutefois, certaines bactéries lactiques appartenant aux genres *Enterococcus* et *Streptococcus* peuvent être considérées comme pathogènes et sont à l'origine de diverses infections animales et humaines (p. ex. endocardite, bactériémie, infection urinaire, péripneumonie, méningite ou carie dentaire) (Rossi et al., 2019).

Les bactéries associées aux produits laitiers fermentés traditionnels appartiennent aux genres *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Bacillus*, *Propionibacterium* et *Bifidobacterium* (Ghosh et al., 2019).

Au cours de la fermentation, les bactéries lactiques peuvent produire plusieurs métabolites dans les aliments, tels que les acides organiques, les bactériocines, ainsi que d'autres molécules comme l'acétaldéhyde, l'acétoïne, l'éthanol, le peroxyde d'hydrogène, le diacétyle, le dioxyde de carbone, la reutérine et la reutéricycline. Ces métabolites ont la capacité d'améliorer la valeur nutritionnelle des aliments et de

développer leurs saveurs (texture, arômes, goût) et de produire des agents antimicrobiens (Girma et Aemiro, 2021; Sieuwerts, 2018).

Les acides organiques sont généralement considérés comme sans danger pour l'humain et empêchent la croissance des bactéries Gram-négatives et Gram-positives, ainsi que des levures et des moisissures dans les aliments. Ils sont produits par diverses espèces telles que: *Lactobacillus acidophilus* (acides acétique et butyrique), *Lactobacillus delbrueckii* subsp. *bulgaricus* (acide lactique), *Lactococcus lactis* (acides formique, propionique et succinique), *Limosilactobacillus reuteri* (acide malique). Ces acides organiques inhibent la croissance d'espèces bactériennes telles que *Clostridium perfringens*, *Escherichia coli*, *Listeria* spp. *Pseudomonas* spp., ou *Salmonella* spp. (Özcelik et al., 2016).

Les bactériocines sont des peptides antimicrobiens synthétisés par des ribosomes et produits par plusieurs bactéries lactiques comme *Lc. lactis* subsp. *lactis* (nisine, lactococcine), *L. delbrueckii* subsp. *bulgaricus*, *L. reuteri*, *Lactobacillus helveticus* ou *Lactobacillus plantarum*. Elles sont généralement actives contre des bactéries apparentées à la souche productrice, mais peuvent aussi agir contre d'autres espèces: *Enterobacter aerogenes, Listeria monocytogenes, Salmonella enterica* sv. Typhimurium ou *Staphylococcus aureus* (Adeniyi et al., 2015; Negash et Tsehai, 2020).

Certaines bactériocines sont utilisées dans les industries alimentaires parce qu'elles sont inodores et incolores, ne modifient pas les caractéristiques organoleptiques des aliments et sont facilement éliminées de l'organisme par des enzymes protéolytiques (Perez et al.,

2014). Elles sont regroupées en quatre grandes classes en fonction de leur origine génétique, spectre d'activité, propriétés biochimiques, mode d'action et poids moléculaire. La première classe regroupe des peptides contenant des lantibiotiques, petits peptides thermostables capables de former des acides aminés tels que la lanthionine et la méthyllanthionine après la traduction, et comprend la nisine. La deuxième classe inclut des bactériocines non lantibiotique à faibles poids moléculaires ou à deux peptides, la troisième classe comprend des bactériocines non lantibiotique à hauts poids moléculaires et la quatrième classe contient des bactériocines complexes (Mokoena, 2017).

Concernant les autres molécules antimicrobiennes, la reutérine (3-hydroxypropionaldéhyde) synthétisée par *L. reuteri*, et le diacétyle sont souvent impliqués dans le contrôle de *Campylobacter* spp. et *E. coli* O157:H7 (Asare et al., 2020; Ibrahim et al., 2021). Quant au peroxyde d'hydrogène (H_2O_2), produit par *Lactobacillus rhamnosus*, il inhibe *in vitro* la croissance de plusieurs bactéries pathogènes comme *E. coli* O157:H7, *L. monocytogenes* ou *Salmonella enterica* (Lin et al., 2002; Ayivi et al., 2020).

Les industries agroalimentaires utilisent plusieurs souches de bactéries lactiques comme cultures *starter* (Wang et al., 2021), parmi lesquelles *L. acidophilus, L. delbrueckii* subsp. *bulgaricus, Lactobacillus casei, L. helveticus, L. plantarum, Lc. lactis*, *Lc. lactis* subsp. *cremoris*, *Leuconostoc* spp., *Streptococcus thermophilus*. *L. bulgaricus* et *S. thermophilus* interviennent dans la fabrication du yaourt, de certains fromages et laits fermentés (Rakhmanova et al., 2018). *L. helveticus*,

connu pour son rôle important dans la production de composés aromatiques spécifiques et *L. casei* sont aussi utilisés comme *starter* dans la production de certains fromages (Widyastuti et Febrisiantosa, 2014).

Outre les bactéries lactiques appartenant aux genres *Lactobacillus*, certaines espèces du genre *Bifidobacterium* (*B. lactis, B. bifidum, B. infantis, B. breve, B. animalis* et *B. adolescentis*) sont aussi couramment utilisées comme probiotiques dans les produits laitiers (Kaur et al., 2022). Une fois ingérées, ces bactéries colonisent le tractus intestinal de l'hôte et peuvent lui apporter des effets bénéfiques (Elshaghabee, 2023) comme la prévention du cancer du côlon, l'atténuation des symptômes d'intolérance au lactose, la prévention d'allergie alimentaire et un soulagement des troubles inflammatoires gastro-intestinaux chroniques (Hendler et Zhang, 2018).

1.2.4. Agents altérants des produits laitiers

Les produits laitiers peuvent aussi contenir des microorganismes capables d'entraîner la détérioration du produit. Ils peuvent provenir des manipulateurs, du pis des vaches, des équipements, de l'environnement, des aliments pour animaux ou de l'eau (Owusu-Kwarteng et al., 2020). Ces altérations peuvent causer un rancissement, des défauts sensoriels de goût, d'arômes, d'apparence ou de texture et réduire ainsi leurs temps de conservation. Les principaux genres identifiés comme flore d'altération sont: *Pseudomonas* spp., les coliformes (*Escherichia* spp. et *Enterobacter* spp.), *Bacillus* spp., *Clostridium* spp., ainsi que certaines levures et moisissures.

Les *Pseudomonas* spp. sont parmi les agents d'altération les plus courants en raison de leur capacité à se multiplier dans les produits laitiers conservés à basse température et surtout dans les fromages frais, à pâte molle (Tirloni et al., 2021). Ce sont des bactéries Gram-négatives, non sporulantes, aérobies, omniprésentes dans les matières premières, le sol, l'eau et les équipements des fermes et des laiteries (Carminati et al., 2019; Machado et al., 2015) d'où elles peuvent contaminer les produits laitiers en fin de production (Rossi et al., 2018). Elles produisent des enzymes lipolytiques et protéolytiques thermotolérantes qui rendent instables et réduisent la qualité et la durée de conservation des produits contaminés (Morandi et al., 2021).

Les *Pseudomonas* spp. montrent également une grande diversité génétique, une grande polyvalence métabolique et la capacité de former des biofilms, ce qui leur permet de survivre dans divers environnements, y compris sur les ustensiles et l'équipement utilisés dans la chaîne de production laitière (Scatamburlo et al., 2015). Ainsi, la métalloprotéase AprX, qui est l'une des enzymes produites par *Pseudomonas* spp., présente une activité spécifique de détérioration de la caséine, ce qui entraîne des modifications importantes des propriétés physico-chimiques et organoleptiques du lait cru (Arslan et al., 2011). De plus, la capacité lipolytique des *Pseudomonas* spp. dépend des lipases qui agissent principalement en conférant au lait un goût amer et un arôme déplaisant, caractéristiques des acides gras à chaîne courte entraînant le rejet catégorique du lait par l'industrie laitière (Capodifoglio et al., 2016). *Bacillus cereus* peut aussi altérer les produits laitiers en produisant des enzymes thermostables, telles que

des lipases souvent associées à une saveur rance, ou des protéases qui donnent une saveur amère au lait (Montanhini et al., 2013).

Certains coliformes (*Citrobacter braakii*, *Citrobacter freundii*, *E. aerogenes*, *E. coli*, *Hafnia alvei*, *Klebsiella aerogenes* ou *Klebsiella oxytoca*), couramment utilisées comme indicateurs des conditions d'hygiène dans la fabrication du fromage, ont été associés au gonflement de certains fromages par production des gaz (Tabla et al., 2016; Martin et al., 2021). De même, certaines bactéries lactiques hétérofermentaires non-*starter*, telles que *Lactobacillus brevis*, *Lactobacillus fermentum* ou *Paucilactobacillus wasatchensis*, peuvent se développer dans du fromage et produire des gaz (Ortakci et al., 2015).

Certaines moisissures (p. ex. appartenant aux genres *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Monilia*, *Mucor* ou *Penicillium*) et certaines levures (*Candida* spp., *Geotrichum candidum*, *Kluyveromyces marxianus*) peuvent se développer dans les produits laitiers surtout à la surface des fromages, en utilisant le lactate, et entraînent des changements indésirables comme la désacidification de surface, qui elle-même favorise la croissance de bactéries moins tolérantes à l'acide (Garnier et al., 2017; Lu et Wang, 2017).

1.2.5. Agents pathogènes bactériens associés aux produits laitiers

Les produits laitiers constituent des milieux favorables pour la croissance et le développement des bactéries pathogènes telles que: *Bacillus* spp., *Brucella* spp., *Campylobacter* spp., *E. coli* toxinogènes,

Klebsiella spp., *L. monocytogenes*, *Mycobacterium* spp., *Salmonella* spp. ou *S. aureus* (Owusu-Kwarteng et al., 2020).

B. cereus sensu lato (*s.l.*), un groupe de bactéries sporulantes, est omniprésent dans la nature, et notamment dans le sol, les fèces, la litière, l'air et les équipements de traite (Stenfors et al., 2008). Ce groupe comprend plusieurs espèces génétiquement proches dont les principales sont *B. cereus sensu stricto*, *Bacillus anthracis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus thuringiensis*, *Bacillus weihenstephanensis*, *Bacillus cytotoxicus* et *Bacillus toyonensis* (Fayad et al., 2019). Certaines souches de *B. cereus* sont des pathogènes alimentaires importants et peuvent causer deux formes distinctes de maladies: le syndrome diarrhéique, lié à des entérotoxines, et le syndrome émétique, causé par la toxine céréulide préformée dans les aliments (Dierick et al., 2005; Vidic et al., 2020).

S. aureus, un microorganisme non sporulé, parfois mobile sur des surfaces d'agar (Pollitt et al., 2016), Gram-positif, oxydase négatif, catalase et coagulase positives, anaérobie facultative qui se développe dans une large gamme de températures et de pH. Il colonise la muqueuse nasale humaine et la peau chez environ 50% de la population en bonne santé, mais il peut également provoquer des infections hématologiques (Abril et al., 2020). *S. aureus* est un microorganisme très important associé aux produits laitiers, ayant la capacité de produire des protéases, lipases et estérases. En outre, il produit des entérotoxines thermostables responsables d'intoxications alimentaires avec pour symptômes des crampes abdominales, des nausées, des vomissements et de la diarrhée (Jiang et al., 2022).

Le genre bactérien *Campylobacter* colonise le tractus intestinal de certains animaux et en particulier des oiseaux d'où il peut être excrété et contaminer l'environnement (Longenberger et al., 2013). Ainsi, *C. jejuni* se retrouve couramment dans les aliments, les fèces et l'eau. Bien qu'il ne se développe pas dans le lait, il est considéré comme l'un des principaux agents pathogènes humains à l'origine de nombreux cas de gastroentérites humaines allant d'une diarrhée aqueuse, non sanglante et non inflammatoire à une diarrhée inflammatoire sévère avec douleurs abdominales, fièvre et malaises (El-Zamkan et Hameed, 2016).

La présence d'*E. coli* dans les produits laitiers est considérée comme un indicateur fiable de la contamination fécale ainsi que de la présence éventuelle d'autres bactéries entéropathogènes et/ou toxinogènes. La plupart des souches d'*E. coli* sont commensales, mais certains pathotypes (entéropathogènes et entérotoxinogènes) sont à l'origine des diarrhées, voire de colites hémorragiques provoquées par les souches entéro-invasives et/ou entérohémorragiques, dont *E. coli* O157:H7 (Hassan et al., 2021). À ce titre, des souches d'*E. coli* toxinogènes ont été signalées dans le lait cru de différents pays africains, dont le Bénin (Farougou et al., 2012).

L. monocytogenes, bactérie Gram-positive non sporulée, anaérobie facultative en forme de bâtonnet, est à l'origine de la listériose, une zoonose se traduisant par des méningites, et chez les femmes enceintes, des accouchements prématurés ou des avortements. Le lait cru peut y être contaminé via les fèces et les équipements de traite (Gonzales-Barron et al., 2023).

Enfin, *Salmonella* spp. est une bactérie Gram-négative en forme de bâtonnet. Elles sont présentes dans le tractus gastro-intestinal des animaux, peuvent se retrouver dans l'environnement et sont à l'origine soit d'infections alimentaires (salmonelloses) ou des fièvres typhoïdes et paratyphoïdes et peuvent provenir d'une pasteurisation inadéquate ou d'une contamination post-traitement (Singh et al., 2018).

1.3. Evaluation de la qualité microbiologique des produits laitiers

1.3.1. Culture-dépendante

L'identification par l'approche « culture-dépendante » est une méthode traditionnelle fondée sur les techniques de dilution en série, mise en culture, détection, énumération et isolement des microorganismes viables (bactéries, levures ou champignons) sur des milieux de cultures complexes ou sélectifs, solides ou liquides. Le principe de cette méthode est basé sur la comparaison de critères morphologiques, phénotypiques, physiologiques et biochimiques du microorganisme à identifier. Cette méthode nécessite une main-d'œuvre importante et est assez chronophage (Porcellato et al., 2018). De plus, les bactéries « viables mais non cultivables » (VNC) ne peuvent être détectées par cette approche. En outre cette méthode manque de précision puisqu'il peut y avoir des propriétés phénotypiques similaires pour des genres et espèces non apparentés (Carraro et al., 2011). De même, pour certaines espèces, une situation de stress survenue au cours de leur manipulation peut modifier ces caractères et fausser leur identification. Une identification basée exclusivement sur le phénotype bactérien peut donc conduire à des résultats erronés.

Une approche moléculaire facilite dans ce cas les identifications. Elle permet une identification via le séquençage complet ou partiel de l'ARNr 16S et sa comparaison avec les séquences contenues dans les bases de données. Elle est désormais utilisée comme alternative ou complémentaire aux méthodes conventionnelles (Petti et al., 2005). L'ARNr 16S est universel chez les bactéries et de nombreuses séquences de ce gène sont disponibles dans des bases de données publiques. Elles sont utilisées comme références pour identifier des séquences inconnues (Clarridge, 2004).

Par ailleurs, l'approche culture-dépendante ne permet pas la description complète de la diversité bactérienne d'un échantillon car elle ne convient qu'aux organismes cultivables, or toutes les bactéries présentes dans les aliments ne peuvent pas être (facilement) cultivées (Weber et al., 2014).

1.3.2. Culture-indépendante

L'approche dite « culture-indépendante », a le potentiel de donner un large aperçu de la diversité microbienne présente dans de divers habitats tels que les sédiments, le sol, les produits alimentaires ou l'eau, que les microorganismes qui y résident soient, ou pas, cultivables (Yeung et al., 2012). Avec l'avènement des techniques de séquençage NGS (*Next-Generation Sequencing*), l'application de méthodes métagénomiques est devenue plus courante dans la recherche sur les produits alimentaires puisqu'elles permettent de déterminer de manière approfondie la composition et la diversité microbienne de ces produits, qui autrefois étaient impossibles à décrire avec les méthodes basées sur la culture (Duru et al., 2018). Cette approche ne nécessite que peu ou

pas de connaissances *a priori* sur les microorganismes et consiste à extraire l'ADN total (métagénomique) directement des échantillons, à étudier par PCR (*Polymerase Chain Reaction* ou réaction en chaîne par polymérase) la diversité des gènes d'intérêt (p. ex. ARN ribosomiques, ADN gyrases ou encore ADN polymérases) et à procéder à une identification par séquençage à haut débit (Xu, 2006; Weber et al., 2014).

L'analyse métagénomique peut être réalisée à travers deux approches: l'une appelée métagénomique sur amplicon ciblé (*barcoding* ou *metabarcoding*) basée sur le séquençage d'un ou plusieurs gènes particuliers, et l'autre appelée métagénomique *shotgun* ou « *Whole Genome Shotgun Sequencing* » qui consiste à séquencer les génomes de tous les organismes (bactéries, virus ou champignons) présents dans l'échantillon à analyser (Quijada et al., 2020). Le processus débute par une extraction d'ADN des échantillons après leur prélèvement. De manière générale, cinq étapes sont suivies pour cette extraction à savoir: l'homogénéisation mécanique, la lyse cellulaire, l'élimination des fragments cellulaires, la précipitation et la purification des acides nucléiques (Quigley et al., 2012). Une fois l'ADN extrait, l'étape suivante consiste à préparer l'ADN de manière à le rendre sous une forme compatible avec le système de séquençage à utiliser. La métagénomique basée sur des amplicons ciblés (la plus utilisée), est basée sur l'amplification par PCR de la séquence complète ou partielle des ADN ribosomiques 16S (gènes 16S rRNA) présents (Mayo et al., 2014).

Dans le cas de la description des communautés bactériennes et fongiques des aliments, les deux technologies de séquençage d'amplicon les plus utilisées sont respectivement le séquençage du gène de l'ARNr 16S pour les bactéries, et le séquençage des ITS (*Internal Transcribed Spacer*) pour les champignons (Caporaso et al., 2011; Schoch et al., 2012). Pour l'étape de séquençage NGS, plusieurs plateformes sont utilisées comme le pyroséquençage Roche 454, SOLiD, Ion Torrent PGM (*Personal Genome Machine*), Illumina (MiSeq, MiniSeq, NextSeq, HiSeq et NovaSeq), PacBio (*Pacific Biosciences*), BGISeq et Nanopore (MinION, GridION et PromethION), toutes avec un protocole spécifique à chacune d'elles (Cardinali et al., 2017). La plateforme Illumina est la plus utilisée dans les études sur la description de la diversité microbienne des produits laitiers.

Une fois le séquençage terminé, des programmes bio-informatiques sont utilisés pour assembler les petits fragments d'ADN issus des données brutes par chevauchement des portions homologues de manière à former de plus grands et ceci après plusieurs étapes de filtrage et de nettoyage de ces séquences. Le traitement des séquences ainsi fait permet d'éliminer les séquences de qualité faible, d'améliorer la précision et d'éviter la surestimation des taxons de l'échantillon (LeMay-Nedjelski et al., 2018). A ce stade, plusieurs pipelines tels que CLARK (Ounit et al., 2015), Greengenes (rRNA sequences - Bokulich et Mills, 2013), QIIME (Hong et al., 2019), MG-RAST (Meyer et al., 2008), mOTU (Sunagawa et al., 2013), NCBI (Quigley et al., 2012), RDP (rRNA sequences - Lusk et al., 2012), SEED (Bhatt et al., 2012) et SILVA (rRNA sequences - Alegría et al., 2012) sont utilisés pour

effectuer un contrôle de qualité des séquences générées par la bio-informatique afin de déterminer la composition taxonomique des microorganismes présents dans un échantillon jusqu'au niveau du genre et, le cas échéant, de l'espèce (Allard et al., 2015; Mosher et al., 2014). Les données de séquençage métagénomique peuvent non seulement offrir une identification taxonomique détaillée du microbiome, mais également comparer l'abondance relative de tous les organismes du microbiome en même temps (Apaliya et al., 2022). Cependant, l'un des inconvénients de l'approche culture-indépendante est qu'elle détecte également l'ADN des cellules mortes qui persiste dans les échantillons même après plusieurs semaines (Ercolini, 2013).

Les produits laitiers hébergent des communautés microbiennes diversifiées, complexes et insuffisamment caractérisées. Ces dernières années, la technique de culture-indépendante est de plus en plus utilisée pour décrire la diversité microbienne des produits laitiers traditionnels. Un aperçu de quelques travaux effectués sur ces produits en Afrique et en Europe avec les techniques de séquençage métagénomique utilisées et les principaux genres détectés est présenté au niveau du Tableau 1.3.

Tableau 1.3: Diversité microbienne des produits laitiers africains.

Produits laitiers	Régions ADN cibles	Plateforme de séquençage	Base de traitement des données *	Principaux genres dominants	Références
Lait cru de vache (Afrique du sud)	ARNr 16S	PacBio	SILVA	***Acinetobacter***, *Enterococcus*, ***Lactococcus***, *Leuconostoc*, *Mycobacterium*, *Pseudomonas*, ***Streptococcus***	Khasapane et al., 2023
***Nunu*: lait de vache fermenté (Ghana)**	ARNr 16S	Illumina MiSeq	SILVA	***Acinetobacter***, *Enterococcus*, ***Escherichia***, *Klebsiella*, ***Lactobacillus***, ***Lactococcus***, *Leuconostoc*, *Macrococcus*, ***Streptococcus,*** *Weissella*	Walsh et al., 2017
***Dhanaan*: lait de chamelle fermenté traditionnel (Ethiopie)**	ARNr 16S (V3-V4)	Ion Torrent PGM	Greengenes	***Acinetobacter***, *Clostridium*, *Enterobacter*, ***Escherichia***, *Klebsiella*, ***Lactococcus***, *Salmonella*, ***Streptococcus,*** *Weissella*	Berhe et al., 2019

Lait cru de vache (Afrique du sud)	ARNr 16S (V3-V4)	Illumina MiSeq	SILVA	***Acinetobacter***, *Bacteroides*, *Brucella*, ***Escherichia,*** *Fusobacterium*, *Pseudomonas*, *Rhodococcus*	Mtshali et al., 2022
Lait de vache fermenté (Bénin, Niger)	ARNr 16S (V4-V5)	Illumina MiSeq	QIIME MG-RAST	*Enterobacter*, ***Lactobacillus***, ***Lactococcus***, *Macrococcus*	Sessou et al., 2023
***Wagashi*: fromage traditionnel à base de lait de vache (Bénin)**	ARNr 16S (V1-V2)	Illumina MiSeq	PEAR	***Acinetobacter***, *Aeromonas*, *Bacillus*, *Enterobacter*, ***Escherichia, Lactobacillus***, *Leuconostoc*, ***Streptococcus***	
***Nono*: lait fermenté (Nigéria)**	ARNr 16S (V3-V4)	Illumina MiSeq	RDP	*Acetobacter*, ***Acinetobacter***, *Bacillus*, *Cronobacter*, *Enterobacter*, *Enterococcus*, ***Escherichia***, *Flavobacterium*, *Klebsiella*, ***Lactobacillus***, ***Lactococcus***, *Pediococcus*, *Pseudomonas*, *Shigella*, ***Streptococcus***, *Weissella*	Fagbemigun et al., 2021

Yaourt et fromage (Afrique du sud)	ARNr 16S (V3-V4)	Illumina MiSeq	RDP SILVA	***Lactobacillus***, *Leuconostoc*, ***Escherichia***, *Pediococcus*, *Staphylococcus*, ***Streptococcus***	Liang et al., 2021
Lait de vache fermenté (Bénin)	ITS	Illumina MiSeq	QIIME	*Kluyveromyces*, *Saccharomyces*, *Candida*, *Sagenomella*	Sessou et al., 2019
Wagashi	ITS	Illumina MiSeq	QIIME	*Kluyveromyces*, *Sagenomella*, *Candida*, *Saccharomyces*	
Halloumi: fromage traditionnel à base de lait de vache (Chypre)	ARNr 16S (V3-V4)	Illumina MiSeq	GreenGenes	***Lactobacillus, Leuconostoc***, *Halomonas*, *Marinilactibacillus*, *Pediococcus*	Kamilari et al., 2020
Plaisentif: fromage traditionnel à base de lait de vache (Italie)	ARNr 16S (V3-V4)	NEXTflex 16S V4 Amplicon-Seq	QIIME MG-RAST	***Lactococcus, Lactobacillus, Streptococcus***	Dalmasso et al., 2016
Fromage traditionnel de la Belgique	ARNr 16S (V1–V3)	Illumina MiSeq	SILVA	***Lactococcus, Streptococcus***	Gérard et al., 2021
Lait cru et pasteurisé de vache (Norvège)	ARNr 16S (V3-V4)	Illumina MiSeq	QIIME	*Acinetobacter*, *Bacillus*, *Chryseobacterium*, ***Lactococcus***, *Pseudomonas*, ***Streptococcus***,	Porcellato et al., 2018

Cheddar: fromage au lait de vache cru et pasteurisé (Irlande)	ARNr 16S (V3-V4)	Illumina MiSeq	QIIME	***Lactobacillus, Lactococcus,*** *Psychrobacter*, *Sphingomonas,* ***Staphylococcus, Streptococcus***	Kamilari et al., 2022
Cheddar: Fromage au lait de vache cru et pasteurisé (Irlande)	ITS1	Illumina MiSeq	QIIME	*Candida*, *Debaryomyces*, *Penicillium*	Kamilari et al., 2022

*MG-RAST: Metagenomics Rapid Annotation using Subsystem Technology; PEAR: Paired-End reAd mergeR; PGM: Personal Genome Machine; QIIME: Quantitative Insights Into Microbial Ecology; RDP: Ribosomal Database Project; SILVA: Sequence Information Library Versions Alignment.

1.3.3. Réglementation et législation des produits laitiers

Les denrées alimentaires insalubres représentent une menace pour la santé du consommateur et pour l'économie des pays car elles font parties des principales causes d'épidémies et pourraient perturber les échanges régionaux et internationaux. Selon la FAO (2021), environ 600 millions de cas de maladies d'origine alimentaire sont dénombrées par an. Aussi, dans plusieurs pays, les systèmes de surveillance rapportent que les épidémies liées à la consommation du lait et des produits laitiers représentent 2 à 6 % des épidémies bactériennes d'origine alimentaire (Saad et al., 2017). Pour cela, la sécurité des produits laitiers constitue un énorme sujet de préoccupation (Montgomery et al., 2020). Dans le but de protéger la santé des consommateurs, plusieurs organismes internationaux ont établi des critères pour réglementer les différentes transactions qui s'effectuent dans le secteur alimentaire. Il s'agit, entre autres, de l'autorité européenne de sécurité des aliments (EFSA), la Commission du *Codex Alimentarius* (CAC), la Commission Européenne (CE), le Comité international de microbiologie et d'hygiène alimentaires (ICFMH), l'Organisation internationale de normalisation (ISO), l'Organisation mondiale de la Santé (OMS), ou encore de l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO).

Au Bénin, l'arrêté 0122/MAEP/D-CAB/SGM/DRH/DP/DE/SA (FAO, 2009) portant sur l'hygiène des denrées alimentaires établit les règles générales à l'intention des exploitants du secteur, avec pour principes la garantie de la sécurité sanitaire alimentaire à toutes les étapes de la chaine alimentaire depuis la production primaire, la responsabilité

première de l'exploitant du secteur alimentaire en matière de sécurité sanitaire alimentaire et l'interdiction d'exposer les denrées alimentaires à température ambiante. Il existe aussi des exigences réglementaires relatives à la sécurité des denrées alimentaires notamment des produits d'origine animale, produits laitiers y compris, destinés à la consommation humaine qui s'appuient sur l'arrêté 074/MAEP/D-CAB/SGM/DRH/DP/DE/SA (FAO, 2009). Ce dernier fixe les règles spécifiques autour des contrôles officiels (inspection des exploitations de production de lait; contrôle du lait cru lors de sa collecte) se rapportant à des établissements des mesures en cas de manquement et des procédures relatives aux importations. Cependant, ces deux arrêtés ne s'appliquent qu'aux produits importés et pas aux petits commerces locaux. Dans la plupart des cas, au plan national, les exigences du secteur alimentaire s'appuient sur les critères européens notamment ceux du règlement CE 2073/2005 (UE, 2005) modifié par le règlement CE 1441/2007 de décembre 2007 (UE, 2007) qui fixe des critères microbiologiques pour les denrées alimentaires et des règles d'hygiène pour les analyses des denrées alimentaires et les interprétations des résultats. Le Tableau 1.4 reprend les exigences de la CE en matière de critères microbiologiques applicables aux produits laitiers.

Tableau 1.4: Critères microbiologiques des produits laitiers provenant du lait de vache (règlement CE 2073/2005).

Produits laitiers	Microorganismes	Limite	n	c	m (ufc/mL)	M (ufc/mL ou /g)
Lait cru destiné à la consommation humaine	*Salmonella* spp.	Absence dans 25 mL	5	0	-	-
	S. aureus	-	5	2	10^2	5 x 10^2
Lait cru destiné à la transformation en produits crus	Germes totaux à 30°C	10^5 ufc/mL				
	S. aureus	-	5	2	5 x 10^2	2 x 10^3
Lait pasteurisé destiné à la consommation	*Salmonella* spp.	Absence dans 25 mL	5	0	-	-
	Enterobacteriaceae	-	5	2	1	5
	B. cereus	-	5	2	10^3	10^4
	Coliformes	-	5	1	0	5
	Germes totaux	-	5	1	5 x 10^4	5 x 10^5
Lait fermenté	*Enterobacteriaceae*	-	5	0	10	10

	L. monocytogenes (dénombrement)	-	5	0	10^2	10^2
	S. aureus	-	5	2	10^2	10^3
Fromages au lait pasteurisé	*Enterobacteriaceae*	-	5	2	10^4	10^5
	E. coli	-	5	2	10^2	10^3
Fromages au lait pasteurisé à pâte molle	*Enterobacteriaceae*	-	5	2	10^4	10^5
	L. monocytogenes (recherche)	Absence dans 25 g	5	0	-	-
	L. monocytogenes (dénombrement)	-	5	0	10^2	5 x 10^2
	Salmonella spp.	Absence dans 25 g	5	0		
	S. aureus	-	5	2	10^2	10^3
	E. coli	-	5	2	10^2	10^3
	Coliformes	-	5	2	10^4	10^5
Fromages au lait pasteurisé à pâte semi-dure	*Enterobacteriaceae*	-	5	2	10^4	10^5
	E. coli	-	5	2	10^2	10^3
	L. monocytogenes (dénombrement)	-	5	0	10^2	10^2
	Salmonella spp.	Absence dans 25 g	5	0	-	-

	S. aureus	-	5	2	10^2	10^3
Fromage au lait cru à pâte semi-dure	*Campylobacter* spp.	-	5	0	10	10
	L. monocytogenes (dénombrement)	-	5	0	10^2	10^2
	S. aureus	-	5	2	10^4	10^5
	Salmonella spp.	Absence dans 25 g	5	0		
	E. coli	-	5	2	10^4	10^5
Fromage au lait cru à pâte molle	*Campylobacter* spp.	-	5	0	10	10
	L. monocytogenes (dénombrement)	-	5	0	10^2	10^2
	S. aureus	-	5	2	10^4	10^5
	Salmonella spp.	Absence dans 25 g	5	0	-	-
	E. coli	-	5	2	10^4	10^5

n = nombre d'échantillons prélevés, c = nombre maximal d'échantillons acceptables dont les valeurs sont comprises entre m et M. Si plus de c échantillons dépassent m mais sont inférieurs ou égaux à M, le lot est rejeté, m = valeur seuil qui représente le niveau microbiologique acceptable pour un critère donné. Les résultats en dessous de cette valeur sont considérés comme satisfaisants, M = valeur maximale acceptable. Les résultats au-dessus de cette valeur sont considérés comme inacceptables et indicatifs d'un problème de sécurité alimentaire ou de qualité.

1.3.4. Méthode de prévention

La conservation des aliments est essentielle à la survie humaine car elle permet d'assurer une disponibilité des denrées alimentaires à tout moment et nécessite l'utilisation des techniques pouvant limiter leur détérioration à travers la croissance des microbes (altérants et pathogènes) (Amit et al., 2017). En effet, la FAO (2011) estime la perte des aliments jusqu'à un tiers de la production mondiale annuelle ce qui représente environ 1,3 milliards de tonnes par an. Les produits laitiers ne sont pas épargnés par cette perte du fait de leur richesse en de nombreux éléments nutritifs qui font d'eux un excellent milieu de croissance pour tous les microorganismes. Ainsi, diverses techniques de conservation sont utilisées pour minimiser la croissance des microorganismes responsables de la détérioration et dans le même temps maintenir les propriétés nutritionnelles desdits produits tout au long de la conservation. Il s'agit des méthodes physiques (p. ex. thermisation, pasteurisation, stérilisation, déshydratation, réfrigération, congélation, utilisation d'atmosphères contrôlées ou modifiées, l'évaporation), chimiques (p. ex. acide propionique, huile essentielle, lysozyme, natamycine, nitrate ou sorbate) et biologiques encore appelées biopréservation. Le choix de l'une de ces méthodes de conservation dépend de la durée de conservation souhaitée, de l'utilisation prévue pour le produit et surtout des ressources disponibles (Sarkar, 2015).

Les méthodes basées sur les traitements thermiques visent la réduction partielle ou totale de la charge microbienne des produits laitiers tout en altérant le moins possible leurs propriétés nutritionnelles et

organoleptiques (Lado et Yousef, 2002). Leur efficacité est liée aux bonnes combinaisons température-temps et à la méthode de chauffage (Sakkas et al., 2014). Ainsi, la pasteurisation du lait permet de réduire les populations microbiennes d'altération et d'agents pathogènes non sporulés, et d'inactiver certaines enzymes, tout en minimisant les dommages causés par la chaleur aux composants du lait. Dans la pasteurisation basse température/longue durée, le lait est chauffé à 63°C/30 min, alors que lors dans la pasteurisation à haute température/courte durée le lait est chauffé à 72°C/15 secondes. Le procédé UHT (Ultra Haute Température, au moins 135°C pendant quelques secondes), utilisé pour le lait et les boissons lactées, permet la destruction totale des microorganismes à l'exception des spores hautement résistantes à la chaleur de quelques rares espèces (p. ex. *Bacillus sporothermodurans*) et doit être suivie d'un conditionnement aseptique du produit traité (Garnier et al., 2017).

D'autres méthodes physiques de conservation des produits laitiers consistent à utiliser des emballages, souvent pour les fromages, dans le but de les protéger et de maintenir leur qualité et sécurité durant la conservation (Costa et al., 2016). Il existe les emballages sous vide et sous atmosphère modifiée. L'emballage sous vide consiste à éliminer l'air autour des aliments, en utilisant un matériau d'emballage peu perméable à l'oxygène et aux autres gaz. Cette méthode permet d'inhiber la croissance des bactéries aérobies, des moisissures et des levures et de réduire les dommages oxydatifs (Miloradovic et al., 2018). La technique utilisant les emballages sous atmosphère modifiée quant à elle consiste à créer autour de la denrée une atmosphère ayant une composition différente de celle de l'air et capable de fournir une

condition optimale pour préserver la qualité et les propriétés sensorielles des denrées pendant leur durée de conservation (Garabal et al., 2010). Les mélanges gazeux utilisés pour le fromage sont le dioxyde de carbone (CO_2), l'azote moléculaire (N_2) et l'oxygène (O_2) sous forme résiduelle. Le CO_2 est le gaz le plus important du point de vue microbiologique car il empêche la croissance de nombreux microorganismes altérants, des bactéries aérobies Gram-négatives, des moisissures et, dans une moindre mesure, des bactéries Gram-positives et des levures (Hotchkiss et al., 2006).

Quant aux méthodes chimiques, l'ajout direct d'additifs aux aliments est l'une des techniques de conservation les plus simples et les plus anciennes utilisées pour prolonger leur durée de conservation. L'acide sorbique (sorbate) fait partie des additifs autorisés par l'Union européenne, mais uniquement dans les fromages préemballés et à des concentrations précises. Il est souvent ajouté aux fromages comme conservateur antimicrobien, en raison de sa capacité à inhiber la croissance des levures et des moisissures (Mazdeh et al., 2017). L'utilisation de l'acide propionique comme conservateur pourrait jouer un rôle important dans l'inhibition de la croissance d'agents pathogènes tels que *L. monocytogenes* pendant l'affinage du fromage (Wemmenhove et al., 2016). Le lysozyme, une enzyme présente en abondance dans le blanc d'œuf dont il est souvent extrait est utilisé pour conserver des fromages en raison de son origine naturelle et de son action bactéricide contre les bactéries Gram-positives, telles que les bactéries lactiques et les clostridies, et dans une moindre mesure contre les bactéries Gram-négatives (Nájera et al., 2021). La natamycine est un additif alimentaire antifongique produit par *Streptococcus natalensis*

et d'autres espèces similaires. Elle s'est montrée très efficace dans le traitement de surface des fromages en agissant contre la croissance des moisissures et des levures (Jalilzadeh et al., 2015).

Actuellement au Bénin, les méthodes de conservation du *Wagashi Gassirè* (WG) sont: le séchage solaire, la conservation dans le lactosérum, dans l'eau simple ou dans l'eau colorée aux panicules de sorgho (*Sorghum vulgaris*), la cuisson journalière dans des plastiques blancs ou noirs de polyéthylène, la friture et le fumage. La conservation dans du lactosérum, dans de l'eau colorée et la cuisson journalière sont les méthodes de conservation les plus pratiquées par des productrices et revendeuses de WG tandis que le fumage, la friture, la réfrigération sont pratiqués par les consommateurs (Sessou et al., 2013).

Cependant ces méthodes empiriques présentent des limites aussi bien qualitatives pour le produit que sécuritaires pour l'homme. En effet, Dossou et al. (2016) ont signalé que les traitements thermiques (cuisson journalière) répétés auxquels le WG est soumis participent à une perte de ses macronutriments (sucres, lipides, protéines), de ses micronutriments tels que les minéraux et à la réduction de sa charge microbienne sans pour autant le rendre conforme aux normes de qualité microbiologique du fromage. Aussi, des travaux de recherche se sont intéressés à la conservation des *Wagashi Gassirè* (WG) par ajout d'additifs chimiques. En effet, Aworh et Egounléty (1985) et Kees (1996) ont montré que les additifs chimiques tels que l'acide propionique et les sorbates ont un impact négatif sur la qualité organoleptique du WG, quand bien même ils allongeaient sa durée de conservation. Sacramento (2008) a testé la conservation du WG par

l'effet combiné du séchage et de l'emballage sous vide et a conclu que la qualité nutritionnelle et sensorielle des WG a été préservée après séchage et que le vide permettait de conserver les WG pendant deux mois. Par ailleurs, il a noté que les dégustateurs avaient mentionné un goût légèrement amer au niveau de certains WG conservés sous vide. D'autres études conduites par Keke et al. (2009) avec la même vision de prolongation de la durée de conservation du produit frais par adjonction d'extraits purifiés de *Sorghum vulgaris* et d'huile essentielle de *Pimenta racemosa* ont montré que l'action combinée des c-hétérosides, des flavonols, des leucoanthocyanes, des stérols et des triterpènes contenus dans les extraits de *S. vulgaris* d'une part et de celles des composés aromatiques et des monoterpènes oxygénés et hydrogénés composant l'huile essentielle d'autre part, ont contribué à éliminer la flore résiduelle contenue dans le fromage frais après l'adjonction des extraits. Ces différentes méthodes menées dans des conditions de laboratoire ne sont, dans l'ensemble, malheureusement, pas transférables aux productrices et transformatrices compte tenu de leur condition de vie.

En ce qui concerne la biopréservation, c'est une méthode de prolongation de la durée de conservation et de la sécurité des aliments par l'utilisation du microbiote naturel et/ou de composés antimicrobiens (Reis et al., 2012). En effet, elle consiste à utiliser des microorganismes sûrs, notamment les bactéries lactiques, dans les aliments pour améliorer la durée de conservation à travers le contrôle des pathogènes (Voir partie 1.2.3.). Les bactéries lactiques sont considérées comme d'excellents bioconservateurs, car elles produisent des bactériocines tels que la pédiocine, la lacticine, l'entérocine, l'endolysine et la nisine

qui sont utilisées pour la conservation des produits laitiers (Ameer et al., 2019).

En Afrique, les méthodes de transformation du lait, bien que traditionnelles, comprennent des opérations (fermentations, traitements thermiques) susceptibles d'améliorer la sécurité des produits laitiers si elles sont rigoureusement appliquées (Owusu-Kwarteng et al., 2020). Selon Jans et al. (2017), diverses mesures de précaution comme les bonnes pratiques d'hygiène et de fabrication doivent en effet être mises en place en évitant la contamination initiale (lait cru), en réduisant les microorganismes pathogènes (transformation et traitement thermique), en évitant la recontamination (transformation et stockage) et en empêchant la croissance des microorganismes (transport et stockage). Les méthodes de conservation relatives aux produits laitiers, surtout dans les pays en développement, doivent aussi être adaptées au contexte de production traditionnelle et doivent être conçues de manière à être facilement accessibles aux acteurs du secteur qui, pour la plupart, sont non scolarisés (Komagbe et al., 2023).

Chapitre 2

2. Objectifs

Le lait caillé (LC) et le fromage traditionnel *Wagashi Gassirè* (WG), très appréciés et consommés au Bénin, constituent des sources de protéines et nutriments essentiels pour les populations à faibles revenus. Toutefois, leurs productions et conservations demeurent artisanales et n'assurent pas toujours la qualité sanitaire et nutritionnelle espérée par les consommateurs. En effet, outre les microorganismes d'importances technologiques impliqués dans les processus de fermentation (bactéries lactiques), ces produits laitiers peuvent aussi contenir des microorganismes d'altérations et/ou pathogènes.

S'agissant des germes d'altération, les plus importants appartiennent aux genres *Bacillus*, *Clostridium*, *Micrococcus* et *Pseudomonas*, dont certaines espèces sont capables de survivre lors de la pasteurisation. Les levures, moisissures et certaines bactéries lactiques peuvent également être associées aux altérations, mais généralement dans une moindre mesure. Parmi les pathogènes, les plus importants sont *B. cereus, Brucella* spp., *Campylobacter jejuni*, *Clostridium botulinum*, *C. perfringens*, *L. monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella* spp., *S. aureus* et les pathotypes de *E. coli*. Ces microorganismes sont couramment rencontrés dans l'eau de rinçage du matériel de traite et de transformation, les aliments et les déjections d'animaux, le sol, la litière, et peuvent causer des toxi-infections alimentaires. Dans de telles conditions, la consommation du LC et du

WG représente un risque pour la santé humaine. Il est donc nécessaire de revoir les techniques de production du LC et du WG actuelles avec beaucoup plus de rigueur et situer les responsabilités pour une meilleure qualité hygiénique desdits produits.

Pour y parvenir, la connaissance de l'écologie microbienne de ces produits laitiers s'avère indispensable. C'est dans cette optique que s'inscrit la présente thèse, dont l'objectif principal est d'évaluer la qualité microbiologique du lait cru, du LC et du WG et, le cas échéant, de les améliorer.

Plus spécifiquement, le travail consistera en une étude préliminaire comparative des communautés bactériennes des fromages WG colorés ou non, prélevés dans les villes de Cotonou et Abomey-Calavi, à travers une approche métagénomique. Ensuite, une synthèse sur les pratiques d'hygiène à travers une enquête auprès des acteurs de la filière lait des communes de Dassa-Zoumé et Nikki sera documentée afin d'identifier les voies de contamination des produits. Les caractéristiques microbiologiques des laits crus, LC et WG échantillonnés dans les campements Peulhs et marchés des communes de Dassa-Zoumé et Nikki seront évaluées à travers des approches de cultures dépendantes et indépendantes. Certaines espèces de bactéries lactiques isolées des LC seront identifiées et leurs propriétés techno-fonctionnelles analysées en vue d'une utilisation pour des essais de productions des LC améliorés. Pour finir, les produits améliorés seront aussi caractérisés.

En somme, ce travail a consisté à l'utilisation des approches métagénomique et conventionnelle pour comparer les communautés bactériennes des laits caillés et des fromages WG, colorés ou non, de différentes régions en fonction du temps, ainsi qu'à l'évaluation des pratiques d'hygiène des acteurs de la filière lait au Bénin. De plus, ce travail propose d'identifier et d'analyser les propriétés techno-fonctionnelles des bactéries lactiques isolées afin de développer des méthodes de production améliorées pour le LC et le WG, améliorant ainsi leur qualité hygiénique et nutritionnelle.

II. RÉSULTATS

Chapitre 3

3. Bacterial community of *Wagashi Gassirè* sold in southern Benin

In this chapter, a preliminary study was conducted as a prelude to the thesis work, and aimed at characterizing the *Wagashi Gassirè* samples collected in markets from Cotonou and Abomey-Calavi (Southern Benin). The culture-independent approach was used, allowing for the observation of the bacterial diversity in these products. This study includes a similar work on curdled milk from Africa and India.

Personal contribution:

I contributed to the research design, collected the cheese samples, performed DNA extraction, and conducted metagenomic analyses. I was also involved in writing the manuscript.

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Chapitre 3

Comparative analyses of the bacterial communities present in the spontaneously fermented milk products of Northeastern India and West Africa

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# Equally contributed to the work.

Abstract

Spontaneous fermentation of raw cow milk without backslopping is in practice worldwide as part of the traditional food culture, including *Doi* preparation in earthen pots in Northeast India, *Kindouri* of Niger and *Fanirè* of Benin prepared in calabash vessels in West Africa. Very few reports are available about the differences in bacterial communities that evolved during the spontaneous mesophilic fermentation of cow milk in diverse geographical regions. In this study, we used high throughput amplicon sequencing of bacterial 16S rRNA gene to investigate 45 samples of naturally fermented homemade milk products and compared the bacterial community structure of these foods, which are widely consumed in Northeast India and Western Africa. The spontaneous milk fermentation shared the lactic acid bacteria, mainly belonging to *Lactobacillaceae* (*Lactobacillus*) and *Streptococcaceae* (*Lactococcus*) in these two geographically isolated regions. Indian samples showed a high bacterial diversity with the predominance of *Acetobacteraceae* (*Gluconobacter* and *Acetobacter*) and *Leuconostoc*, whereas *Staphylococcaceae* (*Macrococcus)* was abundant in the West African samples. However, the *Wagashi* cheese of Benin, prepared by curdling the milk with proteolytic leaf extract of *Calotropis procera* followed by natural fermentation, contained *Streptococcaceae* (*Streptococcus* spp.) as the dominant bacteria. Our analysis also detected several potential pathogens, like *Streptococcus infantarius* an emerging infectious foodborne pathogen in *Wagashi* samples, an uncultured bacterium of *Enterobacteriaceae* in *Kindouri* and *Fanirè* samples, and *Clostridium* spp. in the *Doi* samples of Northeast India. These findings will allow us to develop strategies to address the safety issues related to spontaneous

milk fermentation and implement technological interventions for controlled milk fermentation by designing starter culture consortiums for the sustainable production of uniform quality products with desirable functional and organoleptic properties.

Keywords: Spontaneously fermented milk products, MiSeq amplicon sequencing, *Lactobacillus*, *Lactococcus*, *Gluconobacter*, *Acetobacter*, *Macrococcus caseolyticus*, *Streptococcus infantarius*

1. Introduction

Fermented milk products are an essential component of the traditional food cultures of different ethnic communities worldwide. These fermented milk products are prepared from the raw or boiled milk of cow, buffalo, yak, camel, goat, and sheep through backslopping or spontaneous fermentation. *Kefir* of Russia, *Koumiss* and *Tarang* of Mongolia and China, *Dahi* of India, *Suero Costeno* of Colombia, and *Lait caillé* of sub-Saharan countries are a few examples of well-known traditional fermented milk products (de Melo Pereira et al., 2022). *Dahi* is an analogue of yogurt, a semi-solid ready-to-drink Indian food generally prepared from boiled milk by backslopping a part of the previous batch of successful fermentation as a starter (Dewan and Tamang, 2007; Rai et al., 2016; Mudgal and Prajapati, 2017; Mallappa et al., 2021). Unlike thermophilic yogurt making, *Dahi* is usually

incubated at room temperature (15-30°C) for 1-3 days in mesophilic fermentation (Mudgal and Prajapati, 2017; Mallappa et al., 2021; de Melo Pereira et al., 2022). The Mongoloid ethnic communities in Northeast India mostly prefer the spontaneous fermentation of fresh cow milk in earthen wares without backslopping (Joishy et al., 2019). The local vernacular names of these products are *Doi*, *Dei*, *Dahi*, and *Mishti dahi* in Assam, Tripura, and Meghalaya, or *Sangom afamba* in Manipur (Joishy et al., 2019; Barooah et al., 2020; Wahengbam et al., 2020; Mallappa et al., 2021). Similarly, Fulani communities in Western African countries practice spontaneous fermentation of unpasteurised raw milk in calabash vessels without backslopping, such as *Kindirmou* in Niger and *Fanirè* in Benin (Agyei et al., 2020; Fagbemigun et al., 2021). Backslopping naturally selects well-adapted microbes, resulting in consistent-quality products, whereas spontaneous fermentation results in highly heterogeneous products. Moreover, the microbes present in raw milk of animal origin also influence fermentation, often resulting in poor-quality products (Sun and D'Amico, 2021).

Recent next-generation sequencing (NGS)-based cultivation-independent studies showed a ubiquitous presence of beneficial bacteria, mainly *Lactobacillaceae* (*Lactobacillus delbrueckii*, *Lactobacillus kefiranofaciens*, *Lactobacillus helveticus*, and *Leuconostoc mesenteroides*) and *Streptococcaceae* (*Streptococcus thermophilus and Lactococcus lactis*) in most of the naturally fermented milk products worldwide (Jayashree et al., 2013; Oki et al., 2014; Bokulich et al., 2015; Motato et al., 2017; Shangpliang et al., 2018; Mallappa et al., 2021; de Melo Pereira et al., 2022). The NGS-based studies also highlighted the dominance of acetic acid bacteria,

Acetobacteraceae (mainly *Gluconobacter* and *Acetobacter*), in several spontaneous fermented milk products (Shangpliang et al., 2018; de Melo Pereira et al., 2022). Among yeast, *Kluyveromyces marxianus*, *Geotrichum candidum*, and *Saccharomyces cerevisiae* are recorded across naturally fermented milk products (Bokulich et al., 2015; Sessou et al., 2019; Von Gastrow et al., 2020; Tenorio-Salgado et al., 2021). The substrate-specific adaptive evolution during backslopping of boiled milk and mesophilic fermentation shapes a particular group of bacterial communities, resulting in uniform quality products, whereas spontaneous fermentation of raw milk results in high variability in taste, flavor, and texture due to variations in geography, milk source, local climatic conditions, quality, and composition of milk (Zhong et al., 2016; Peng et al., 2021; Zhang et al., 2021; Zhao et al., 2021; Tamang, 2022). At the same time, such spontaneous fermentation also results in products displaying unique tastes and aromas, such as *Khoormog* and *Airag* of Mongolia and China (Oki et al., 2014; de Melo Pereira et al., 2022), *Churpii* of India (Shangpliang et al., 2018), and *Wagashi* cheese of Benin (Sessou et al., 2019), which deserve geographical indicator tagging. Therefore, there is a need to study the bacterial community structure and safety of these products by culture-independent NGS analysis to understand the presence/absence of beneficial microbes and unwanted potential pathogens (Tamang et al., 2021; de Melo Pereira et al., 2022; Rai and Tamang, 2022). This understanding will allow us to design starter culture consortia for sustainable industrial production of quality and safe fermented milk products with health benefits (Agyei et al., 2020).

In this study, we aimed to understand how spontaneous fermentation of raw cow milk without backslopping shapes the bacterial diversity in two geographically separated regions of two continents by analyzing homemade milk products, namely, *Doi*, *Sangom afamba*, and *Mishti dahi* of Northeast India; and *Kindirmou* and *Fanirè* of Western Africa, by using 16S rRNA gene amplicon sequencing.

Doi is traditionally prepared from fresh cow milk without backslapping by keeping it in an earthen pot wrapped with banana leaves and allowing it to ferment for 2 days (Joishy et al., 2019). *Sangom afamba* is also prepared from fresh cow milk, similar to *Doi*, in a traditional earthen pot, but a spoonful of the previous fermented batch is added and covered with a muslin cloth and allowed to stand for 2-3 days. In the *Mishti dahi*, cow milk is boiled with up to 10% sugar, cooled, and poured into small earthen pots. A starter from the previous batch was added and kept at room temperature for 1-2 days. Traditionally, *Doi*, *Sangon afamba*, and *Misthi dahi* are consumed raw and taken along with steamed rice. *Sangom afamba* is essential for performing rituals in certain traditional religious ceremonies, such as the *na-hutpa* (ear-piercing ceremony of children) in Manipur.

Fulani communities of Western African countries practice spontaneous fermentation of unpasteurised fresh cow milk without backslopping in calabash vessels for 1 day at room temperature to allow spontaneous fermentation (Sessou et al., 2019), such as *Kindirmou* in Niger and *Fanirè* in Benin, which are commonly used as a dessert or refreshment by people in these countries.

In addition, we studied a unique West African traditional cheese, *Wagashi*, with no report available on its associated bacterial communities. *Wagashi* (*Gassirè* in the local *Fulfuldé* language) is a soft, fresh cheese from Benin produced from cow milk. In the traditional preparation, 1L of boiled cow milk is mixed with ~0.5L of fresh milk with the extract of *Calotropis procera* leaves (10-15g). The mixture is kept at a warm temperature (60-70°C) until coagulation is achieved, and then the curd and whey are separated. The curd portion is drained, moulded without pressing, and incubated at room temperature. The pink coloured *Wagashi* cheese is prepared by soaking in a Sorghum (*Sorghum bicolor*) leaf extract brine for the pink colour formation. Unlike conventional cheeses, *Wagashi* is prepared by curdling cow milk with proteolytic *Calotropis procera* leaf extract (Akogou et al., 2018; Sessou et al., 2019).

We compared the bacterial community structure of both uncoloured and coloured *Wagashi* cheese samples from Benin using 16S rRNA gene amplicon sequencing and related its bacterial community to similar cheeses reported from earlier studies (Shangpliang et al., 2018; Zhao et al., 2021). In addition, we aimed to assess the safety of these spontaneously fermented milk products by detecting potential foodborne pathogens.

2. Materials and methods

2.1. Sampling and homogenisation

The spontaneously fermented milk product samples from Northeast India, Niger, and Benin were collected under aseptic conditions (Table 3.1). Samples of uncoloured and coloured *Wagashi* cheese prepared traditionally at home and locally marketed in different towns in Benin were also collected. The samples were transported in ice-cooled boxes and stored in the laboratory at -80°C for further analysis. A volume of 10 mL of each fermented milk sample was homogenized with 90 mL of 2% sodium citrate solution, while 25 g of each cheese sample was homogenized with 45 mL of buffered peptone water (Bio-Rad, pH 7.0 ± 0.2) at 200 rpm for 2 min using a Stomacher 400 Circulator (Seward, United Kingdom). After allowing the large debris to settle down, the resulting clear homogenate was used for metagenomic DNA extraction.

Table 3.1. Features of naturally fermented cow milk product samples collected in Northeast India and West Africa.

Region	Local name	Place of collection	Coordinates	Altitude (msl)	Temperature range (°C)	Pre-treatment	Backslopping	Number of samples
Northeast India	*Doi*	Guwahati, Assam	26.1792° N, 91.7533° E	55	11-33	No	No	3
	Doi	Silchar, Assam	24.8207° N, 92.8018° E	22	11-33	No	No	2
	Doi	Jorhat, Assam	26.7477° N, 94.2132° E	116	11-31	No	No	3
	Doi	Agartala, Tripura	23.8419° N, 91.2820° E	13	10-34	No	No	4
	Doi	Shillong, Meghalaya	25.5811° N, 91.8865° E	1525	4-23	No	No	3
	Sanggom afamba	Imphal, Manipur	24.8072° N, 93.9341° E	786	5-30	No	Yes	3
	Mishti Dahi	Agartala, Tripura	23.8419° N, 91.2820° E	13	10-34	Addition of 10% sugar and boiling	Yes	3
West Africa	*Kindirmou*	Niger (Tillaberi, Dosso, Niamey)	14.2061° N, 1.4580° E; 13.0505° N, 3.2081° E 13.5116° N, 2.1254° E	180-228	18-40	No	No	8

	Fanirè	Benin (Agouna, Pehunco, Parakou Djougou, Houeyogbe)	9.3467° N, 2.6090° E 10.2283° N, 2.0019° E 9.3466° N, 2.609° E 6.5321° N, 1.8708° E	234-444	17-40	No	No	5
	Uncoloured *Wagashi* cheese	Benin (Abomey-Calavi, Cotonou)	6.3719° N, 2.4348° E 6.4503° N, 2.3468° E	51-54	24-32	Yes, boiling and *Calotropis* leaf extract addition	No	5
	Coloured *Wagashi* cheese	Benin (Abomey-Calavi, Cotonou)	6.3719° N, 2.4348° E 6.4503° N, 2.3468° E	51-54	24-32	Yes, boiling, *Calotropis* leaf extract addition and Sorghum leaf extract coloration	No	5

2.2. Metagenomic DNA extraction

Two different extraction methods were independently used to extract DNA from fermented milk and cheese samples. Metagenomic DNA of fermented milk samples was extracted according to method V, described earlier by Keisam et al. (2016). Briefly, 1.5 mL of homogenate was transferred to a sterile 2-ml screw-cap tube containing zirconia/silica beads and centrifuged. The resulting pellets were treated with enzymes (50KU lysozyme and 25U mutanolysin) and incubated at 37°C for 1 h, incubated with proteinase-K at 65°C for 1 h, and treated with GES reagent (5 M guanidine thiocyanate, 100 mM EDTA, and 0.5% sarkosyl). The samples were further treated with ammonium acetate, purified with chloroform: isoamyl alcohol (24:1), and precipitated with ethanol. The precipitated DNA pellets were dissolved in 50 µl of TE buffer. The metagenomic DNA of *Wagashi* cheese samples was extracted as described earlier (Anihouvi et al., 2021) using the NucleoSpin® Food (Macherey-Nagel GmbH&Co.) following the manufacturer's instructions. Qualitative (A260/280) and quantitative estimations of the extracted DNA of both food types were performed using a spectrophotometer (NanoDrop ND-1000, United States). The DNA was stored at -20°C for subsequent 16S rRNA gene amplicon sequencing.

2.3. Barcoded Illumina MiSeq sequencing and data processing

Barcoded Illumina MiSeq amplicon sequencing was used for in-depth bacterial community structure analyses. Two independent approaches were performed for the spontaneously fermented milk products and traditional *Wagashi* cheese. For fermented milk products, the V4-V5

region of the 16S rRNA gene was targeted with the forward primer F563-577 (5′-AYTGGGYDTAAAGNG-3′) and reverse primer R924-907 (5′-CCGTCAATTCMTTTRAGT-3′) with barcodes for sample multiplexing as described earlier (Romi et al., 2015). The PCR-amplified DNA was purified using a QIAquick gel extraction kit (Qiagen, New Delhi, India) and quantified with a Qubit dsDNA BR Assay Kit in a Qubit 2.0 fluorometer (Invitrogen) for multiplexing in equimolar proportion. The DNA pool was sequenced on the Illumina MiSeq platform (Xcelris, Ahmedabad), and the sequence data were processed through the QIIME v1.8.0 bioinformatics pipeline (Caporaso et al., 2012) for adapter sequence removal, paired-end read generation, and sample de-multiplexing. A further MG-RAST pipeline was used at a 97% similarity threshold against the M5RNA database for the generation of Operational Taxonomic Unit (OTU) tables at different taxonomic levels. For the *Wagashi* cheese samples of Benin, the V1-V2 region of the 16S rRNA gene was targeted using the forward primer 28F (5′-GAGTTTGATCNTGGCTCAG-3′) and reverse primer 388R (5′-TGCTGCCTCCCGTAGGAGT-3′) with Illumina adapter and barcodes (Anihouvi et al., 2021). The target was PCR amplified in an ABI Verti-thermocycler, purified, pooled in equimolar proportion after quantification using a Qubit assay, and sequenced on Illumina MiSeq at RTL Genomics (Lubbock, TX, United States) as described earlier (Anihouvi et al., 2021). The sequence data were processed by PEAR sequence merger, USEARCH clustering and alignment algorithm, and UPARSE algorithm for OTU generation at different taxa levels.

2.4. Eubacterial-specific qPCR assay

A SYBR green-based qPCR assay targeting the SSU rRNA gene V3 region was performed for total bacterial load quantification. The PCR reaction containing 0.25 µM of each primer, with forward primer 338f 5′-ACTCCTACGGGAGGCAGCAG-3′ and reverse primer 518r 5′-ATTACCGCGGCTGCTGG-3′ (Ampe et al., 1999), and 1 × EXPRESS SYBR GreenER qPCR Supermix (Invitrogen), was used according to the manufacturer's instructions. The Applied Biosystems 7500 was used to carry out the PCR amplification at 95°C, 5 min, which consisted of 40 cycles of denaturation, annealing, and extension at 95°C for 15 s; 62°C for 30 s, and 68°C for 45 s, respectively (Keisam et al., 2016). A melt curve was generated for each assay from 60°C to 95°C using the default conditions to check the assay's specificity. The SSU rRNA gene (1×10^1 to 1×10^8 copies) derived from the strain type *Lactiplantibacillus plantarum* ATCC 8014 was used for the standard curve preparation.

2.5. Statistical analysis

The relative abundance data of the bacterial Operational Taxonomic Units (OTUs) at the different taxonomic positions were used for statistical analysis. The significant differences in the relative abundance (%) of taxa between the study groups were calculated by Student's two-tailed t-test and ANOVA. Principal coordinate analysis (PCoA) was performed using the Bray-Curtis dissimilarity (PAST v3.22) (Hammer and Harper, 2008). The PERMANOVA test with 10,000 permutations was used to observe the significance of the difference in the bacterial taxa in the samples between two geographical regions and express it as a Bonferroni-corrected p-value. For calculating the alpha diversity

indices (Chao species richness and Shannon diversity index) between two geographical regions, the compare_alpha_diversity.py script was used in the QIIME pipeline (Morris et al., 2014). The observed differences were visualized as boxplots using BoxPlotR (http://shiny.chemgrid.org/boxplotr/). The Wilcoxon test using "svDialogs" in the R package (v3.5.2) was conducted to show the significance of the difference in the bacterial species between two geographical regions and express it as a Benjamini-Hochberg (BH)-corrected p-value (Tuikhar et al., 2019). The hierarchically clustered heat map to show the species-level significant difference between two geographical regions was visualized using "gplots" in R. The OTU data, with a relative abundance of more than 0.1% and a significance of $p < 0.001$, was used for the heatmap generation.

2.6. Data availability

The sequence data associated with this study are available on the MG-RAST server: https://www.mg-rast.org/mgmain.html?mgpage=project&project=mgp87174 and MG-RAST ID mgp104874.

3. Results

3.1. Spontaneously fermented milk products from Northeast India and West Africa display distinct bacterial diversity

We performed a cultivation-independent bacterial community analysis of spontaneously fermented milk products from Northeast India (n=21, Doi/*Sangom afamba*/*Mishti dahi*) and West Africa (n=13, *Kindrmou* of Niger and *Fanirè* of Benin) by Illumina MiSeq amplicon sequencing of the 16S rRNA gene from the DNA of fermented milk samples. *Bacillota* (relative abundances of 72 and 61%) and *Pseudomonadota* (relative abundances of 17 and 3%) were the dominant bacterial phyla present in these naturally fermented milk products of India and West Africa, respectively. The mesophilic spontaneously fermented milk products of India analyzed in this study comprised *Lactobacillaceae* (34.7%), mainly *Lactobacillus* (*L. delbrueckii*) and *Leuconostoc* (*L. mesenteroides*); *Acetobacteraceae* (14.7%), mainly *Gluconobacter* and *Acetobacter* spp.; and *Streptococcaceae* (8.3%), mainly *Lactococcus* (*L. lactis*) and *Enterobacteriaceae* (8.3%, uncultured bacterium related to *Enterobacter* spp.) as core bacteria (Fig. 3.1). However, the spontaneously fermented milk products of West Africa studied here contained *Staphylococcaceae* (34.6%), mainly *Macrococcus* (*Macrococcus caseolyticus*), *Enterobacteriaceae* (32.6%, uncultured *Enterobacter* sp.), *Streptococcaceae* (8.4%, *L. lactis*), and *Lactobacillaceae* (8%, *L. delbrueckii*) as the dominant bacteria.

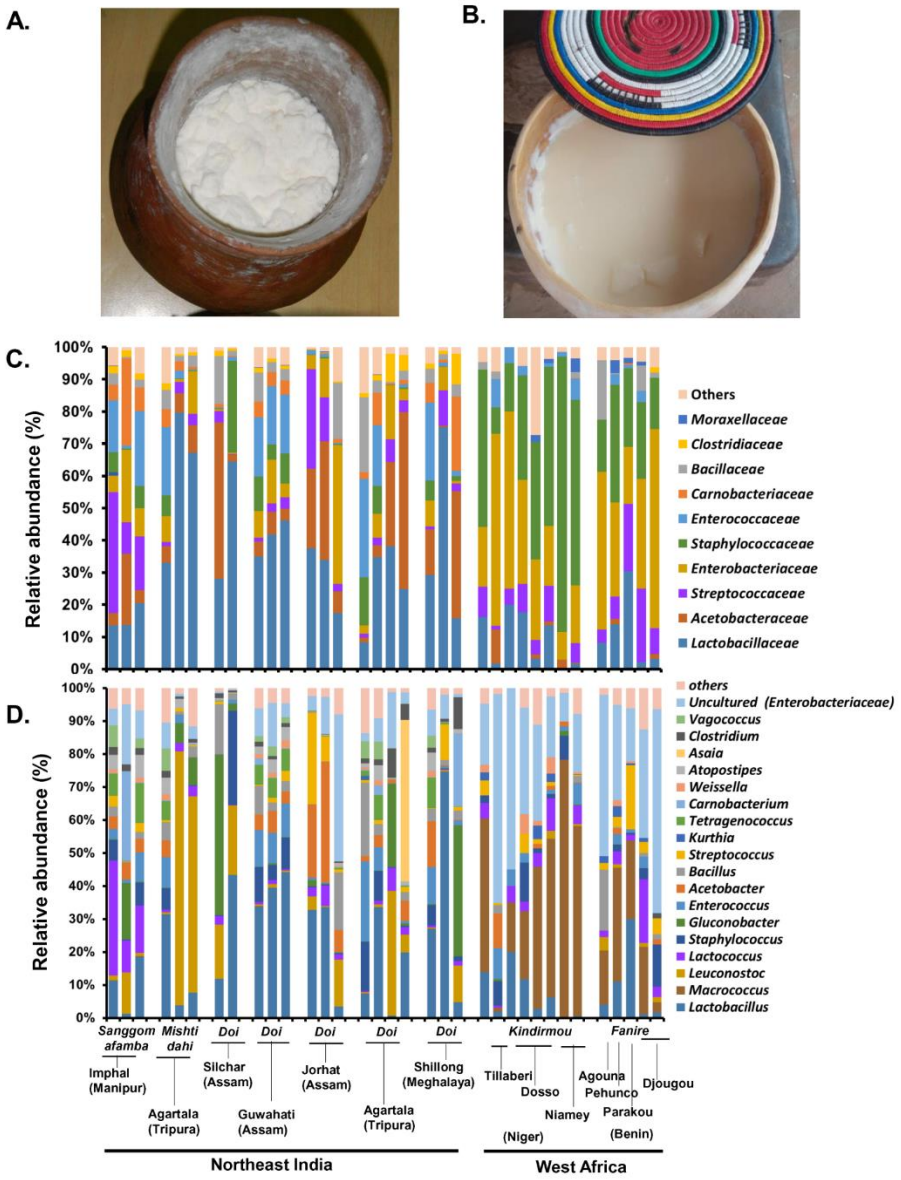

Figure 3.1. The bacterial communities compositional difference in the spontaneously fermented milk products of Northeast India and West Africa. Spontaneously fermented milk product *Doi* in the traditional earthen pot (A) in Northeast India and *Fanirè* in the traditional calabash vessel (B) in the Fulani camp of Benin are shown here. The taxon bar

chart shows the family level (C) and genus level (D) differences in the relative abundance (%) of predominant bacteria present in the fermented milk products of Northeast India and West Africa. The sample details are available in Table 3.1.

An unweighted principal coordinate analysis (PCoA) based on the Bray-Curtis distance matrix using species-level relative abundance showed a distinct separation of the fermented milk samples of India from West Africa (PERMANOVA, $p < 0.0001$) (Fig. 3.2A). The PCoA biplot displayed that *M. caseolyticus*, uncultured *Enterobacteriaceae*, *L. mesenteroides*, *G. frateurii*, and *Tetragenococcus halophilus* were the key ecological drivers that shaped up the overall bacterial community structure difference in the fermented milk products of two geographical regions.

The fermented milk products of India had significantly higher bacterial species richness (Chao1) and diversity (Shannon index) than the West African products (p = 2.34 E-07, Student's t-test, two-tailed) (Fig. 3.2B). The total bacterial load of fermented milk samples analyzed using a eubacteria-specific qPCR assay resulted in a 9.47-11.02 log10 bacterial load per gram of the samples, without any significant difference between Indian and African samples.

While comparing the two geographical regions, *Acetobacteraceae* (*G. frateurii* and *Acetobacter* spp.) (p = 0.007) and *L. mesenteroides* (p = 0.014) were abundant in fermented milk samples from India, while *Staphylococcaceae* (*M. caseolyticus*) (p = 9.72 E-07) and uncultured *Enterobacteriaceae* were present abundantly in West African fermented milk samples (p = 1.23 E-05). In addition, *Tetragenococcus*,

Atopostipes, and *Vagococcus* were present only in Indian fermented milk products (Fig. 3.3). Among the beneficial Actinobacteria present in fermented milk products (Parker et al., 2018), *Bifidobacterium* was detected in only two samples from India. Within Indian samples, *Streptococcaceae* (21.4%, mainly *L. lactis*) were abundant (p = 0.014, Student's t-test, two-tailed) in the *Sangom afamba* samples, where backslopping was practiced with unpasteurised raw milk.

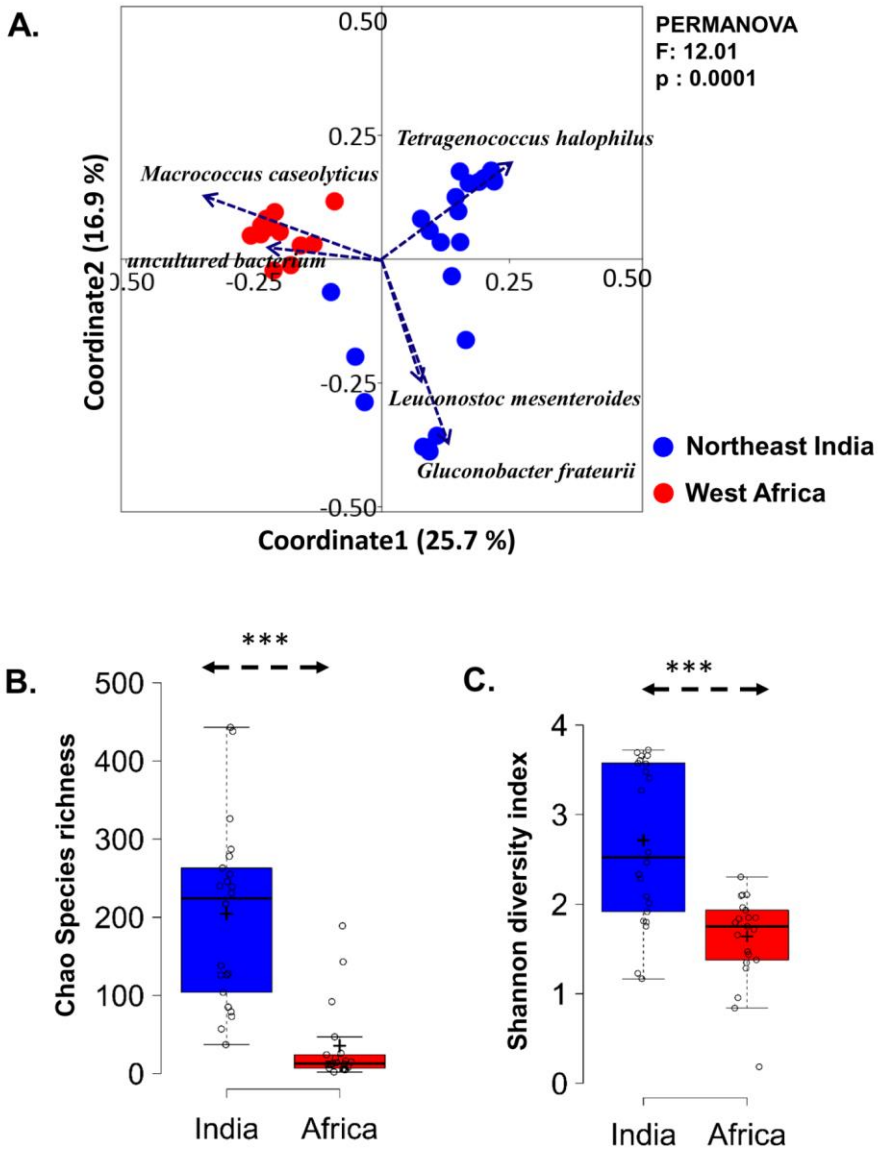

Figure 3.2. Differences in bacterial diversity in the spontaneously fermented milk products of Northeast India and West Africa. (A) PCoA biplot based on Bray-Curtis dissimilarity of the species-level OTUs shows a significant difference in the overall bacterial community

structure between the spontaneously fermented milk products of the two continents. The significance of the difference is expressed as a Bonferroni-corrected p-value (q = 0.0001, F = 12.0, PERMANOVA). (B, C) The boxplot shows higher bacterial diversity in Indian fermented milk products (Chao species richness and Shannon diversity index) than in West African samples. The significance of the difference was calculated using Student's t-test and indicated as ****p<0.0001*.

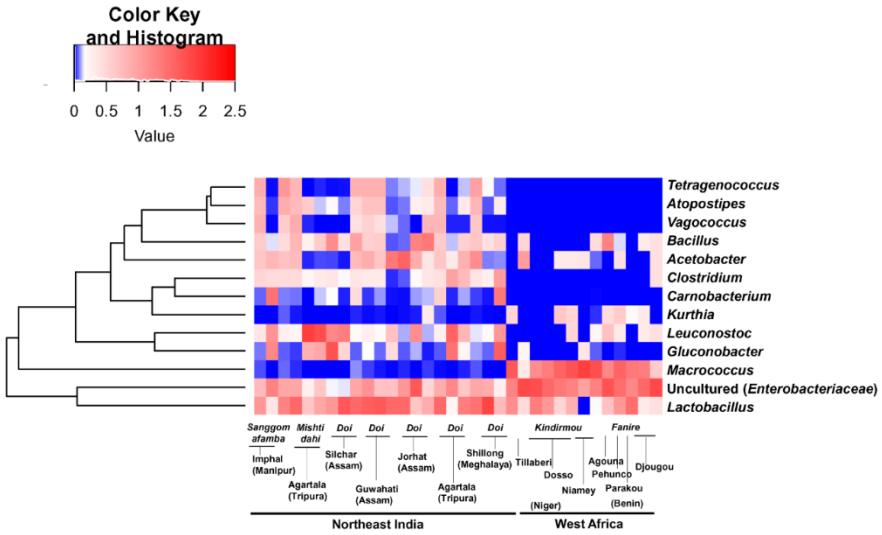

Figure 3.3. A hierarchically clustered heat map shows the significantly differing bacterial genera between the spontaneously fermented milk samples of Northeast India and West Africa. The bacterial genus with a relative abundance of more than 1 % and significantly different between the two continents (Wilcoxon test, $q<0.001$, BH corrected) are listed here. The abundance difference is shown as a red and blue colour gradient key.

3.2. Bacterial community differences in the coloured and uncoloured *Wagashi* cheese

The bacterial community structure of uncoloured and coloured *Wagashi* cheese of Benin (n=10) prepared by spontaneous fermentation of boiled cow milk was analyzed by Illumina amplicon sequencing and compared with other reported traditional cheeses. At the phylum level, *Bacillota* (relative abundance of 85.1 and 76.2%) and *Pseudomonadota* (14.8 and 23.9%) were dominant in the uncoloured and coloured *Wagashi* cheese samples, respectively. *Lactobacillaceae* (55.4%), mainly *Lactobacillus* (*L. delbrueckii*), and *Streptococcaceae* (31.9%), mainly *Streptococcus* spp., were dominant in the uncoloured *Wagashi* cheese. However, the coloured *Wagashi* cheese was detected mainly with *Streptococcaceae* (72.9%) and low *Lactobacillaceae* (1.6%). Although no significant difference in the overall bacterial diversity (Chao species richness and Shannon diversity index) was visible between these two cheese types, we noticed a drastic change in the abundance of *Lactobacillus* (*L. delbrueckii*, p=0.01, Students t-test, paired two-tailed) after soaking with Sorghum (*Sorghum bicolor*) leaf extract in the coloured *Wagashi* cheese (Fig. 3.4).

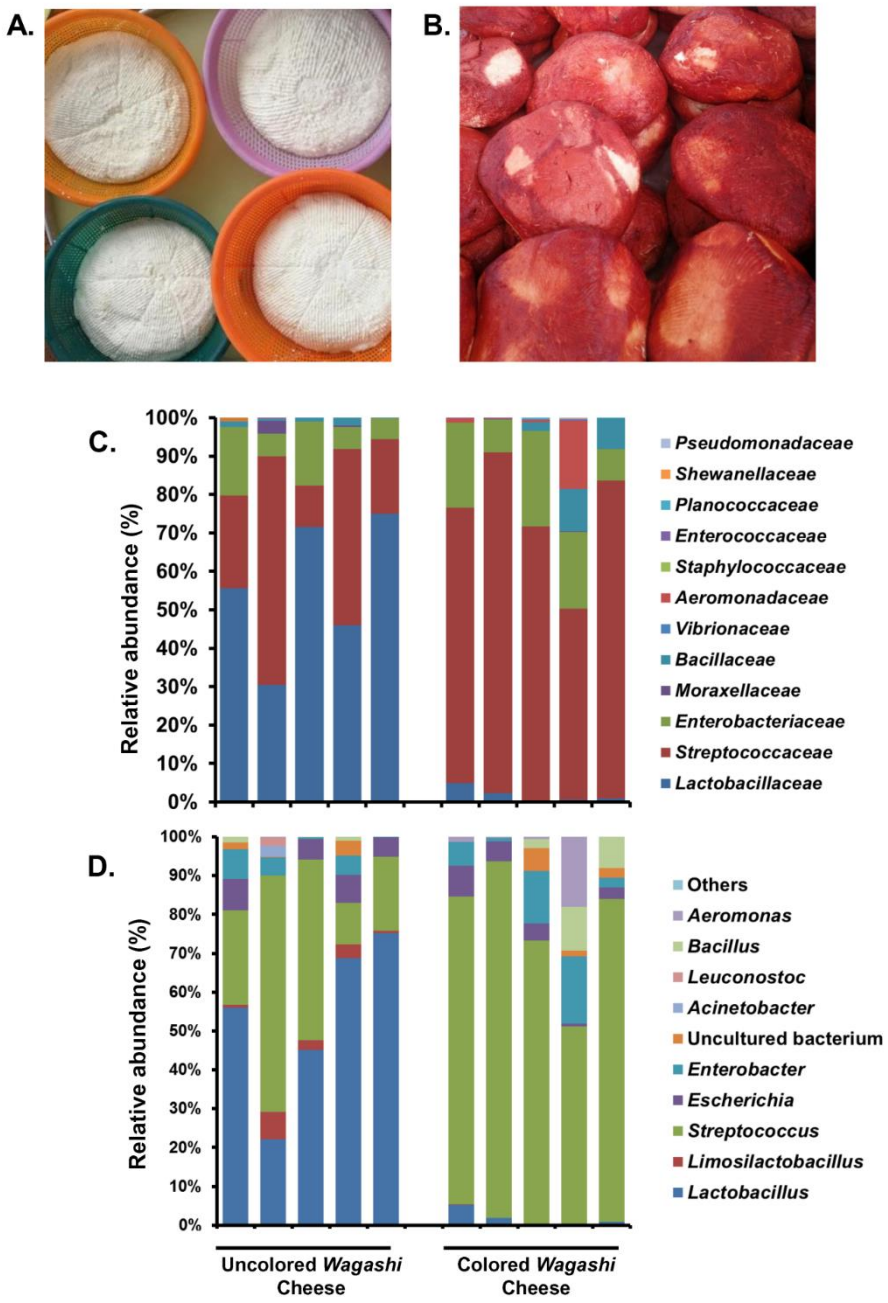

Figure 3.4. The difference in the overall bacterial community structure in the uncoloured (A) and coloured *Wagashi* cheese (B) marketed in

Benin. The taxon bar chart shows the difference in the relative abundance (%) of predominant bacteria present in the uncoloured *Wagashi* cheese and coloured *Wagashi* cheese at the family level (C) and genera level (D).

3.3. Safety of the spontaneously fermented milk products of Northeast India and Western Africa

In addition to the fermenting beneficial bacteria, the 16S rRNA gene amplicon sequencing-based in-depth analysis effectively detected the presence of several unwanted potential pathogens in the fermented milk samples. Our study detected *Clostridium* spp. in all the *Doi* samples up to 4% of relative abundance, particularly in the samples collected from Tripura and Meghalaya of Northeast India, whereas none of the West African fermented milk samples detected *Clostridium*. The *Wagashi* cheese samples from Benin contained *Streptococcus infantarius*, with up to 80% relative abundance in some samples. In addition, we found an uncultured *Enterobacteriaceae* bacterium with <96% similarity with the 16S rRNA sequence of *Enterobacter* spp. in West African and Indian fermented milk products. On average, *Kindrmou* and *Fanirè* samples contained the uncultured bacterium related to *Enterobacter* with a 32% relative abundance, and *Wagashi* cheese samples had a 15% relative abundance of *Enterobacter*. In addition, *Wagashi* cheese contained, on average, more than 4% relative abundance of *Escherichia coli*. Among the members of *Staphylococcus*, we detected *Staphylococcus aureus* in West African fermented milk products and *Staphylococcus epidermis* in Indian *Doi* samples. While compared to the uncoloured *Wagashi* cheese, *Bacillus cereus* was abundantly

present, with more than 5% average relative abundance in the coloured, processed *Wagashi* cheese samples.

4. Discussion

Unlike thermophilic *yoghurt* fermentation in which *L. delbrueckii* and *S. thermophilus* dominate (Bokulich et al., 2015; de Melo Pereira et al., 2022; Parker et al., 2018), the mesophilic spontaneously fermented milk products of Northeast India comprised mainly *Lactococcus, Leuconostoc*, *Gluconobacter*, *Acetobacter* and *Enterobacter*, in addition to *Lactobacillus*. Most of the NGS-based earlier studies reported such dominance of *Lactococcus* in naturally fermented milk products prepared by backslopping (Von Gastrow et al., 2020; Jans et al., 2017; Moonga et al., 2020; Shangpliang et al., 2018). Similarly, *L. mesenteroides,* which can tolerate high concentrations of sugar compared to other lactic acid bacteria (Fellows, 2017), was abundantly present in the *Mishti Dahi*, prepared by boiling milk with 10 % sugar and allowed mesophilic fermentation by backslopping (Mallappa et al., 2021).

The NGS-based earlier studies showed the dominance of *L. lactis*, *L. helveticus*, *L. mesenteroides*, and *Acetobacter* spp. in the *Dahi* prepared by backslopping in the high-altitude Himalayan region (de Melo Pereira et al., 2022; Shangpliang et al., 2018). At the same time, the *Dahi* prepared in Southern India had *L. delbrueckii* as the dominant bacteria (Jayashree et al., 2013; Joishy et al., 2019; Mallappa et al., 2021). However, *Streptococcus* spp. and *Enterococcus* spp. the most abundant in the *Dahi* samples of Bangladesh (Nahidul-Islam et al., 2018) and Bhutan (Shangpliang et al., 2017), respectively *Enterococcus durans*

predominates in the naturally fermented milk of cow and yak products of Arunachal Pradesh in India, such as *mar*, *chhurpi* and *churkam* (Shangpliang and Tamang, 2021). Among the beneficial Actinobacteria reported in the fermented milk products (Parker et al., 2018), only two *Doi* samples were detected with *Bifidobacterium*.

On the contrary, the spontaneously fermented raw milk products of West Africa studied here contained *Macrococcus* and *Enterobacter* as the dominant bacteria. Unlike other species of *Staphylococcaceae*, *M. caseolyticus*, abundantly present in the fermented milk products of West Africa, is not considered a human pathogen and has been used as a starter culture for aroma and flavour production in several fermented foods (Ramos et al., 2021). The unique characteristic property of Fulani fermented milk products prepared in calabash containers may be linked with such bacterial association (Fagbemigun et al., 2021; Groenenboom et al., 2020; Moonga et al., 2020). However, earlier NGS studies did not report *M. caseolyticus* as a primary bacterium in other African fermented milk products like *Nono* (Fagbemigun et al., 2021) and *Mabisi* (Moonga et al., 2020). The bacterial diversity of fermented foods inferred from metagenomic studies differs by the method of DNA extraction used. In this study, we utilised an enzymatic lysis method which recovers a high bacterial diversity from fermented milk products (Keisam et al., 2016). We speculate the DNA extraction method adopted here may be one reason for the above differences, in addition to the environmental factors during the sampling (Zhao et al., 2021).

Earlier NGS analysis of similar spontaneously fermented unpasteurised cow milk *Nono* prepared in Nigeria, by mesophilic fermentation in

calabash container without backslopping, contained mainly *Lactobacillus* spp. and *Acetobacter* spp. (Fagbemigun et al., 2021). Another Zambian traditionally fermented milk, *Mabisi,* prepared similarly was dominated by *L. lactis* (Groenenboom et al., 2020; Moonga et al., 2020). On the contrary, *S. thermophilus* and *L. helveticus*, reported worldwide as dominant bacteria in several naturally fermented milk products (Bokulich et al., 2015; Oki et al., 2014; Peng et al., 2021; Shangpliang et al., 2018; Yu et al., 2021) were rare (less than 2% relative abundance) in the spontaneously fermented milk products of this study. The mesophilic spontaneous fermentation, without backslopping, might be the reason for such a difference in the predominant bacterial communities. Another observation of total bacterial load analysed by culture-independent qPCR assay is much higher than those reports based on the cultivation-dependent analysis of similar fermented milk products with 6.0 -9.0 log 10 population load per gram (Dewan and Tamang, 2007; Shangpliang et al., 2017), which support the possible presence of unculturable strains of dominant bacterial species in natural milk fermentation.

The presence of *Lactobacillus* (*L. delbrueckii*) and *Streptococcus* as dominant bacteria in the uncoloured *Wagashi* cheese, is similar to the Mexican Poro Cheese (Aldrete-Tapia et al., 2014), traditional cheeses of China, Mongolia, and Russia (Zhao et al., 2021; Zhong et al., 2016). The drastic change in the main bacterial abundance in the coloured *Wagashi* cheese is linked with the Sorghum leaf extract used for colouring and further drying. The Sorghum leaf extract mainly contains apigeninidin pigment and phenolic compounds, normally used for prolonging the shelf life (Akogou et al., 2018). The antimicrobial

activity of sorghum leaf extract (Akogou et al., 2018; Schnur et al., 2021) and further processing like drying may have favoured the *Streptococcus* spp. Contrary to the cheese types produced worldwide from cow milk through natural fermentation, *L. lactis* and *L. mesenteroides* (Delcenserie et al., 2014; Rocha et al., 2021; Shangpliang et al., 2017; Sun and D'Amico, 2021; Zhang et al., 2021) were not predominant in *Wagashi* cheese, while *Streptococcus* spp. dominated. The traditional production method by curdling with proteolytic leaf extract of *Calotropis procera* and thermophilic incubation at 60-70° C (Akogou et al., 2018; Sessou et al., 2019) might have favoured *Streptococcus* spp. in *Wagashi* cheese of Benin.

Clostridium in naturally fermented milk products is a safety concern in Northeast India, where the Indian Council of Medical Research (ICMR) has already reported high incidences of foodborne diseases and intestinal infections (ICMR report, 2017). Earlier NGS-based studies also detected a similar presence of *Clostridium* in the *Dahi* samples of Bangladesh (Nahidul-Islam et al., 2018). Our study detected *Clostridium* in the *Doi* samples collected from Tripura and Meghalaya, which are Indian states bordering Bangladesh. Several earlier reports also showed the presence of *Clostridium* in other fermented foods (particularly in fermented pork, fish and soybean products) marketed in Northeast India (De Mandal et al., 2018; Deka et al., 2021; Keisam et al., 2019).

The *Wagashi* cheese samples of Benin contained *Streptococcus infantarius*, possibly related to *S. infantarius* subsp. *infantarius* (Sii), belonging to *Streptococcus bovis*/*Streptococcus equinus* complex

(SBEEC), which is recently been reported as an emerging infectious foodborne pathogen in African countries (Jans et al., 2017). Attempts on selective isolation and characterisation of an uncultured *Enterobacteriaceae* bacterium found in West African and Indian fermented milk products could result in novel species. The earlier NGS studies also reported such dominance of *Enterobacter* spp. in the African naturally fermented milk *Mabisi* (Moonga et al., 2020) and *Kefir* of Turkey (Dertli and Çon, 2017). Similar to our result, several reports based on 16S rRNA gene sequencing account *E. coli* presence in traditional cheese produced by natural fermentation (Fuka et al., 2013).

Most West African fermented milk products are prepared from raw, unpasteurised milk by spontaneous fermentation (Leone et al., 2022). Usually, unpasteurised raw milk contains *Staphylococcaceae* members abundantly (Joishy et al., 2019; Sun and D'Amico, 2021), which supports the dominance of *M. caseolyticus* in the spontaneously fermented raw cow milk in West African countries. Moreover, several traditionally fermented milk products have been reported to contain *M. caseolyticus* (Fuka et al., 2013). Few other studies detected *M. caseolyticus* in foods of animal origin (Aldrete-Tapia et al., 2014; Ramos et al., 2021). However, it is not prioritised as a human pathogen and has been used as a starter culture in several fermented foods (Ramos et al., 2021). The traditional milk curdling with proteolytic leaf extract of *Calotropis procera,* thermophilic incubation at 60-70° C, and further processing by colouration and drying might have favoured the *B. cereus* growth in the coloured *Wagashi* cheese. Lowering the moisture content by drying and adding 3% NaCl in the coloured *Wagashi* cheese would

reduce the presence of *B. cereus* (Raevuori and Gnigeorgis, 1975; Rukure and Bester, 2001). These findings will allow us to understand the safety issues related to spontaneous milk fermentation and to develop strategies to overcome them by identifying the critical control points of pathogen entry. Moreover, technological intervention by designing the starter culture consortiums for a controlled fermentation of boiled or pasteurised milk will improve the safety and quality of the fermented milk products in both the regions studied (Agyei et al., 2020; de Melo Pereira et al., 2022; Mallappa et al., 2021).

Natural milk fermentation is highly reproducible across geographical regions under similar processes and conditions (Wolfe et al., 2014). Such substrate and conditions-specific adaptive evolution of microbiota are responsible for the unique properties of different fermented foods (Tamang et al., 2021). In our study, natural mesophilic fermentation of unpasteurised cow milk, without back slopping in two geographically separated regions, shape up different bacterial community structure. In contrast to the thermophilic spontaneous milk fermentation by back slopping commonly practised worldwide, the mesophilic milk fermentation without back slopping favoured a combination of lactic acid bacteria (*Lactobacillus* and *Lactococcus)* and acetic acid bacteria (*Gluconobacter* and *Acetobacter*) in Indian *Doi* samples. Whereas *Macrococcus* and uncultured *Enterobacteriaceae* in *Fanirè* and *Kindirmou* samples of Benin and Niger, respectively. Such differences in the microbiota evolved during natural fermentation may relate to the microbiota of raw cow milk used and the indigenous production process (in traditional earthen pots or calabash containers) in practice in both regions. Moreover, we report for the first time the bacterial

communities of *Wagashi* cheese of Benin and their difference during processing (colouring). This understanding will allow us to go for controlled production through technological intervention by designing the starter culture consortiums, which improve the safety and quality of the traditional fermented milk and cheese products in India and Africa, where natural fermentation is in practice.

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6. References

Agyei, D., Owusu-Kwarteng, J., Akabanda, F., Akomea-Frempong, S., (2020) Indigenous African fermented dairy products: Processing technology, microbiology and health benefits. *Crit. Rev. Food Sci. Nutr.* 60, 991-1006.

Akogou, F.U.G., Den Besten, H.M.W., Polycarpe Kayodé, A.P., Fogliano, V., Linnemann, A.R. (2018) Antimicrobial evaluation of red, phytoalexin-rich sorghum food biocolorant. *PLoS One* 13, 1-15.

Aldrete-Tapia, A., Escobar-Ramírez, M.C., Tamplin, M.L., Hernández-Iturriaga, M. (2014) High-throughput sequencing of microbial communities in Poro cheese, an artisanal Mexican cheese. *Food Microbiol*. 44, 136-141.

Ampe, F., Omar, N.B., Moizan, C., Wacher, C., Guyot, J.P. (1999) Polyphasic study of the spatial distribution of microorganisms in Mexican pozol, a fermented maise dough, demonstrates the need for cultivation-independent methods to investigate traditional fermentations. *Appl. Environ. Microbiol.* 65, 5464-5473.

Anihouvi, D.G.H., Henriet, O., Kpoclou, Y.E., Scippo, M.L., Hounhouigan, D.J., Anihouvi, V.B., Mahillon, J. (2021) Bacterial diversity of smoked and smoked-dried fish from West Africa: A metagenomic approach. *J. Food Process. Preserv*. 45, 1-12.

Barooah, M., Bora, S.S., Goswami, G. (2020) Ethnic fermented foods and beverages of Assam. In: Tamang, J.P. (eds) Ethnic fermented foods and beverages of India: Science history and culture. Springer Nature Singapore Pte Ltd 152, 85-104.

Bokulich, N.A., Amiranashvili, L., Chitchyan, K., Ghazanchyan, N., Darbinyan, K., Gagelidze, N., Sadunishvili, T., Goginyan, V., Kvesitadze, G., Torok, T., Mills, D.A. (2015) Microbial biogeography of the transnational fermented milk matsoni. *Food Microbiol*. 50, 12-19.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1-5.

De Mandal, S., Singh, S.S., Muthukumaran, R.B., Thanzami, K., Kumar, V., Kumar, N.S. (2018) Metagenomic analysis and the functional profiles of traditional fermented pork fat 'sa-um' of Northeast India. *AMB Express* 8, 1-11.

de Melo Pereira, G.V., de Carvalho Neto, D.P., Maske, B.L., De Dea Lindner, J., Vale, A.S., Favero, G.R., Viesser, J., de Carvalho, J.C., Góes-Neto, A., Soccol, C.R. (2022) An updated review on bacterial community composition of traditional fermented milk products: what next-generation sequencing has revealed so far? *Crit. Rev. Food Sci. Nutr*. 62, 1870-1889.

Deka, P., Mehetre, G.T., Lalnunmawii, E., Upadhyaya, K., Singh, G., Hashem, A., Al-Arjani, A.B.F., Abd_Allah, E.F., Singh, B.P. (2021) Metagenomic analysis of bacterial diversity in traditional fermented foods reveals food-specific dominance of specific bacterial taxa. *Ferment.* 7, 1-17.

Delcenserie, V., Taminiau, B., Delhalle, L., Nezer, C., Doyen, P., Crevecoeur, S., Roussey, D., Korsak, N., Daube, G. (2014) Microbiota characterisation of a Belgian protected designation of origin cheese, Herve cheese, using metagenomic analysis. *J. Dairy Sci*. 97, 6046-6056.

Dertli, E., Çon, A.H. (2017) Microbial diversity of traditional kefir grains and their role on kefir aroma. *LWT* 85, 151-157.

Dewan, S., Tamang, J.P. (2007) Dominant lactic acid bacteria and their technological properties isolated from the Himalayan ethnic fermented milk products. *Antonie van Leeuwenhoek* 92, 343-352.

Fagbemigun, O., Cho, G.S., Rösch, N., Brinks, E., Schrader, K., Bockelmann, W., Oguntoyinbo, F.A., Franz, C.M.A.P. (2021) Isolation and characterisation of potential starter cultures from the Nigerian fermented milk product nono. *Microorganisms* 9, 1-20.

Fellows, P.J. (2017) 6 - Food biotechnology. In: Fourth, E. (eds) Food science, technology and nutrition. *Woodhead Publishing* pp. 387-430.

Fuka, M.M., Wallisch, S., Engel, M., Welzl, G., Havranek, J, Schloter, M. (2013) Dynamics of bacterial communities during the ripening process of different Croatian cheese types derived from raw ewe's milk cheeses. *PLoS ONE* 8, e80734.

Groenenboom, A.E., Shindano, J., Cheepa, N., Smid, E.J., Schoustra, S.E. (2020) Microbial population dynamics during traditional production of *Mabisi*, a spontaneous fermented milk product from Zambia: a field trial. *World J. Microbiol. Biotechnol.* 36, 1-14.

Hammer, Ø., Harper, D.A.T. (2008) Paleontological data analysis. *John Wiley & Sons Ltd*.

ICMR Report (2017) Indian Council of Medical Research, Public Health Foundation of India, and Institute for Health Metrics and Evaluation. India: Health of the Nation's States - The India State-Level Disease Burden Initiative. New Delhi: ICMR, PHFI, and IHME.

Jans, C., Meile, L., Kaindi, D.W.M., Kogi-Makau, W., Lamuka, P., Renault, P., Kreikemeyer, B., Lacroix, C., Hattendorf, J., Zinsstag, J., Schelling, E., Fokou, G., Bonfoh, B. (2017) African fermented dairy products - Overview of predominant technologically important microorganisms focusing on African *Streptococcus infantarius* variants and potential future applications for enhanced food safety and security. *Int. J. Food Microbiol*. 250, 27-36.

Jayashree, S., Pushpanathan, M., Rajendhran, J., Gunasekaran, P. (2013) Microbial diversity and phylogeny analysis of buttermilk, a fermented milk product, employing 16S rRNA-Based pyrosequencing. *Food Biotechnol*. 27, 213-221.

Joishy, T.K., Dehingia, M., Khan, M.R. (2019) Bacterial diversity and metabolite profiles of curd prepared by natural fermentation of raw milk and back sloping of boiled milk. *World J. Microbiol. Biotechnol*. 35, 1-12.

Keisam, S., Romi, W., Ahmed, G., Jeyaram, K. (2016) Quantifying the biases in metagenome mining for realistic assessment of microbial ecology of naturally fermented foods. *Sci. Rep*. 6, 155.

Keisam, S., Tuikhar, N., Ahmed, G., Jeyaram, K. (2019) Toxigenic and pathogenic potential of enteric bacterial pathogens prevalent in the traditional fermented foods marketed in the Northeast region of India. *Int. J. Food Microbiol.* 296, 21-30.

Leone, C., Thippareddi, H., Ndiaye, C., Niang, I., Diallo, Y., Singh, M. (2022) Safety and quality of milk and milk products in Senegal-a review. *Foods* 11, 3479.

Mallappa, R.H., Balasubramaniam, C., Nataraj, B.H., Ramesh, C., Kadyan, S., Pradhan, D., Muniyappa, S.K., Grover, S. (2021) Microbial diversity and functionality of traditional fermented milk products of India: Current scenario and future perspectives. *Int. Dairy J*. 114, 104941.

Moonga, H.B., Schoustra, S.E., Van den Heuvel, J., Linnemann, A.R., Samad, M.S., Shindano, J., Smid, E.J. (2020) Composition and diversity of natural bacterial communities in *Mabisi*, a traditionally fermented milk. *Front. Microbiol.* 11, 1-12.

Morris, E.K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T.S., Meiners, T., Müller, C., Obermaier, E., Prati, D. Socher, S.A. (2014) Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. *Ecol. Evol.* 4, 3514-3524.

Motato, K.E., Milani, C., Ventura, M., Valencia, F.E., Ruas-Madiedo, P., Delgado, S. (2017) Bacterial diversity of the Colombian fermented milk "*Suero Costeño*" assessed by culturing and high-throughput sequencing and DGGE analysis of 16S rRNA gene amplicons. *Food Microbiol*. 68, 129-136.

Mudgal, S.P., Prajapati, J.B. (2017) *Dahi*-An Indian naturally fermented yogurt. In: Shah, N.P. (eds) Yogurt in health and disease prevention. *Elsevier Inc*. pp. 353-369.

Nahidul-Islam, S.M., Kuda, T., Takahashi, H., Kimura, B. (2018) Bacterial and fungal microbiota in traditional Bangladeshi fermented milk products analysed by culture-dependent and culture-independent methods. *Food Res. Int*. 111, 431-437.

Oki, K., Dugersuren, J., Demberel, S., Watanabe, K. (2014) Pyrosequencing analysis of the microbial diversity of airag, khoormog and tarag, traditional fermented dairy products of Mongolia. *Biosci. Microbiota Food Heal*. 33, 53-64.

Parker, M., Zobrist, S., Donahue, C., Edick, C., Mansen, K., Nadjari, M.H.Z., Heerikhuisen, M., Sybesma, W., Molenaar, D., Diallo, A.M., Milani, P., Kort, R. (2018) Naturally fermented milk from northern Senegal: Bacterial community composition and probiotic enrichment with *Lactobacillus rhamnosus*. *Front. Microbiol*. 9, 2218.

Peng, C., Sun, Z., Sun, Y., Ma, T., Li, W., Zhang, H. (2021) Characterisation and association of bacterial communities and non-volatile components in spontaneously fermented cow milk at different geographical distances. *J. Dairy Sci.* 104, 2594-2605.

Raevuori, M., Genigeorgis, C. (1975) Effect of pH and sodium chloride on growth of *Bacillus cereus* in laboratory media and certain foods. *Appl. Microbiol*. 29, 68-73.

Rai, R., Shangpliang, H.N.J. Tamang, J.P. (2016) Naturally fermented milk products of the Eastern Himalayas. *J. Ethn. Foods* 3, 270-275.

Rai R., Tamang J.P. (2022) In vitro and genetic screening of probiotic properties of lactic acid bacteria isolated from naturally fermented cow-milk and yak-milk products of Sikkim, India. *World J Microbiol Biotechnol*. 6, 25.

Ramos, G.L.P.A., Vigoder, H.C., Nascimento, J.S. (2021) Technological applications of *Macrococcus caseolyticus* and its impact on food safety. *Curr. Microbiol*. 78, 11-16.

Rocha, R., Velho, M.V., Santos, J., Fernandes, P. (2021) *Serra da estrela pdo* cheese microbiome as revealed by next generation sequencing. *Microorganisms* 9, 2007.

Romi, W., Ahmed, G., Jeyaram, K. (2015) Three-phase succession of autochthonous lactic acid bacteria to reach a stable ecosystem within 7 days of natural bamboo shoot fermentation as revealed by different molecular approaches. *Mol. Ecol.* 24. 13237.

Rukure, G., Bester, B.H. (2001) Survival and growth of *Bacillus cereus* during *Gouda* cheese manufacturing. *Food Control*. 12, 31-36.

Schnur, S.E., Amachawadi, R.G., Baca, G., Sexton-Bowser, S., Rhodes, D.H., Smolensky, D., Herald, T.J., Perumal, R., Thomson, D.U., Nagaraja, T.G. (2021) Antimicrobial activity of sorghum phenolic extract on bovine foodborne and mastitis-causing pathogens. *Antibiotics* 10, 1-12.

Sessou, P., Keisam, S., Tuikhar, N., Gagara, M., Farougou, S., Jeyaram, K. (2019) High-throughput Illumina Miseq amplicon sequencing of yeast communities associated with indigenous dairy products from republics of Benin and Niger. *Front. Microbiol*. 10, 594.

Shangpliang, H.N.J., Rai, R., Keisam, S., Jeyaram, K., Tamang, J.P. (2018) Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing. *Sci. Rep*. 8, 1532.

Shangpliang, H.N.J., Sharma, S., Rai, R., Tamang, J.P. (2017) Some technological properties of lactic acid bacteria isolated from *Dahi* and *Datshi*, naturally fermented milk products of Bhutan. *Front. Microbiol*. 8, 116.

Shangpliang, H.N.J., Tamang, J.P. (2021) Phenotypic and genotypic characterisation of lactic acid bacteria isolated from exotic naturally fermented milk (cow and yak) products of Arunachal Pradesh, India. *Int. Dairy J.* 118, 105038.

Sun, L., D'Amico, D.J. (2021) Composition, succession, and source tracking of microbial communities throughout the traditional production of a farmstead cheese. *mSystems* 6, 1-18.

Tamang, J.P. (2022) "Ethno-microbiology" of ethnic Indian fermented foods and alcoholic beverages. *J. Appl. Microbiol*. 133, 145-161.

Tamang, J.P., Jeyaram, K., Rai, A.K. Mukherjee, P.K. (2021) Diversity of beneficial microorganisms and their functionalities in community-specific ethnic fermented foods of the Eastern Himalayas. *Food Res. Int*. 148, 110633.

Tenorio-Salgado, S., Castelán-Sánchez, H.G., Dávila-Ramos, S., Huerta-Saquero, A., Rodríguez-Morales, S., Merino-Pérez, E., Roa de la Fuente, L.F., Solis-Pereira, S.E., Pérez-Rueda, E., Lizama-Uc, G. (2021) Metagenomic analysis and antimicrobial activity of two fermented milk *kefir* samples. *MicrobiologyOpen* 10, e1183.

Tuikhar, N., Keisam, S., Labala, R.K., Ramakrishnan, P., Arunkumar, M.C., Ahmed, G., Biagi, E. Jeyaram, K. (2019) Comparative analysis of the gut microbiota in centenarians and young adults shows a common signature across genotypically non-related populations. *Mech. Ageing Dev.* 179, 23-35.

Von Gastrow, L., Madec, M.N., Chuat, V., Lubac, S., Morinière, C., Lé, S., Santoni, S., Sicard, D., Valence, F. (2020) Microbial diversity associated with Gwell, a traditional French mesophilic fermented milk inoculated with a natural starter. *Microorganisms* 8, 982.

Wahengbam, R., Thangjam, A.S., Keisam, S., Asem, I.D., Ningthoujam, D.S., Jeyaram, K., (2020) Ethnic fermented foods and alcoholic beverages of Manipur. In: Tamang, J.P. (eds) Ethnic fermented foods and beverages of India. *Sci. History Cult*. 5, 349-519.

Wolfe, B.E., Button, J.E., Santarelli, M. Dutton, R.J. (2014) Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. *Cell* 158, 422-433.

Yu, Z., Peng, C., Kwok, L.Y., Zhang, H. (2021) The bacterial diversity of spontaneously fermented dairy products collected in northeast Asia. *Foods* 10, 2321.

Zhang, K., Jia, M., Guo, Z., Li, Y., Li, B., Li, X. (2021) Evaluation of bacterial diversity of traditional cheese in Tarbagatay Prefecture, China, and its correlation with cheese quality. *Food Sci. Nutr.* 9, 3155-3164.

Zhao, Z., Ning, C., Chen, L., Zhao, Y., Yang, G., Wang, C., Chen, N., Zhang, Z., Li, S. (2021) Impacts of manufacture processes and geographical regions on the microbial profile of traditional Chinese cheeses. *Food Res. Int.* 148, 110600.

Zhong, Z., Hou, Q., Kwok, L., Yu, Z., Zheng, Y., Sun, Z., Menghe, B., Zhang, H. (2016) Bacterial microbiota compositions of naturally fermented milk are shaped by both geographic origin and sample type. *J. Dairy Sci.* 99, 7832-7841.

Chapitre 4

4. Livestock farming and milk processing practices in Benin: risks of contamination in curdled milk and *Wagashi Gassirè*

A survey was conducted among stakeholders in the dairy sector in Nikki and Dassa-Zoumé to gather relevant insights into breeding, production, and preservation methods for raw milk, curdled milk, and *Wagashi Gassirè*. This study highlighted hygiene deficiencies that could compromise product quality.

Personal contribution:

I was involved in the conceptualization of the study, data collection, analysis and the preparation and revision of the original draft. Additionally, I managed the manuscript submission and followed up during the review process.

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State of the art of breeding, milking, and milk processing for the production of curdled milk and *Wagashi Gassirè* in Benin: Practices favoring the contamination of its dairy products

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Abstract

This study aimed to identify the factors favouring the contamination of raw cow's milk, curdled milk, and *Wagashi Gassirè* cheese during their production and preservation in order to develop strategies to improve their quality. A cross-sectional survey of 401 randomly selected stakeholders encompassing all levels of the dairy production chain in the Nikki and Dassa-Zoumé communes of Benin was conducted. The data obtained were analysed using the SAS software for the calculation of frequencies and the R software for classifying the stakeholders based on the hygiene practices they adopted during the production and conservation of raw cow's milk, curdled milk, and *Wagashi Gassirè*. The study identified three types of dairy farmers based on how they medically treated their cattle and implemented hygiene practices, including farmers who (1) relied on themselves or received help from veterinarians trained in animal husbandry and milking to monitor the animals on their farms; (2) relied only on veterinarians; and (3) relied only on themselves. The majority of these dairy farmers felt that hygienic milking practices were very restrictive and difficult to implement. In addition, three groups of *Wagashi Gassirè* producers were identified: (1) producers trained in good hygiene practices who did not boil or sundry the cheese; (2) producers lacking the infrastructure to protect from weather exposure who used all parts of *Calotropis procera* for coloured *Wagashi Gassirè* production; and (3) producers who did not often filter the milk and boiled the *Wagashi Gassirè* in bags before immersion in simple water or whey. The sanitary quality of milk and milk products is influenced by the diverse handling

practices employed by producers. These practices must be considered according to the types of farmers and processors when suggesting improved intervention policies.

Keywords: Milk, cheese, hygiene, quality, Benin

1. Introduction

Like most West African countries, Benin's economy is based on agricultural production (Okry et al., 2017). The agricultural sector employs about 70 percent of the labor force, contributes nearly 23.3 percent to GDP, and provides about 75 percent of export earnings and 15 percent of government revenue (MPD, 2022). This sector is considered to have numerous potentialities that must be judiciously exploited to support national economic growth and thus contribute to effectively fight poverty. Most importantly, agriculture ensures Benin's food security (MAEP, 2017). In the agricultural production sector, livestock occupies a predominant place and contributes to the livelihood and food security of the population (Sounon et al., 2019). In 2012, the live-stock subsector, which plays an important role in the incomes of northern Beninese people, generated a revenue of USD 180.6 million (2.4% of GDP). In 2013, the national livestock population was estimated to be 4.6 million head, including 2.1 million cattle and 2.5 million small ruminants (MPD, 2022). However, the output of this subsector is plagued by the strong influences of various constraints,

such as climatic, nutritional, sanitary, and genetic factors, that are at the root of major problems, including the low productivity of livestock (Soule et al., 2017). To overcome these obstacles and increase production, the government has implemented a series of projects including the Milk and Meat Sector Support Project (PAFILAV), which is part of the Growth Strategy for Poverty Reduction and the implementation of which has helped to increase production in the milk industry (Gouvernement du Bénin, 2022a; Groupes de la Banque Africaine de Développement (GBAD), 2022). Indeed, the average number of lactating cows in Benin increased from about 495,000 in 2011 to more than 580,000 in 2015, and milk production increased from 102,000 tons in 2011 to 113,000 tons in 2015 (FAO, 2020). To boost milk production, the Government of Benin through its actions program for the period from 2021 to 2026, under pillar 2 relating to the pursuit of the structural transformation of the economy has planned to increase milk production through the development of the milk sector and the promotion of livestock farming enterprises (Gouvernement du Bénin, 2022b). With this program, the livestock sector is flourishing and interest in milk production has increased (Groupes de la Banque Africaine de Développement (GBAD), 2022). Although milk production is increasing and makes a significant contribution to economic growth and to the food and nutritional security of the population, the marketed and sanitary quality of the milk is not always satisfactory and milk spoils rapidly under the influence of high environmental temperature in Benin (Sessou et al., 2013). To circumvent its quick and high adulteration, milk produced in Benin is often transformed into curdled milk and *Wagashi Gassirè* cheese using

traditional processes. These products are important sources of protein and other essential nutrients and could help to eliminate protein deficiency in low-income populations (Dossou et al., 2016). They are part of the dairy products recognized worldwide as excellent sources of nutritional components (proteins, minerals, vitamins, lipids, and carbohydrates) and important part of the human diet (Verruck et al., 2019). Raw cow milk as well as curdled milk obtained via the spontaneous fermentation of raw cow milk are often consumed without any treatment by people in Benin in order to benefit from their nutritional elements (Farougou et al., 2012; Boko et al., 2016; Sessou et al., 2019). The traditional cheese *Wagashi Gassirè* is one of the most widespread dairy products in both rural regions and big cities and is widely appreciated by consumers in the Republic of Benin. It is also produced and consumed in many other West African countries, including Burkina Faso, Ghana, Nigeria, and Togo (Dossou et al., 2016; Zannou et al., 2018; Sessou et al., 2019). While these milk and traditional dairy products are enjoyed by consumers, they are also important in contributing to the nutritional health status of the population. In contrast to these potential benefits, it should also be recognized that these dairy products are perceived to be a major public health risk. Indeed, the practices generally used for the production and preservation of milk and milk derivatives do not always ensure their sanitary and nutritional quality. Contamination, particularly microbiological contamination, which leads to poor sanitary, nutritional, and marketable qualities, is commonly observed (Sessou et al., 2013). Since the demand for these pastoral dairy products, including *Wagashi Gassirè* and curdled milk, in local markets is increasing

(Chabi Toko et al., 2015), it is essential to develop strategies to provide consumers with products of high sanitary and nutritional quality. Designing efficient strategies to improve the quality of these products requires knowledge of the different production and preservation practices adopted by all stakeholders in the milk production chain. Here, with this perspective, we analyzed the production and preservation practices that can cause the contamination of milk and milk products in two Beninese communes, Nikki and Dassa-Zoumé.

2. Materials and methods

2.1. Survey of dairy farmers, dairy producers, and consumers in the Beninese milk sector

A cross-sectional survey of dairy farmers, dairy producers, and consumers of the milk sector in the Beninese communes of Dassa Zoumé and Nikki was conducted between May and July 2020 (Fig. 4.1). The choice of these two areas was done since they are among the main cattle-breeding areas located in the center and north of Benin, respectively. A total of 401 participants, including cattle dairy farmers, dairy producers, traders, and consumers of curdled milk and *Wagashi Gassirè*, were surveyed (Table 4.1). The producers of fermented milk and cheese were predominantly women. Data were collected through guided interviews with each randomly selected participant using a structured questionnaire. This random approach was used as described in our previous paper (Dossou et al., 2022).

The structured questionnaire was implemented using the Epicollect5 software. Field information concerning the hygienic practices implemented at all steps, i.e., milking, the production of cheese and curdled milk, preservation, and marketing, was obtained. Different questions specific to the production step were also asked. For instance, dairy farmers were asked questions regarding the hygiene measures implemented during milking and the therapeutic interventions used to treat sick cattle. The questionnaires included a list of various diseases in the local Fulani language; the questions and the answers were translated to French by trained veterinarians. The questions asked to dairy producers, traders, and consumers identified the existing production conditions and the product preservation methods being employed. During the study, the participation of respondents was voluntary, and participants provided informed consent before attempting the questionnaire (Dossou et al., 2022).

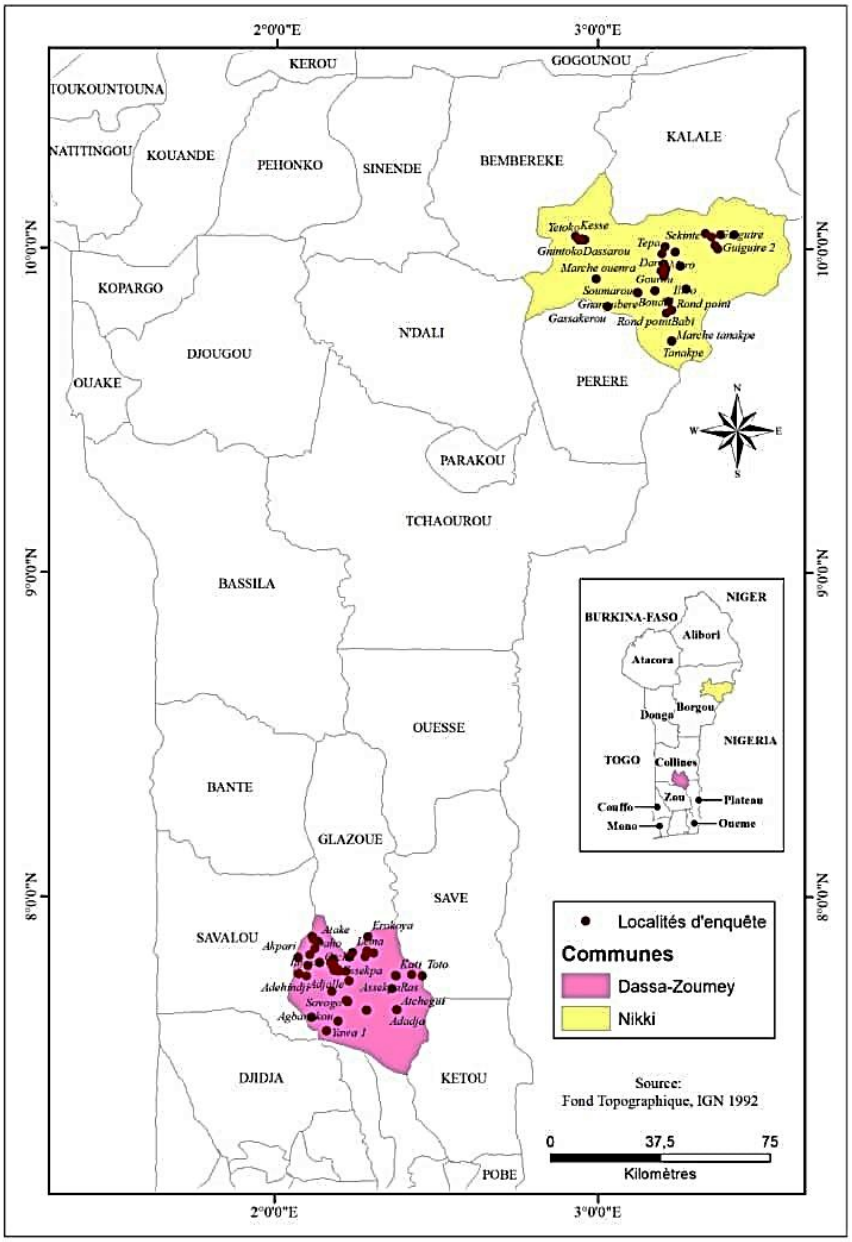

Figure 4.1: Map of the two study areas: Nikki in northern Benin and Dassa-Zoumé in central Benin.

Table 4.1: Distribution of respondents according to stakeholder category in each commune.

	Commune		
Stakeholder category	**Dassa-Zoumé**	**Nikki**	**Total**
Dairy farmers	54	30	84
***Wagashi Gassirè* producers**	90	75	165
Curd producers	02	09	11
Traders of *Wagashi Gassirè* and curd	35	18	53
Consumers	56	32	88
Total	**237**	**164**	**401**

2.2. Statistical analysis

The collected data were analyzed using the SAS (SAS, 2013) and R4.0.2 software. The PROC FREQ procedure of SAS was used to calculate frequencies, and the Chi2 test to specify the significance of the common factor for the variables considered. For each relative frequency, a 95% confidence interval (CI) was calculated using the following formula: $CI=1.96\sqrt{([P(1-P)]/N)}$, where P is the relative frequency and N is the sample size. The multiple correspondence analysis (MCA) function of R's FactoMineR library was used for multiple correspondence analysis (Husson et al., 2017; Cornillon et al., 2018) on a contingency table crossing the individuals in row and the variables in column. The variables considered are: for farmers (application of sanitary measures, implementation of hygienic measures during milking, hygienic measures observed during milking, hygiene training, availability of processing materials), for *Wagashi* producers

(ethnicity, marital status, hygiene training, boiling and drying practices, immersion in whey, colouring of cheese, dye used, preservation method, filtering of milk, coagulant used), for traders (ethnicity, equipment, preservation methods and situation of sale point), for consumers (level of education, level of satisfaction and preservation methods). A hierarchical ascending classification (HAC) of the MCA components was then performed using the hierarchical clustering on principal components (HCPC) function. Stakeholder categories were then identified and characterized. The correspondence analysis (CA) function in R's FactoMineR library was used for factorial correspondence analysis (FCA) (Husson et al., 2017; Cornillon et al., 2018) on a contingency table crossing individuals in a row with variables in the column. The variables considered for the FCA are the level of education, ethnicity, and level of training on hygiene of the actors. The individuals are represented by the breeders, traders, *Wagashi* producers and consumers.

3. Results

3.1. Sociodemographic characteristics of the surveyed actors in the milk sector

The responses showed that milk farming is practiced exclusively by men (100%), the majority of whom are married and belong to the Fulani (87%) and Gando (13%) sociocultural groups in the two communes. Cattle rearing is therefore primarily carried out by the Fulani in the two communes surveyed. Overall, 93% of farmers were uneducated (91%

in Dassa-Zoumé and 97% in Nikki). Women were the exclusive *Wagashi Gassirè* producers in both communes. In the Dassa-Zoumé commune, these women belonged to the Peulh (95%), Idaatcha (3%), and Fon (2%) sociocultural groups. In the Nikki commune, they belonged to the Gando (77%) and Peulh (23%) sociocultural groups. Curd production was carried out mostly by women of the Peulh sociocultural group, with 100 and 89% in Dassa-Zoumé and Nikki, respectively. The survey revealed that the female producers were all uneducated, which could negatively influence the sanitary quality of their milk products due to a lack of knowledge on the safe handling of milk and dairy products. The analysis of the data showed that the *Wagashi Gassirè* traders belonged to the Baatonou, Dendi, Peulh, Fon, and Idaatcha tribes. In Nikki, 83% of traders were exclusively uneducated, whereas 43 and 31% of the traders in Dassa-Zoumé were uneducated and had a primary level education, respectively.

3.2. Dominant diseases in cattle identified by dairy farmers and their in-farm treatment strategies

As shown in Table 4.2, several diseases were found to be prevalent in the cattle farms surveyed. Arranged in descending order of importance, the dominant diseases were foot and mouth disease (FMD; 87%), pasteurellosis (62%), dermatophilosis (27%), trypanosomiasis (27%), lumpy skin disease (21%), and contagious bovine pleuropneumonia (18%). A comparison of the results obtained in the two communes (Table 4.2) revealed that pasteurellosis was significantly more prevalent ($p < 0.05$) in the commune of Nikki than in Dassa-Zoumé.

These diseases were identified during the survey when a question was asked about the diseases often encountered on the participants' farms. The majority (64%) of dairy farmers reported that they diagnosed diseases themselves before administering treatment to the animals. When faced with these different diseases, the farmers adopted different treatment methods (Table 4.2). The majority (64%) of cattle dairy farmers treated their sick animals themselves, compared to 36% who relied solely on veterinarians. Some of the cattle dairy farmers who diagnosed and treated their animals themselves claimed to be self-taught, having learned either from their peers or from veterinarians, and 36% of cattle dairy farmers said that they had received training in treating animals.

Two treatment types were identified in the survey: modern treatments and traditional treatments using bark and plant leaves (Table 4.3). All the dairy farmers surveyed used modern treatments and obtained drugs from veterinary clinics by describing the symptoms of their animals to the veterinarians or pharmacists. Of these, 68% combined modern and traditional treatments. An inventory of the antibiotics used to treat animals based on the medicine labels found in the dairy farmers' homes showed that the most commonly used antibiotics were penicillin, streptomycin, oxytetracycline, tylosin, and sulphonamides. These antibiotics were also purchased from veterinary pharmacies, as the quality of products on the black market is doubtful. A total of 59% of the respondents estimated the weight of the animal and the dosage without referring to the manufacturer's instructions, and the rest (41%) determined the dosage according to the manufacturer's instructions by estimating the weight of the animal. In addition, all respondents did not

respect the post-treatment waiting period for milking. The majority (98%) of the surveyed dairy farmers said that milk from cows treated with antibiotics was used either for direct human consumption or for processing into *Wagashi Gassirè* or curdled milk, in contrast to the remaining 2% who rejected this type of milk (Table 4.4). Compared to Nikki, the Dassa-Zoumé commune had a significantly higher proportion of dairy farmers who treated animals without veterinary help and used milk collected during the treatment period. Consequently, the probability of having dairy products contaminated with drug residues is higher in Dassa-Zoumé than in Nikki.

Table 4.2: Dominant diseases identified in farm animals and disease diagnosis in the communes of Dassa-Zoumé and Nikki.

Variable	Both communes			Dassa-Zoumé			Nikki			Chi² test
	N	%	CI	N	%	CI	N	%	CI	
Dairy cattle diseases (local dialect)										
Foot and mouth disease (*Tchabou*)	73	86a	8	46	91	8	27	78	16	NS
Pasteurellosis (*Hinrin*)	73	62b	11	46	52	14	27	78	16	*
Nodular dermatosis (*Borla*)	73	21c	9	46	26	13	27	11	12	NS
Dermatophilosis (*Goungnan*)	73	27c	10	46	22	12	27	37	18	NS
Brucellosis (*Konedjé*)	73	7d	6	46	9	8	27	4	7	NS
PPCB (*Otel*)	73	18c	9	46	24	12	27	7	10	NS
Trypanosomiasis (*Samorin*)	73	27c	10	46	30	13	27	22	16	NS
Calf plague (*Tchibel*)	73	1d	3	46	2	4	27	0	0	NS
Others	73	7d	6	46	7	7	27	7	10	NS
Diagnosed and treated by										
Dairy farmer	84	64b	10	54	72	12	30	50	18	*
Veterinarian	84	36a	8	54	28	10	30	50	15	NS

Legend: * $p<0.01$; N: number; NS: not significant; %: percent of respondents; CI: confidence interval; a, b, c, d: values from the same column followed by the same letter were not significantly different at the 5% level. Chi^2- tests were performed for variables from both communes in the same line.

Table 4.3: Mode and type of treatment of listed livestock diseases and sources of drugs.

Variable	Both Communes			Dassa-Zoumé			Nikki			Chi^2 test
	N	%	CI	N	%	CI	N	%	CI	
Basis for treatment choice by dairy farmers who diagnose and treat diseases themselves										
Self-taught	54	19b	10	39	17	12	15	7	13	NS
Professionally trained	54	36ab	13	39	28	14	15	43	25	NS
Mixed (self-taught and professionally trained)	54	45a	13	39	55	15	15	50	25	NS
Type of treatment used										
Modern only	54	32b	12	39	31	15	15	33	24	NS
Modern and traditional	54	68a	12	39	69	15	15	67	24	NS
Supply sources of drugs										
Veterinary office	54	87a	10	39	85	11	15	93	13	NS
Informal source	54	13b	9	39	15	11	15	7	13	NS
Noncompliance with waiting period	54	100	0	39	100	0	15	100	0	NS
Choice of timeframe										
Self-taught	54	74a	12	39	72	14	15	80	20	NS
Veterinarian's instructions	54	17b	10	39	15	11	15	20	20	NS
Mixed (self-taught and veterinarian's instructions)	54	9b	8	3	13	11	15	0	0	NS

Legend: N: number; NS: not significant; %: percent of respondents; CI: confidence interval; a, b, c, d: values followed by the same letter were not significantly different at the 5% level. Chi2 tests were performed for variables from both communes in the same line.

Table 4.4: Use of milk from cows treated with antibiotics without respecting the withdrawal period.

Variable	Both Communes			Dassa-Zoumé			Nikki			Chi² test
	N	%	CI	N	%	CI	N	%	CI	
Consumption/transformation	42	98a	4	31	97	6	11	100	0	NS
Rejection	42	2b	4	31	3	6	11	0	0	NS

Legend: N: number; NS: not significant; %: percent of respondents; CI: confidence interval; a, b: values followed by the same letter were not significantly different at the 5% level, while means followed by different letters were significantly different at the 5% level. Chi2 tests were performed for variables from both communes in the same line.

3.3. Hygiene measures implemented by dairy farmers during milking

In Benin, all farmers use a hand milking procedure. The hygiene measures implemented by dairy farmers during milking are shown in Table 4.5. The analysis of the data on milking equipment revealed that most of the respondents (96%) used milking equipment only for milking. Milking equipment was either stored in rooms, hung on trees, or placed on open racks, exposing it to environmental microorganisms. Plastic equipment was used more frequently ($p< 0.001$) (Table 4.6) in Dassa-Zoumé than in Nikki. In contrast, calabashes were used more ($p< 0.001$) frequently in Nikki than in Dassa-Zoumé. All dairy farmers washed the milking utensils after milking, whereas only a minority (2-15%) washed them just before milking. Only 38% of the dairy farmers surveyed claimed to wash their hands before milking. Approximately 11% of the dairy farmers claimed to be trained in good milking hygiene practices, but only 5% implemented these practices. The majority who did not implement them felt that these practices were very restrictive and difficult to implement (Table 4.5).

Table 4.5: Milking hygiene measures implemented on farms.

Variable	Both Communes			Dassa-Zoumé			Nikki			Chi² test
	N	%	CI	N	%	CI	N	%	CI	
Training in milking hygiene	84	11	7	54	13	9	30	7	9	NS
Implementation of hygiene practices	84	55	5	54	29	10	30	100	0	*
Hand washing before milking	73	38	11	46	37	14	27	41	19	NS
Other use of milking materials	84	4	4	54	4	5	30	3	6	NS
Washing of utensils after use	73	100	0	46	100	0	27	100	0	NS
Washing of utensils just before use	73	7	6	46	2	4	27	15	13	*

Legend: * $p<0.05$; N: number; NS: not significant; %: percent of respondents; CI: confidence interval. Chi² tests were performed for variables from both communes in the same line.

Table 4.6: Types of equipment used for milking and curd production.

Variable	Both Communes			Dassa-Zoumé			Nikki			Chi² test
	N	%	CI	N	%	CI	N	%	CI	
Milking material										
Plastic	73	67a	11	46	96	6	27	19	15	***
Calabash	73	29b	10	46	2	4	27	74	17	***
Plastic and calabash	73	3c	4	46	0	0	27	7	10	NS
Stainless steel	73	1c	2	46	2	4	27	0	0	NS

Legend: *** $p<0.001$; N: number; NS: not significant; %: percent of respondents; CI: confidence interval; a, b, c: values followed by the same letter were not significantly different at the 5% level. Chi2 tests were performed for variables from both communes in the same line.

3.4. *Wagashi Gassirè* production practices

The unit operations of the *Wagashi Gassirè* production process that could influence its sanitary quality were investigated. Only 28 and 8% of the cheese producers in Dassa-Zoumé and Nikki, respectively, had cheese-making rooms that were laid out for improved protection from weather. Consequently, although the *Wagashi Gassirè* cheese produced in both communes was exposed to external pollutants, including microbiological and physical hazards, it was exposed to a significantly greater extent in the Nikki commune ($p< 0.01$). While 72% of the cheese producers in Dassa-Zoumé filtered the milk used for cheese production, only 59% implemented this step in the Nikki commune. *Calotropis procera*, which is used for milk coagulation, was procured from markets (or known clients) in 72% of cases in Dassa-Zoumé. The majority of Nikki cheese producers obtained *Calotropis procera* directly from the fields (99%). After moulding, *Wagashi Gassirè* is stored immersed in either water or whey, and 92 and 50% of the producers in the Dassa-Zoumé and Nikki communes, respectively, followed the immersion in whey method of storing. In addition, 50% of the female producers immersed the cheese in plain water after moulding. Generally, the cheese is coloured using different techniques and raw materials. In Dassa-Zoumé, the colouring of *Wagashi Gassirè* cheese was mostly performed (69.33%) using sorghum panicles procured from markets, while in Nikki, sorghum ears were used for colouring. Touhi bark or teak leaves (Tectona grandis) were also used for colouring by many female producers. For colouring, sorghum panicles added to potash (followed by 67% of producers in Dassa-Zoumé) or to salt/bicarbonate (followed by a smaller proportion of

producers) were used in either hot (cooking) or cold (trituration of the dye in water) methods. Importantly, very few cheese producers (9% in Dassa-Zoumé and 9% in Nikki) were trained in hygienic *Wagashi Gassirè* production practices (Data not shown).

3.5. Conditions of curd production

The milk used for curd production was mostly filtered in both communes. Curd producers in Dassa-Zoumé exclusively used plastic equipment for fermentation (Table 4.5). In contrast, in the Nikki commune, stainless steel or enamel equipment was also used in addition to plastic. Moreover, the fermentation equipment was not solely reserved for curd making; this multipurpose could lead to the cross-contamination of the dairy products.

3.6. Market conditions for selling *Wagashi Gassirè*

The results showed that *Wagashi Gassirè* was sold by traders untrained in good hygiene practices. They also lacked the infrastructure to protect the cheese from environmental contamination. As a result, it was exposed to microbiological, physical, and chemical hazards. This study considered consumers with varying levels of education and of both sexes. In both Dassa-Zoumé and Nikki, most respondents were educated. According to the educated respondents, the hygiene conditions at the time of sale of *Wagashi Gassirè* were less than satisfactory (Table 4.7). In contrast, the uneducated consumers responded that hygiene conditions at the time of sale of *Wagashi Gassirè* were satisfactory. After buying, the consumers in the commune of Nikki made significant use ($p < 0.001$) of sun drying to pre-serve

Wagashi Gassirè, compared to daily heating in water, which was utilized by consumers in Dassa-Zoumé. Other methods used by consumers to preserve cheese included refrigeration, frying, and hot smoking. The level of microbiological contamination in *Wagashi Gassirè* would therefore depend on the preservation method used, especially because sun drying exposes the cheese to more microbes (Table 4.7).

Table 4.7: Consumer assessment of hygiene conditions at the time of sale and preservation methods.

Variable	Both Communes			Dassa-Zoumé			Nikki			Chi² test
	N	%	CI	N	%	CI	N	%	CI	
Hygiene conditions during the sale										
Satisfactory	88	42b	10	56	45	13	32	38	17	
Unsatisfactory	88	58a	10	56	55	13	32	62	17	NS
Preservation method										
Cooking	86	48a	11	54	59	13	32	28	16	**
Refrigeration	86	7d	5	54	11	8	32	0	0	NS
Frying	86	30bc	10	54	37	13	32	19	14	NS
Smoking	86	41ab	10	54	43	13	32	38	17	NS
Cooking in salted water	86	21c	9	54	19	10	32	25	15	NS
Sun drying	86	33bc	10	54	11	8	32	69	16	***

Legend: ** $p<0.01$; *** $p<0.001$; N: number; NS: not significant; %: percent of respondents; CI: confidence interval; a, b, c, d: values followed by the same letter were not significantly different at the 5% level. Chi2 tests were performed for variables from both communes in the same line.

3.7. Classification of actors according to hygiene practices

3.7.1. Classification of dairy cattle farmers

This study characterized the dairy cattle farmers and classified them into different groups. The first three axes were used to interpret the MCA results. The total inertia of the three axes was 57.97%, with 27.24% on the first axis, 21.39% on the second axis, and 9.34% on the third axis (Fig. 4.2). Group 1 dairy cattle farmers treated their animals themselves or with help from veterinarians. These farmers also practiced milking hygiene measures. For example, they washed and dried the collection utensils and washed their hands before milking. Group 2 consisted of dairy cattle farmers who used veterinarians to monitor their animals and who had received training in animal husbandry and milking, which they implemented on their farms. Group 3 consisted of dairy farmers who treated their animals themselves, without help from veterinarians. These individuals had not received any training in milking hygiene. Training in good hygiene practices should be implemented for all farmers, but especially for the latter group, and a rigorous follow-up of the application of these good practices should be carried out to improve the sanitary quality of the milk produced in these breeding areas.

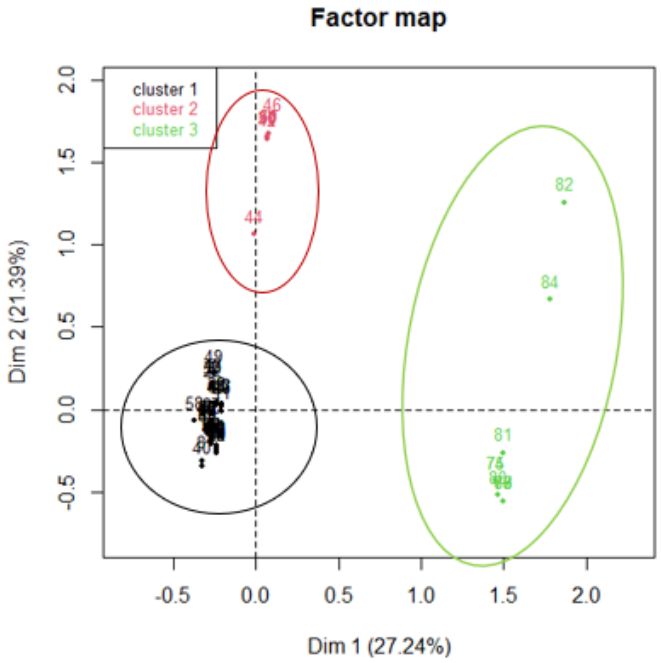

Figure 4.2: Classification of cattle dairy farmers in Dassa-Zoumé and Nikki.

3.7.2. Classification of female *Wagashi Gassirè* producers

This study identified three groups of female *Wagashi Gassirè* producers. The first three axes were used to interpret the MCA results. The total inertia of the three axes was 36.98% with 21.98%, 10.38%, and 4.61% on the first, second, and third axes, respectively (Fig. 4.2). Axis 1 contrasts the female producers in Groups 1 and 3 with those in Group 2. Axis 2 compares female producers in Group 3 to those in Groups 1 and 2 (Fig. 4.3). Group 1 consisted of women who were trained in good production hygiene practices. They did not scald, or sun dry their cheese. Most also did not immerse the cheese in whey and did

not colour it. In addition, they put the cheese in salted water but did not smoke it. Group 2 consisted of single female producers who did not have infrastructure, such as properly laid out cheese-making rooms, to protect the cheese from weather exposure. They used filtered milk to make *Wagashi Gassirè* by coagulating the milk with the leaves, stems, and sap of *Calotropis procera*. They mainly produced red cheese. The cheese was coloured with residues of sorghum (panicles and ears) and teak (Touhi bark and leaf). These producers were of the Gando and Peulh ethnic groups, and they also sun-dried the cheese. Group 3 consisted of female producers from the Idaatcha ethnic group. They often skipped the milk filtration step and sometimes used papaya leaves to coagulate milk. They used vegetables and plastic strainers for draining. They preserved the *Wagashi Gassirè* by boiling in bags and storing the cheeses immersed in plain water and whey. They also smoked the *Wagashi Gassirè* for better preservation. Therefore, it is important to assess the sanitary quality of products according to these groups and to initiate training on good hygiene practices for these groups according to their individual needs.

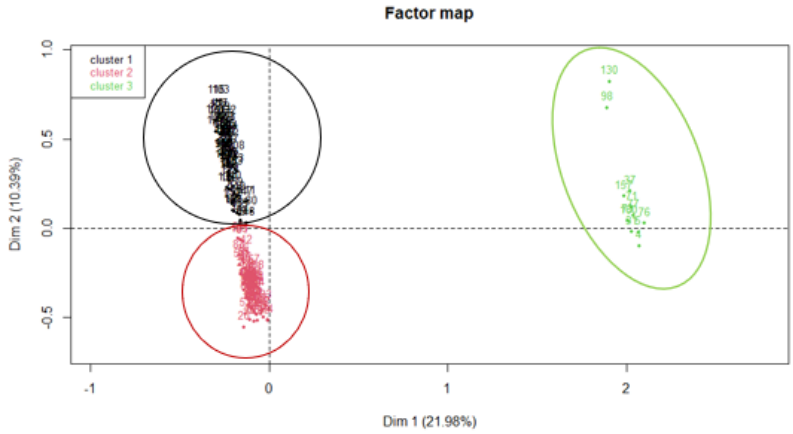

Figure 4.3: Classification of female *Wagashi Gassirè* producers in Dassa-Zoumé and Nikki.

3.7.3. Classification of female *Wagashi Gassirè* traders

This study identified two groups of female *Wagashi Gassirè* traders. The first two axes were used to interpret the MCA results. The total inertia of the three axes was 22.79%, with 12.72% on the first axis and 10.07% on the second axis (Fig. 4.4). The female traders were opposed on axis 2. The first group comprised female traders from the Baatonou, Fon, Mahi, and Peulh tribes who worked either on the edge of the streets, at crossroads, or as street vendors. They preserved cheese by drying it in the sun and cooking it daily in salted water. The products sold by these groups could therefore be contaminated by environmental microorganisms and chemical contaminants from vehicles on the streets and intersections. The second group consisted of vendors from the *Idaatcha*, *Yoruba*, *Dendi*, and *Mina* tribes. They preserved *Wagashi Gassirè* by boiling it either enclosed in or without plastic bags.

Contamination from the plastics used for cooking is possible under these conditions, affecting the sanitary quality of these products at the chemical level and weakly at the microbiological level.

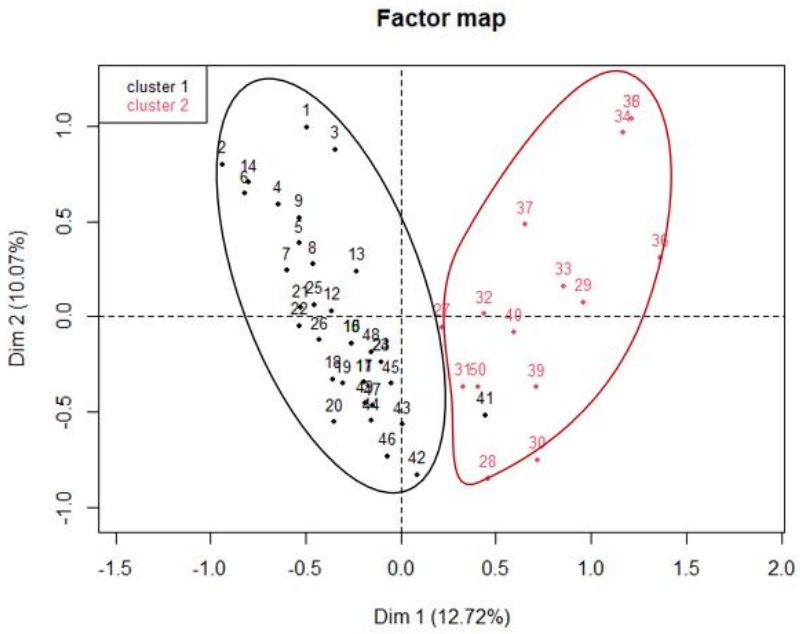

Figure 4.4: Classification of *Wagashi Gassirè* traders in Dassa-Zoumé and Nikki.

3.7.4. Classification of consumers

The first three axes were used to interpret the MCA results. The total inertia of the three axes was 44.10 with 17.52%, 15.12, and 11.45% on the first, second, and third axes, respectively (Fig. 4.5). Axis 2 contrasted the female consumers in Group 3 with those in Groups 1 and 2 (Fig. 4.5). Group 1 consisted of secondary-school-level educated consumers who practiced frying and sun-drying *Wagashi Gassirè*. Group 2 consisted of university-level educated consumers who cooked

and refrigerated *Wagashi Gassirè* for preservation and were not satisfied with the hygienic conditions under which the *Wagashi Gassirè* was sold. Group 3 consisted of uneducated consumers who smoked and cooked the cheese in salted water for storage; they were satisfied with the hygiene conditions of the market. Therefore, the appreciation of the level of hygiene depends on the education level of the consumers; however, abnormally, the uneducated consumers fortunately seem to adopt practices for treating the cheese that support the destruction of microbiological contaminants.

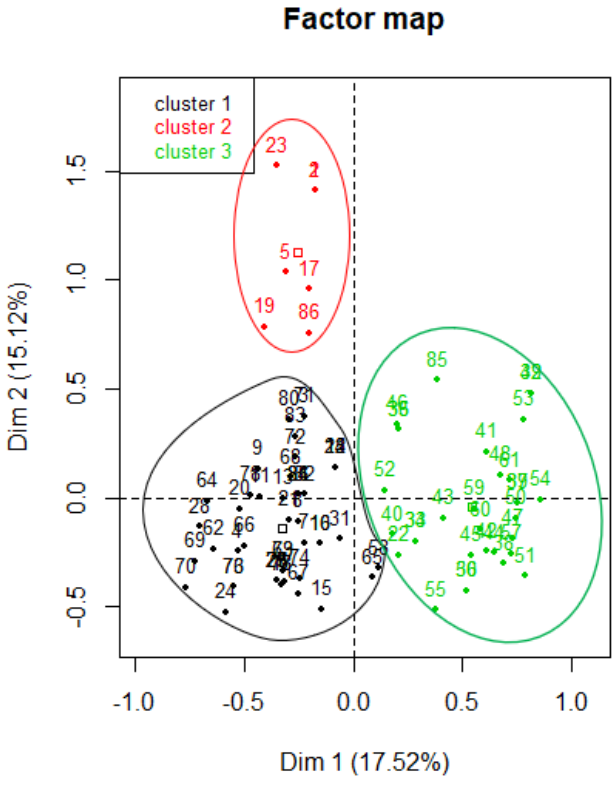

Figure 4.5: Classification of *Wagashi Gassirè* consumers from Dassa-Zoumé and Nikki.

3.8. Presentation of stakeholders according to level of education, ethnicity, and hygiene training

The analysis of the dairy stakeholders according to their sociocultural group as well as their level of education and training on hygiene practices during the milking, production, and preservation of curdled milk and *Wagashi Gassirè* revealed that the *Wagashi Gassirè* trade was practiced by the Dendi and Baatonou in Nikki and the Fon and Idaatcha in Dassa-Zoumé. Milk farming was practiced by mostly uneducated Fulani farmers, some of whom had received training in hygiene, while the female producers of *Wagashi Gassirè* were mostly uneducated and from the Fulani and Gando tribes.

4. Discussion

The husbandry and hygiene practices adopted in the production, processing, and distribution of milk not only affect the quality of the raw milk and its byproducts, but also directly affect human and animal health. Moreover, they also have economic implications (Singh and Ramachandran, 2020). Here, the various stakeholders in the dairy chain were characterized and factors that may affect the sanitary quality of milk and its derivatives in Benin were identified. This study revealed that cattle rearing is practiced mostly by uneducated men of the Fulani group in the two communes considered. In agreement with these results, Dahouda et al. (2019) reported that the majority (62.73%) of cattle dairy farmers in Nikki were of the Fulani ethnic group. The lack of education among most farmers acts as a handicap in the implementation of good husbandry and hygiene in milking practices. According to Gökdai et al. (2020), the education level of stakeholders influences the observance of

good hygiene practices on the farms; uneducated farmers fail to master good hygiene and husbandry practices and lack an understanding of the risks associated with bad practices.

Several diseases, mainly foot and mouth disease, pasteurellosis, dermatophilosis, trypanosomiasis, lumpy skin disease, contagious bovine pleuropneumonia, brucellosis, and calf plague, were found to be prevalent in the surveyed cattle farms, corroborating the findings of Kpodékon et al. (2015), who showed that foot and mouth disease is the dominant viral disease in cattle farms in northern Benin. Sow (2014) also observed that (arranged in decreasing order of importance) foot and mouth disease, pasteurellosis, trypanosomiasis, botulism, lumpy skin disease, mastitis, and symptomatic anthrax were the dominant diseases encountered in dairy cattle farms in the regions of Thiès and Diourbel in Senegal. These diseases cause significant economic losses in terms of milk production and skin condition in the affected animals. Apart from these economic consequences, some of these diseases encountered in animals, such as brucellosis, can affect the quality of milk and meat and consequently the health of consumers. They are diagnosed and treated by the farmers themselves, mostly with antibiotics purchased from pharmacies or veterinary clinics.

Consistently, in another study, 76.7% of farmers used antibiotics without veterinary assistance for the treatment of diseases encountered on cattle farms in northern Benin (Mensah et al., 2019). Similarly, Dognon et al. (2018) observed that only 49% of farmers used the veterinary services in northern Benin for the treatment of their animals. While 59% of farmers obtained veterinary drugs from local markets,

41% procured them from veterinary pharmacies. Here, the antibiotics commonly used to treat animals were penicillin, streptomycin, oxytetracycline, tylosin, and sulphonamides. These results agree with the reports of Dognon et al. (2018) and Mensah et al. (2019), who observed that tetracyclines, penicillin, sulphonamides (47%), and macrolides (17%) were the antibiotic families most used by livestock keepers in northern Benin for treating sick cattle. Most respondents in this study did not follow the manufacturer's instructions when estimating weight and the dosage of the medicine. This proves the existence of the rampant misuse of antibiotics on cattle farms in the surveyed communes.

The guidelines for the post-treatment waiting period were disregarded by all respondents, and the milk from antibiotic-treated cows was used either for direct human consumption or for processing into *Wagashi Gassirè* or curdled milk. These practices suggest an increased likelihood of the contamination of dairy products with antibiotic residues and antibiotic-resistant bacteria, which can adversely affect consumer health (Dognon, 2018). The practice of the unhindered consumption or processing of raw milk contaminated in this manner by concerned stakeholders could possibly be explained by a lack of understanding of the associated health risks by the high number of uneducated respondents in this survey.

Plastics and calabashes are the most commonly used materials for milking equipment. The use of calabashes favours the formation of microbial biofilms that can participate in the rapid fermentation of raw milk. Unlike plastic, which can be properly cleaned when soap and

drinking water are available, calabashes cannot be disinfected easily (Gagara et al., 2019). According to Kebede et al. (2007), the use of different types of containers for milk collection significantly influences the diversity of the flora present in curdled milk and the characteristics of the resulting milk product. Only a minority of the responders complied with many hygiene measures; in particular, hand washing procedures were not followed during the production and preservation of raw milk, curdled milk, and *Wagashi Gassirè*. Group 3, which consisted of dairy cattle farmers who treated their animals themselves without help from veterinarians and had no training in milking hygiene, was the most targeted as not following good hygiene practices. The absence of good hygiene practices reported by majority of respondents and the non-washing of hands, which are reservoirs of microorganisms including *Staphylococcus* in wound infections and faecal coliforms, highlighted that the probability of the contamination of milk and dairy products by pathogenic germs is very high; this was confirmed in the works of Farougou et al. (2012), Boko et al. (2016), Gouissi et al. (2017), Sessou et al. (2019), and Djobo et al. (2021). Indeed, the work of Djobo et al. (2021) showed that raw cow milk from Benin was of unsatisfactory quality, with 1.8×10^8 CFU/mL for total aerobic mesophilic flora (TMC), 4.0×10^7 CFU/mL for faecal coliforms (FC), 3.5×10^7 CFU/mL for *Escherichia coli*, 2.8×10^7 CFU/mL for total coliforms (TC), 2.1×10^7 CFU/mL for faecal Streptococci (FS), 1.6×10^7 CFU/mL for yeasts and molds (YM), 1.7×10^7 CFU/mL for sulfur-reducing anaerobic bacteria (SRA), and 1.2×10^7 CFU/mL for *Staphylococcus* spp. The same observations were recorded by Farougou et al. (2012), where raw cow milk samples collected from Dassa-Zoumé

were determined to be unsatisfactory regarding the total mesophilic aerobic flora (1.1×10^8 CFU/ mL), faecal coliforms (9.2×10^2 CFU/mL1), *Escherichia coli* (0.4×10^1 CFU/mL), and *Staphylococcus aureus* (4.0×10^1 CFU/mL).

Boko et al. (2016) revealed that the average faecal coliforms in curdled milk made from raw cow milk varied from $11.31 \pm 13 \times 10^3$ CFU/mL at 30°C to $0.98 \pm 1.228 \times 10^3$ CFU/mL, while the average *Escherichia coli* count was 0.34 ± 0.89 CFU/mL. Gouissi et al. (2017) and Sessou et al. (2019) showed that *Wagashi* cheese was of poor microbial quality when some pathogens were present. The presence of *Escherichia coli* used as reliable indicator of faecal contamination indicates possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. The low proportion of stakeholders who implement hygiene measures indicates a lack of awareness regarding the risks associated with non-compliance. Therefore, increasing stakeholders' awareness of good hygiene practices in milking and milk handling is necessary to obtain safe, high-quality dairy products.

The results obtained regarding preservation methods corroborate the findings of Sessou et al. (2013), who inventoried the methods studied here and found limitations in *Wagashi Gassirè* production and preservation methods, reflected by their low sanitary quality. The existence of stakeholder subcategories indicates diversity in the employed husbandry and hygiene practices, and consequently diversity in the sanitary and nutritional quality of the target dairy products. In view of these findings, the products targeted may present potential risks

for public health during their consumption, given their contamination with the above-mentioned microorganisms and findings reported in other studies (Michael and Mullan, 2019; Idland et al., 2022). Indeed, several studies have identified milk borne pathogens including Shiga-toxin producing *Escherichia coli* (STEC), *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella* spp. and *Yersinia enterocolitica* in dairy products. The presence of these pathogenic bacteria in milk emerged as major public health concerns, especially for those individuals who still drink raw milk or eat these uncooked dairy products. These bacterial pathogens are a substantial source of health loss globally (Ahmadi, 2019; Idland et al., 2022).

To improve the safety quality and shelf-life of dairy products, factors that contribute to microbiological contamination must be considered at the level of every stakeholder cluster in the dairy chain. The critical points found in this study will be used to improve hygiene quality of dairy products in Benin. Although this study has provided valuable information on the milk sector in Benin for the improvement of dairy products, there are some limitations to our study due to the selection approach of our participants; there was an uneven distribution of participants from different area and level of education.

Future studies should use more rigorous sampling methods, for example in a nationally representative household survey, specifying balanced and adequate representation of important demographic groups. On the other hand, the reluctance of farmers to report truthful status could bias the information obtained.

5. Conclusions

This study assessed the production and storage practices of raw cow's milk, curdled milk, and *Wagashi Gassirè* in two Beninese communes. Several factors were found to affect the sanitary quality of dairy products in these regions. These included the misuse of antibiotics on farms, the non-implementation of good hygiene practices by stakeholders, the sale of products in the open air (as a source of contamination), and the non-availability of coagulants, especially in the Dassa-Zoumé area. This study also confirmed the prevalence of several diseases on cattle farms in these regions, which can influence the quality of their dairy products. A large cluster of dairy cattle farmers treated their animals themselves, without help from veterinarians. These individuals had not received any training in milking hygiene. Training in good hygiene practices should be implemented for all farmers, and a rigorous follow-up of the application of these good practices should be carried out to improve the sanitary quality of the milk produced in these breeding areas.

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7. Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.1050592/full#supplementary-material

8. References

Ahmadi, E. (2019) Potential public health risk due to consumption of contaminated bovine milk with aflatoxin M1 and *Coxiella burnetii* in the West of Iran. *Int. J. Dairy Technol.* 73, 479-485

Boko, C., Adje, Y., Atindogbe, G., Hounmanou, G.M.Y., Dossa, F., Sessou, P., Farougou, S. (2016) Evaluation of the production technologies and the microbial and physico-chemical qualities of curdled milk produced in Benin. *J. Appl. Biosci*. 104,10019 -10033.

Chabi Toko, R., Adegbidi, A., Lebailly, P. (2015) Valorisation des produits laitiers dans les ménages Peul du Nord-Est du Bénin. *Int. J. Biol. Chem. Sci.* 9, 2716-2726.

Cornillon, P.A., Guyader, A., Husson, F., Jégou, N., Josse J., Klutchnikoff, N., Matzner-Lober, E., Rouvière, L., Thieurmel, B., (2018) R pour la statistique et la science des données. *Saic. Rennes*, 420 p.

Dahouda, M., Mama Yero Boubacar, A., Dossa, L.H., Dotche, O.I., Ahounou, G.S., Kiki, P., Youssao, A.K.I. (2019) Stratégies d'alimentation et gestion des ressources alimentaires dans les

élevages bovins des Communes de Nikki, Kalalé et N'Dali au Nord Est Bénin. *Rev. Int. Sci. Appl*. 2, 48-70.

Djobo, O., Sina, H., Kombienou, D.P., Djenontin, M.W., Ahyi, V., Youssao, I.A.K., Adjanohoun, A., Rendueles, M., Baba-Moussa, L. (2021) Uses and microbiological quality of fresh cow's milk sold in three markets in South Benin. *Afr. J. Food Sci*. 15, 345-352.

Dognon, S.R. (2018) Évaluation de l'impact de l'utilisation de molécules bioactives en agriculture et en élevage sur la qualité sanitaire des produits carnés: cas des résidus d'antibiotiques et de pesticides dans la viande bovine consommée au Nord-Est du Bénin. Thèse de doctorat en Sciences Vétérinaires de la Faculté de Médecine Vétérinaire de l'Université de Liège, Belgique, 245p. Available online at: https://hdl.handle.net/2268/229529

Dognon, S.R., Antoine-Moussiaux, N., Douny, C., Gustin, P., Moula, N., Scippo, M.L., Youssao, A.K.I. (2018) The use of antibiotics in cattle in North-East Benin: pharmaceutical inventory and risk practices of cattle breeders. *Trop. Anim. Health Prod.* 50, 1683-1699.

Dossou, J., Atchouké, G.D., Dabadé, D.S., Azokpota, P., Montcho, J. K. (2016) Évaluation comparative de la qualité nutritionnelle et sanitaire du lait de différentes races de vaches de quelques zones d'élevage du Bénin. *Eur. Sci. J.* 12, 141-159.

Dossou, W.A., Seko Orou Baké, M.T., Komagbe, G., Sessou, P., Youssao, A.K.I., Farougou, S., Hounhouigan, D.J., Mahillon, J., Mongbo, R., Poncelet, M., Madode, Y.E., Douny, C., Scippo, M-L., Clinquart, A., Azokpota, P. (2022) Processing and preservation

methods of *Wagashi Gassirè*, a traditional cheese produced in Benin, *Heliyon*, 8, e10605.

FAO (2020) Deuxième partie: Plan National d'investissements Agricoles et de Sécurité Alimentaire et Nutritionnelle de seconde génération (PNIASAN, 2017-2021). Consulté le 14 Mai 2020 à l'adresse suivante: https://www.fao.org/faolex/results/details/fr/c/LEX-FAOC184003/

Farougou, S., Sessou, P., Yehouenou, B., Dossa, F. (2012) Microbiological quality of raw milk processed from cows raised under extensive system in the Republic of Benin. *Res. J. Microbiol*. 7, 337-343.

Gagara, M.H., Sessou, P., Dossa, F., Ahounou, S., Azokpota, P., Youssao, I., Gouro, A.S., Farougou, S. (2019) A study on the indigenous methods of processing milk in Niger. *Curr. Agric. Res. J.* 7, 213-223.

Gökdai, A., Sakarya, E., Contiero, B., Gottardo, F. (2020) Milking characteristics, hygiene, and management practices in Saanen goat farms: a case of Canakkale Province, Turkey. *Italian J. Animal. Sci*. 19, 213-221.

Gouissi, F.M., Assongba, H., Assogba, G.M. (2017) (2017) Evaluation of the microbiological quality of the cheese-based of cow's milk sold in the markets of Porto-Novo (Benin). *The Int. J. Sci. Tech*., 5, 67-70.

Gouvernement du Bénin (2022) Performances agricoles au Bénin : Des progressions en 2020 et de nouvelles ambitions. Accessed online 4 July 2022 at https://www.gouv.bj/actualite/1436/performances-agricoles-benin-progressions-2020-nouvelles-ambitions/

Gouvernement du Bénin (2022) Programme d'Actions du Gouvernement (PAG 2021-2022): présentation générale, 62p. Accessed on 17 July 2022 at file:///C:/Users/home/Downloads/presentation_pag-2021-2026-seance-appropriation-06-01-2021.pdf%20(5).pdf

Groupes de la Banque Africaine de Développement (GBAD) (2022) Bénin - Projet d'appui aux filières lait et viande (PAFILAV). Accessed online 15 July 2022 at https://projectsportal.afdb.org/dataportal/VProject/show/P-BJ-AB0-005?lang=fr

Husson, F., Lê, S., Pagès, J. (2017) Exploratory multivariate analysis by Example Using R, *CRC Press: New York* 15, 77p.

Idland, L., Bø-Granquist, E.G., Aspholm, M., Lindbäck, T. (2022) The ability of Shiga toxin-producing *Escherichia coli* to grow in raw cow's milk stored at low temperatures. *Foods* 11, 3411.

Kebede, A., Viljoen, B.C., Gadaga, T.H., Narvhus, J.A., Lourens-Hattingh, A. (2007) The effect of container type on the growth of yeast and lactic acid bacteria during production of Sethemi, South African spontaneously fermented milk. *Food Res. Int.* 40, 33-38.

Kpodékon, T.M., Ogni, C.A., Dassou, H., Dougnon, T.J., Boko, C., Koutinhouin, G.B., Goussanou, J.S., Akoegninou, A., Youssao, I. (2015) Dominant viral pathologies in the extensive and semi-intensive animal breeding and their treatment mode in ethno veterinary medicine in Benin. *Vet. World* 8, 1424-1434.

MAEP (2017) Synthèse sur le Plan Stratégique de Développement du Secteur Agricole (PSDSA) 2025 et le Plan National d'investissements Agricoles et de Sécurité Alimentaire et

Nutritionnelle PNIASAN 2017-2021 ainsi que leurs documents connexes. République du Bénin, 21p.

Mensah, S.E.P., Sessou, P., Nata, C., Adjahoutonon, K., Yélindo, K.B., Lahamy, O., Farougou, S. (2019) Mode d'utilisation des antibiotiques dans les élevages de bovin laitier sédentaires au nord-ouest du Bénin. *J. Anim. Plant Sci.* 42, 7198-7206.

Michael, W. Mullan, A. (2019) Are we closer to understanding why viable cells of *Mycobacterium avium* subsp. *paratuberculosis* are still being reported in pasteurised milk? *Int. J. Dairy Technol.* 73, 332 -344 doi: 10.1111/1471-0307.12617

MPD (2022) Plan national de développement 2018-2025. Accessed on 15 August 2022 at file:///C:/Users/home/Downloads/mpd_plan-national-developpement_2018-2025_final_14_janv.pdf.pdf

Okry, F., Kpenavoun Chogou, S., Fanou-Fogny, N., Moumouni, I., Hounhouigan, J. (2017) Local governance of a dairy sector in Benin Republic: actors, roles, power relationships and perception of interdependency. *Asian J. Agric. Ext. Econ. Sociol*. 21, 1-14.

SAS (2013) Base, 2nd Edition, SAS ® 9.4 Procedures Guide: Statistical Procedures. *Cary, NC, USA: SAS Institute Inc.*

Sessou, P., Farougou, S., Azokpota, P., Youssao, I., Yèhouenou, B., Ahounou, S., Sohounhloue, K.C.D. (2013) Endogenous methods for preservation of *Wagashi*, a Beninese traditional cheese. *Afr. J. Agric. Res*. 8, 4254-4261.

Sessou, P., Keisam, S., Tuikhar, N., Gagara, M., Farougou, S., Jeyaram, K. (2019) High-throughput Illumina MiSeq amplicon sequencing of yeast communities associated with indigenous dairy products from republics of Benin and Niger. *Front. Microbiol.* 10, 594.

Singh, A., Ramachandran, A. (2020) Assessment of hygienic milking practices and prevalence of bovine mastitis in Small Dairy farms of peri-urban area of Jaipur. *Indian J. Community Med*. 45, S21-S25.

Soule, H.A., Djènontin, J.P.A., Kpera, G.N., Mensah, G.A. (2017) Production laitière bovine au Nord-Bénin. Bulletin de la recherche Agronomique du Bénin 81, 18-25.

Sounon, K.L.S.A., Aho, E., Lesse, P., Ickowicz, A., Messad, S., Lesnoff, M., Houinato, M.R.B. (2019) Evaluation de la valeur économique directe de l'élevage bovin au nord-Bénin. *Int. J. Biol. Chem. Sci.* 13, 8-17.

Sow, E. (2014) Caractéristiques et dominantes pathologiques des élevages bovins laitiers dans les régions de Thiès et de Diourbel. Thèse de doctorat en Médecine Vétérinaire de l'Université Cheick Anta Diop de Dakar, École Inter Etats des Sciences et Médecine Vétérinaires: Sénégal, 112p.

Verruck, S., Balthazar, C.F., Rocha, R.S., Silva, R., Esmerino, E.A., Pimentel, T.C., Freitas, M.Q., Silva, M.C., da Cruz, A.G., Prudencio, E.S. (2019) Dairy foods and positive impact on the consumer's health. *Adv. Food Nutr. Res*. 89, 95-164.

Zannou, O., Agossou, D.J., Koca, I. (2018) Traditional dairy products in the Republic of Benin: *Wagashi* and dèguè. *Global Sci. J.* 6, 630-650.

Chapitre 5

5. Microbiological quality of Beninese dairy products and techno-functional properties of dominant lactic acid bacteria from *Fanirè*

This chapter presents a microbiological characterization of raw milk, fermented milk (*Fanirè*), and *Wagashi Gassirè* produced in the North (Nikki) and Center (Dassa-Zoumé) of Benin. This analysis was conducted using the culture-dependent approach and provided insight into the sanitary quality of the products, particularly on potential microbiological changes that occurred over time when they were stored at room temperature (30°C), without any preservation treatment.

Personal contribution:

I was involved in conceptualizing the study, collecting samples, and conducting microbiological analyses. I also drafted the original manuscript and handled its revision.

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Chapitre 5

Microbiological quality assessment of dairy products from Benin and techno-functional properties of dominant lactic acid bacteria isolated from *Fanirè*, a naturally fermented milk

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Abstract

Milk and its derivatives, like *Fanirè*, a naturally fermented milk called also “lait caillé”, and *Wagashi Gassirè* cheese from Benin, are essential in the Beninese diet. However, their nutrient-rich composition makes them susceptible to microbial growth that could affect their safety and marketability. The present study evaluated both the microbiological quality of traditional dairy products from Dassa-Zoumé and Nikki in Benin, and the antibiotic susceptibility of *Escherichia coli* and *Salmonella enterica* strains isolated from these dairy products. Additionally, the techno-functional properties of dominant lactic acid bacteria (LAB) found in fermented milk samples were investigated. To this end, microbiological analyses were performed on 70 Fulani camps and market samples, including a three-day preservation monitoring (Nikki commune). Results showed high microbial loads of LAB in raw milk (8.7 $\log_{10}$ CFU/mL), fermented milk (9.2 $\log_{10}$ CFU/mL), and *Wagashi Gassirè* cheese (5.3-7 $\log_{10}$ CFU/g). *E. coli* levels exceeded EC regulation 2073/2005 standards (2-3 $\log_{10}$ CFU/g) in raw milk (5.7 $\log_{10}$ CFU/mL), fermented milk (5.8 $\log_{10}$ CFU/mL), and stained *Wagashi Gassirè* (4.1 $\log_{10}$ CFU/g), while *Staphylococcus aureus* levels (<1-2.5 $\log_{10}$ CFU/mL) were within acceptable limits for the vast majority of samples, except for the raw and fermented milk samples from Dassa-Zoumé. *S. enterica* and *Bacillus cereus* were isolated in 10 and 14% of samples, respectively. No significant differences were found between samples from Fulani camps and markets indicating that both types of samples are equally contaminated. Antibiotic susceptibility tests showed that *E. coli* and *S. enterica* isolates were resistant to tylosin and penicillin G. Of the four LAB strains tested, only

Lactococcus lactis strain LBB001 had the potential to acidify milk within 24h at 30°C. Overall, the findings suggest unsatisfactory microbiological quality in the samples and could pose, due to pathogens identified, significant health risks to consumers.

Keywords: *Bacillus cereus,* Dairy products, *Escherichia coli*, *Lactococcus lactis*, microbiological quality, *Salmonella enterica*.

1. Introduction

Agriculture contributes significantly to Benin's economy, accounting for an average of 32.7% of GDP, 75% of export earnings, 15% to government revenue and provides around 70% of jobs. Above all, it also plays a crucial role in ensuring the country's food security (MAEP, 2017). Within this sector, livestock contributes to 13% of agricultural GDP, with a capital value estimated at 242 billion FCFA, including a value of on-farm production of live animals of 67 billion FCFA (GBAD, 2023). Among the products derived from livestock farming in Benin, cow milk plays a major role in the diet and economy of pastoral and other communities, since it helps create jobs and income for the population. Cow milk production in Benin experienced a notable increase, from 118,903 tonnes in 2017 to 128,415 tonnes in 2020 (FAOSTAT, 2022). This increase coincided with a consistent growth in milk marketing and consumption across the nation. However, despite

these advancements, the demand for milk and dairy products within the population remains unfulfilled, both in terms of quality and quantity. This deficit is compensated by imports of milk and dairy products, worth over 7.5 billion FCFA in 2009 (FAO, 2010).

Milk is a food of high nutritional value thanks to its richness in proteins, minerals, vitamins, lipids and carbohydrates (Verruck et al 2019). However, because of its high water and nutrient content, milk undergoes rapid deterioration under the high temperatures typical of tropical countries (Sessou et al., 2013). In the absence of a cold chain, players in the dairy sector resort to different preservation methods and processing techniques adapted to their socio-economic and environmental context (Kèkè et al., 2009). In Benin, milk is processed into various dairy products such as "*Fanirè*", a spontaneous fermented milk obtained by natural fermentation, yoghurt and the traditional cheese "*Wagashi Gassirè*" (Sessou et al., 2023), which is the most widespread and widely consumed.

Despite the social, economic, and nutritional importance of these products in Benin, the conditions under which they are produced do not always guarantee the required safety and wholesomeness and, by extension, consumer safety (Sessou et al., 2013, Gouissi et al., 2017, Dossou et al., 2019). To improve the quality of fermented milk and *Wagashi Gassirè*, it is essential to understand their sanitary quality and the evolutionary dynamics of microbial populations during their production and preservation. The present study aimed to carry out the microbiological characterization of raw milk, *Fanirè* and *Wagashi Gassirè* produced in the communes of Dassa-Zoumé and Nikki in Benin

and to evaluate the techno-functional properties of the dominant lactic acid bacteria (LAB) isolated from fermented milk and the antibiotic-resistant profile of the pathogen bacteria isolated from these products.

2. Materials and methods

2.1. Sampling

During the study, a total of 70 samples were collected, with 35 samples obtained from each commune (Dassa-Zoumé and Nikki) where 5 dairy farms were targeted to supply raw milk from the day's milking mix. Samples of these milks were taken for analysis. To ensure product traceability, a production follow-up was carried out where each raw milk taken from the farms was used to produce fermented milk, unstained (WGB) and stained *Wagashi Gassirè* (WGR). In addition, 5 samples of fermented milk, 5 of WGB and 5 of WGR were also taken from markets in each commune for quality assessment. The samples were stored in a cooler equipped with cold packs and then transported to the laboratory for immediate analysis.

2.2. Enumeration of microflora and pH of the dairy products

The microbiological quality of the samples was assessed using standardized methods to enumerate *Bacillus cereus* (MYP, ISO 7932:2004), *Escherichia coli* (TBX, ISO 16649-1:2018), LAB (MRS Agar, ISO 15124:1998), *Listeria monocytogenes* (alternative method for the enumeration of *L. monocytogenes* using Rapid'L mono, validated in accordance with ISO 16140-2:2016), *Salmonella* spp. (XLD and Rapid *Salmonella*, ISO 6579-1: 2017), *Staphylococcus*

aureus (Baird-Parker + RPF, ISO 6888-3: 2003) and yeasts (ISO 21527-1:2008). The pH of the samples was taken using a HANNA pH meter, previously calibrated with pH 7,4, and 10 buffer solutions, following the method reported by Sessou et al. (2016).

2.3. Culture dependant dynamics of dairy microflora

To appreciate the evolution of the above-mentioned microorganisms and the microbial changes that may occur in these products, a three-day microbial follow-up of raw milk, fermented milk, and *Wagashi Gassirè* was carried out on samples collected in the commune of Nikki only.

2.4. Biochemical and molecular characterizations of bacterial isolates

For a more comprehensive characterization of LAB and yeast for their selection as starters, their biochemical profiles were assessed using API50 CH gallery (Bio Mérieux, France) for LAB and API20C AUX for yeasts, according to the manufacturer's instructions, including the APIWEB software. These steps were followed by the molecular identification of representative LAB, after their grouping according to their biochemical profiles, and the isolated pathogenic bacteria based on their 16S rRNA sequences. The PCR amplification used the Univ1 and Bact4 were respectively 5′-ACTCCTACGGGAGGCAG-3′ and 5′-GGCGTGTGTACAAGGCCCGG-3′, following the method described by Anihouvi et al. (2019). The identification of bacteria was carried out by comparing their 16S rRNA sequence with those from the NCBI databases.

2.5. Antibiotic susceptibility of isolates

The sensitivity of strains of *E. coli* and *Salmonella enterica* was assessed against antibiotics commonly used in the treatment of bacterial infections in animal farms in Benin, including streptomycin (300 µg/disk), tylosin (30 µg/disk), penicillin G (1.4 µg/disk), oxytetracycline (30 µg/disk), sulfamethoxazole-trimethoprim (25 µg/disk), and sulfonamide (300 µg/disk) (Komagbe et al., 2023). The disk diffusion method was employed for this purpose. Blank disks were used as negative controls. Tests were conducted following the Clinical and Laboratory Standards Institute (CLSI) method using Mueller Hinton Agar (MHA) as the culture medium. A bacterial suspension of 0.5 McFarland scale was prepared for each of the pathogenic bacteria in the Mueller Hinton broth. Using a sterile swab, the microbial suspension was spread on the surface of Mueller-Hinton agar. After 15 min, commercial antibiotic-loaded discs were placed on the surface using a disk dispenser. Incubation was carried out at 37°C for 24h, and readings were taken by measuring the inhibition zones' diameters (Prabhurajeshwar and Chandrakanth, 2017). Results were interpreted based on EUCAST (2019) normative values.

2.6. Determination of techno-functional properties of LAB isolates

Following the identification of LAB strains (see below), four strains including *Lactococcus lactis* (LBB001), *Leuconostoc mesenteroides* (LBB007), *Lactobacillus plantarum* (LBB130) and *Lactobacillus fermentum* (LBB275) were selected and characterized for their techno-functional properties. This evaluation encompassed their acidifying and coagulation capacities in milk, proteolytic and lipolytic properties,

antimicrobial and haemolytic activities, as well as their ability to grow at different temperatures and NaCl concentrations, with the perspective of their use as starter cultures and bio-preservatives for improved curd production.

2.6.1. Acidification and coagulation

These tests were performed using methods described by Ribeiro et al. (2014) and Shangpliang et al. (2017). Acidification activity measured by pH variation over time in skimmed milk (Oxoid). Similarly, the coagulation capacity of LAB was evaluated. For this, isolates were grown overnight (O/N) in Man Rogosa and Sharpe (MRS) broth at 30°C. Tubes containing 10 mL of 10% skimmed milk were inoculated with 1% v/v (0.1 mL) of revitalized isolates and incubated at 30°C. pH was measured at 0, 2, 4, 6, 12 and 24h using a portable HANNA pH meter, and coagulation was assessed visually. All experiments were conducted in duplicate.

2.6.2. Proteolytic and lipolytic activities

Proteolytic activity was determined according to the method described by Ribeiro et al. (2014) by spreading LAB suspensions on the surface of skimmed milk agar prepared by adding 10% (w/v) powdered skimmed milk to PCA medium (Oxoid, 450 ml PCA + 50 ml milk). Plates were incubated at 30°C, and after 72h, they were flooded with 1% HCl. Proteolytic activity was indicated by a clear zone around the colonies.

For assessing the lipolytic activity, a colony was picked with a loop and diluted in 9 mL of buffered peptone water (BPW). A quantity of 0.5 mL of this dilution was used to deeply inoculate 200 mL of Nutrient Agar supplemented with 2% Tween 80. After 72h of incubation, lipolytic activity was detected by a clear zone surrounding the colonies in the nutrient agar (Ketrouci et al., 2021).

2.6.3. Haemolytic activity

To evaluate haemolytic activity, Sheep Blood Agar Base (Oxoid) supplemented with 5% of fresh sheep blood was used. Activated LAB cultures were inoculated onto plates containing the medium and incubated at 30°C for 48h. Hemolytic activity was assessed by the degradation of red blood cells in the medium (Sharma et al., 2018).

2.6.4. Growth at different temperatures and NaCl concentrations

This experiment was conducted according to the modified protocol described by Ribeiro et al. (2014). Five mL of MRS broth supplemented with bromocresol purple at a concentration of 0.12 g/l was dispensed into tubes. A volume of 0.1 mL of fresh culture with a turbidity equal to 0.5 McFarland scale was inoculated into screw-cap tubes and incubated for 4-6 days at variable temperatures (4, 30 and 45°C). A qualitative colour change from purple to yellow during the incubation period indicated microbial growth. Similarly, isolates were tested for their tolerance to different NaCl concentrations (4 and 6.5% NaCl), with the incubation procedure being similar as above.

2.6.5. Antimicrobial activity of LAB against *E. coli* and *S. enterica*

To assess antimicrobial activity against potential pathogens, the disk diffusion method was employed. LAB isolates were cultured on MRS agar at 37°C for 48h. A colony was picked and inoculated into 5 mL of MRS broth, then incubated for 24h at 37°C. Likewise, a colony of each pathogenic strain cultured on nutrient agar for 24h at 37°C was used to prepare bacterial suspensions in Mueller Hinton broth. A sterile swab was then used to inoculate the surface of Mueller Hinton Agar (MHA) with the *E. coli* or *S. enterica* pathogens strains isolated from dairy products. Disks of 6 mm diameter were impregnated with 50 µL of LAB suspension and placed on the surface of MHA. Incubation was carried out at 37°C for 24h, and the inhibition zone around the disks was measured using calipers (Nami et al., 2019).

2.7. Statistical Analysis of Data

Statistical analyses of microbiological parameters and pH data were conducted using SAS9.4 (SAS Institute Inc., Cary, NC, USA) and R. The generalized linear models (GLM) procedure of SAS was used for analysis of variance, and Fisher's F test was used to test the significance of factors such as products, origin, and time on the variables studied. Pairwise comparisons between means were conducted using Student's t-test at a significance level of 5%.

3. Results

3.1. Microbiological characteristics of the dairy products from Fulani camps

The samples collected from the Fulani camps in the two municipalities are compared here with each other only at the first analysis time 0h (n=70, with 35 per municipality) to assess the potential differential microbiological quality between the municipality. There is no significant difference (p>0.05) between the average microbial loads and pH of raw milk from the two municipalities of Nikki and Dassa-Zoumé (Fig. 5.S1). The average pH of raw milk samples ranged from 6 (Dassa-Zoumé) to 6.2 (Nikki), while the LAB counts in raw milk from Dassa-Zoumé (8.2 ± 0.9 $\log_{10}$ CFU/mL) are not significantly different to those from Nikki (9.2 ± 1.3 $\log_{10}$ CFU/mL). Similarly, the average yeast loads in samples from Dassa-Zoumé (5.9 ± 0.7 $\log_{10}$ CFU/mL) did not significantly differ from those of Nikki (5.8 ± 0.4 $\log_{10}$ CFU/mL) and *E. coli* was enumerated at levels of 5.4 ± 0.5 *versus* 6 ± 0.9 $\log_{10}$ CFU/for Dassa-Zoumé and Nikki samples, respectively. Of note, the observed counts for *E. coli* for both municipalities and *S. aureus* for Dassa-Zoumé (3.9 ± 0.8 $\log_{10}$ CFU/mL) were all higher than the microbiological criteria applicable to raw milk, except for *S. aureus* in samples from Nikki (1.1 ± 0.8 $\log_{10}$ CFU/mL) that complies with the normative EC regulation 2073/2005 requirements (2-3 $\log_{10}$ CFU/mL) (Fig. 5.S1A).

Regarding samples of fermented milk, the analysed fermented milks showed no significant difference regardless of their origin. The LAB counts in fermented milk samples were 9.3 ± 0.7 $\log_{10}$ CFU/mL for

Dassa-Zoumé samples and 9.2 ± 0.7 $\log_{10}$ CFU/mL for Nikki samples. The *S. aureus* counts in the fermented milks from both municipalities were below the detection limit, hence compliant with this microbiological criterion, unlike those for *E. coli* (6.7 ± 0.7 $\log_{10}$ CFU/mL in Dassa-Zoumé and 4.9 ± 0.7 $\log_{10}$ CFU/mL in Nikki). The average pH of the fermented milks was 4.4 and 4.5, for samples from Dassa-Zoumé and Nikki, respectively (Fig. 5.S1B).

Concerning the quality of cheese samples, no significant difference was observed between the white cheese (WGB) of the two study municipalities. However, the average load of LAB in white cheese from Dassa-Zoumé was higher (5.9 ± 2.7 $\log_{10}$ CFU/mL) compared to that of Nikki samples (4.7 ± 2.8 $\log_{10}$ CFU/mL). The white (unstained) *Wagashi Gassirè* had an average pH of 6.9 ± 0.1 $\log_{10}$ CFU/mL (Fig. 5.S1C). The stained cheese (WGR) from both study areas showed no significant difference at the 5% level for the parameters counted, except for yeast. Indeed, the yeast counts in samples from Dassa-Zoumé (5.3 ± 2.6 $\log_{10}$ CFU/mL) were significantly (p<0.05) different from those of Nikki samples (3.2 ± 2.4 $\log_{10}$ CFU/mL). Finally, the stained cheese (WGR) from Dassa-Zoumé had an average LAB load of 7.3 $\log_{10}$ CFU/mL, while those from Nikki had an average load of 6.8 $\log_{10}$ CFU/mL. The pH (6.5) of the stained cheese remained constant in both study areas (Fig. 5.S1D).

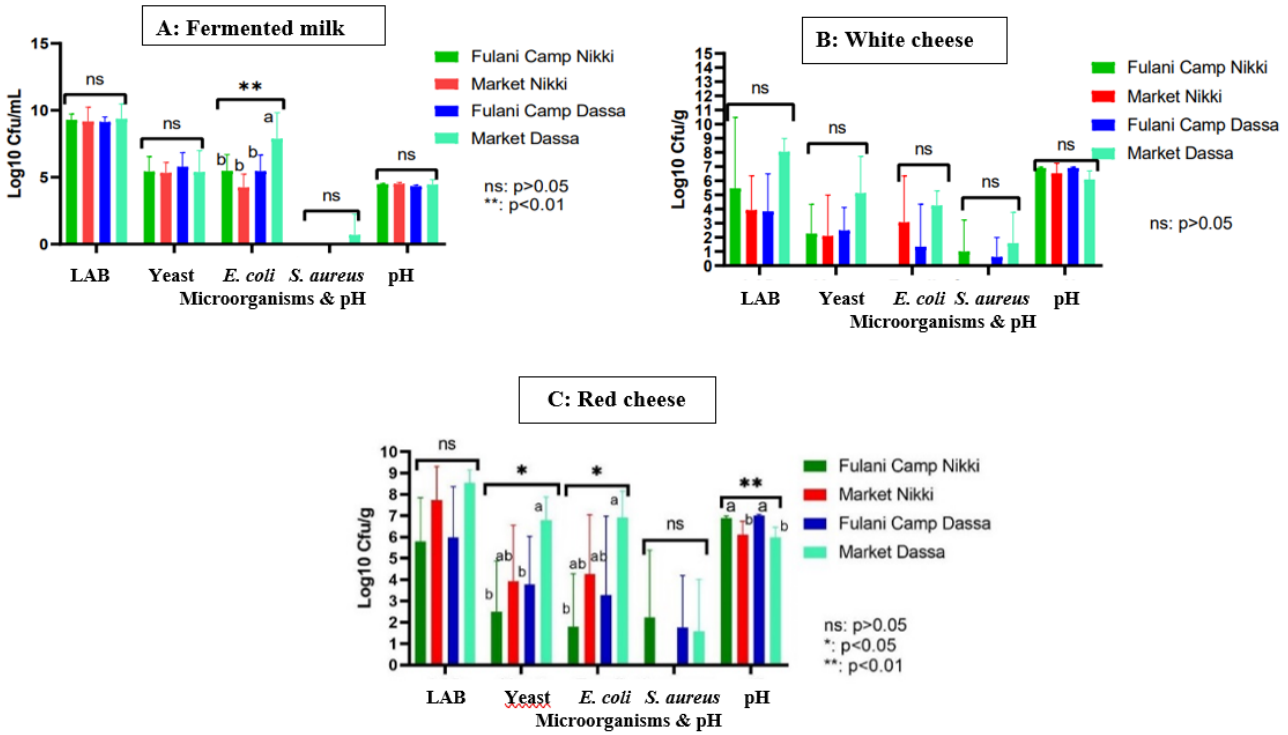
3.2. Dairy product microbiological characteristics: Fulani camps *vs*. markets

The dairy products were collected in Fulani camps and markets and their microbiological features compared. As indicated in Fig. 5.1A, no significant difference was observed between the fermented milks from the two municipalities, whether they originated from the Fulani camps or the markets, when considering LAB, yeast, and *S. aureus*. However, the fermented milk samples from the markets of Dassa-Zoumé were highly contaminated (7.9 ± 1.9 $\log_{10}$ CFU/mL) with *E. coli* and significantly different (p<0.01) from those originating from Fulani camps (5.5 ± 1 $\log_{10}$ CFU/mL). No difference was observed between *E. coli* microbial loads of fermented milk samples from the Fulani camps (5.5 ± 1.2 $\log_{10}$ CFU/mL) and markets of Nikki (4.2 ± 1 $\log_{10}$ CFU/mL). For the unstained *Wagashi Gassirè* (Fig. 5.1B), there was no significant difference between the samples from the two municipalities regardless of the collection location. However, the stained *Wagashi Gassirè* (Fig. 5.1C) showed a significant difference (p< 0.05) for yeast, with a higher load in samples from the market in Dassa-Zoumé (6.8 ± 1.1 $\log_{10}$ CFU/mL). The same observation was made with *E. coli*, where the highest load (6.9 ± 1.3 $\log_{10}$ CFU/mL) was observed in samples from the markets of Dassa-Zoumé (p< 0.05). Additionally, the average pH values of the samples from the Fulani camps were similar and significantly different (p< 0.01) from those of the market samples, which were also similar to each other.

3.3. Dairy product global quality, based on microbiological criteria.

The results obtained from the microbiological analyses of the products were compared to EC regulation 2073/2005 criteria. Table 5.1 presents the percentage of samples with satisfactory quality considering *B. cereus, E. coli, L. monocytogenes, S. aureus, S. enterica*. Results showed that the quality of all raw and fermented milk samples obtained from Nikki camps and markets were substandard. Similar findings were observed for samples collected from Dassa-Zoumé camps, except in the market of this commune, where 80% of fermented milk samples failed to meet quality standards. Additionally, 40% of *Wagashi Gassirè* samples from Nikki Fulani camps were deemed of poor quality, compared to 40% of unstained *Wagashi Gassirè* and 80% of stained *Wagashi Gassirè* in markets within the same commune. In Dassa-Zoumé, 20% of unstained *Wagashi Gassirè* samples from Fulani camps and 40% from markets were found to be unsatisfactory, while 40% from Fulani camps at Dassa-Zoumé and 100% from markets of stained *Wagashi Gassirè* were also of inferior quality. Of note, *L. monocytogenes* was not detected in the samples.

In sum, among the samples, none of the 10 raw milk samples meet the satisfaction criteria, while only one of the 20 fermented milk samples is satisfactory. In contrast, 11 of the 20 unstained *Wagashi Gassirè* (WGB) samples are deemed satisfactory, as well as 7 of the 20 stained *Wagashi Gassirè* (WGR) samples.



a, b, c: The intraclass graphs followed by different letters differ significantly at the threshold of 5%.

Figure 5.1: Comparison of microbial density of the different dairy products, originating from the Fulani camps and markets of the Nikki and Dassa-Zoumé communes.

Table 5.1: Microbiological quality of dairy products according to the EC regulation 2073/2005 Guidelines (2022).

Pathogens	**Percentage of samples with satisfactory microbiological quality**														
		Nikki							**Dassa-Zoumé**						
		Fulani camp				**Market**			**Fulani camp**				**Market**		
	Limit (CFU g^{-1})	RM	FM	WGB	WGR	FM	WGB	WGR	RM	FM	WGB	WGR	FM	WGB	WGR
		n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5	n=5
E. coli	(*) 10^2 - 10^3 (**) <10	0	0	100	60	0	60	20	0	0	80	60	20	20	0
S. aureus	10^2 - 10^3	80	100	80	60	100	100	100	20	100	100	60	20	60	80
S. enterica	Absence in 25 g or mL	100	80	60	100	80	80	60	100	100	100	100	100	100	100
L. monocytogenes	10^2	100	100	100	100	100	100	100	100	100	100	100	100	100	100
B. cereus	10^5	100	100	80	100	80	80	80	80	80	100	80	100	100	60

Concentration limit admitted for raw milk and cheese (*) and for fermented milk (**). RM: Raw milk, FM: Fermented Milk, WGB: unstained *Wagashi Gassirè*, WGR: stained *Wagashi Gassirè*.

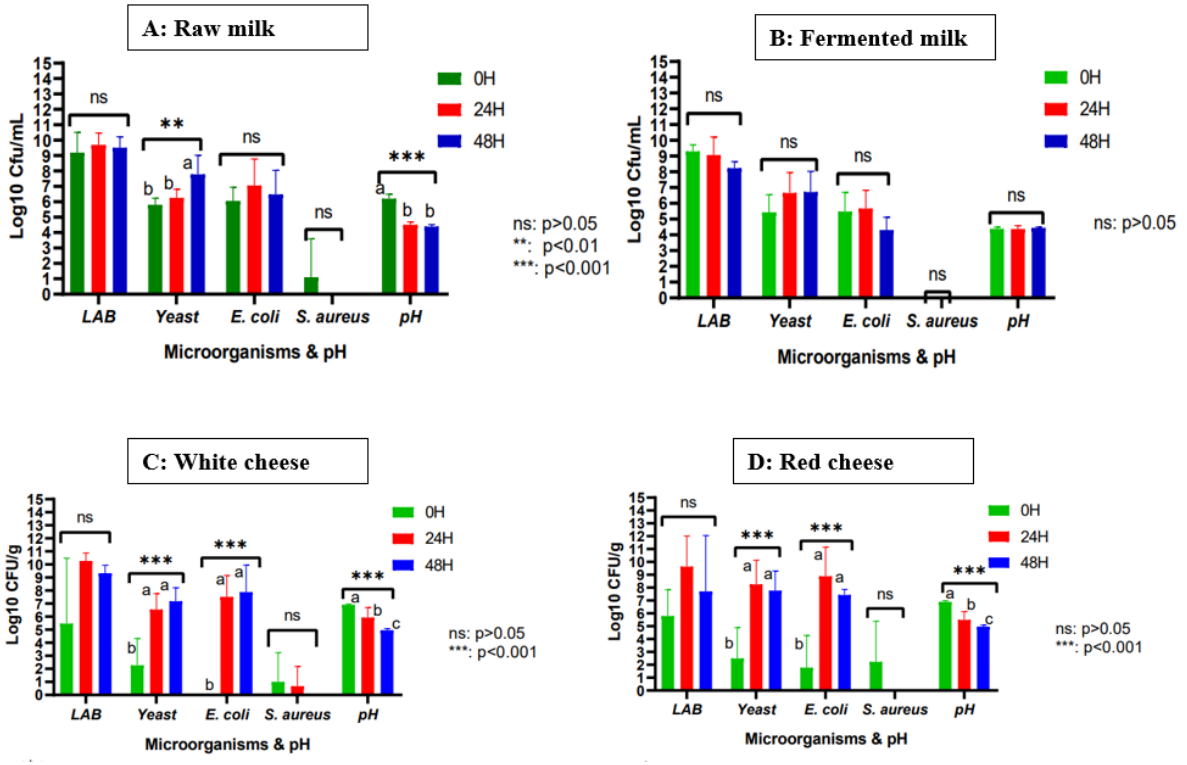
3.4. Dynamics of microbial populations and pH of dairy samples

The products collected from Nikki were analyzed at three time points spaced 24h apart (0, 24 and 48h) to assess the evolution of different parameters when the products are stored at room temperature without heat treatment.

For raw milks (Fig. 5.2A), the average counts of LAB, *E. coli*, and *S. aureus* did not vary significantly between the three time points, unlike those of yeast. Indeed, at the third analysis time (48h), the yeast count in raw milks was higher (7.8 ± 1.2 log10 CFU/mL) and significantly different ($p<0.01$) from those obtained at 0 and 24h. The average pH of the raw milk samples at 0h (pH = 6.2 ± 0.3) was significantly different from those at 24h (pH 4.5 ± 0.2) and 48h (pH 4.4 ± 0.1). About fermented milks (Fig. 5.2B), the average counts obtained at the three analysis times showed no significant difference ($p>0.05$) for all the counted parameters. The same observation was made for the pH, which remained constant. The *Wagashi Gassirè* (stained and unstained) displayed no significant difference ($p>0.05$) over time for LAB and *S. aureus*, unlike yeast, *E. coli*, and pH, which differed significantly ($p<0.001$) (Fig. 5.2C and 5.2D).

3.5. Nature of dominant isolates from fermented milk

To identify dominant LAB and yeast isolates in the fermented milks and confirm the nature of the isolated pathogenic microorganisms from these dairy products, several tools or tests were conducted. Five dominant isolates were selected per sample. In total, fifty isolates of LAB were obtained and classified into 13 groups based on their biochemical profiles using API 50CH gallery. The most dominant LAB in decreasing order were *L. mesenteroides* (64%), *Streptococcus lutetiensis* (18%), *L. lactis* (14%), *Lb. plantarum* (2%), and *Lb. fermentum* (2%). Representative isolates of these groups of LAB previously identified by gallery API were subjected to 16S RNA sequencing. Results showed that *L. lactis, S. lutetiensis, L. mesenteroides, Lb. plantarum* and *Lb. fermentum* were, in decreasing order, the most dominant LAB (Table 5.2). As for the yeast, the 100 identified species were grouped into 23. The dominant species were *Candida* species *(C. colliculosa, C. famata, C. glabrata, C. guillermondi, C. krusei, C. kefyr, C. lusitaniae, C. parapsilosis, C. rugosa, C. tropicalis* and *C. utilis*), *Cryptococcus* species (*C. humicola, C laurentii, C. terreus*), *Geotrichum klebahnii, Kadamaca ohmeri, Rhodotorula mucilaginosa, Saccharomyces cerevisiae, Trichosporon asahii* and *Trichosporon mucoides*.



a, b, c: The intraclass graphs followed by different letters differ significantly at the threshold of 5%.

Figure 5.2: Dynamic of microbial populations and pH over time in studied dairy product samples.

T**able 5.2:** Strains identified by 16S rRNA sequencing.

Bacteria (Number of the isolates)	**Closest reference strain (Identity > 99%)**
LAB (n=13)	
LBB007, LBB009, LBB014, LBB060, LBB086, LBB114	*Leuconostoc mesenteroides* NBRC 100496
LBB001, LBB133, LBB260	*Lactococcus lactis* NBRC 100933
LBB004, LBB006	*Streptococcus lutetiensis* NEM 782
LBB130	*Lactobacillus plantarum* JCM 1149
LBB275	*Lactobacillus fermentum* CIP 102980
***E. coli* (n=14)**	
ECB001 - ECB007, ECB036 - ECB042	*E. coli* NBRC 102203
***Salmonella* spp. (n=7)**	
SAL03 - SAL08, SAL10	*S. enterica* subsp. *enterica* strain LT2
***B. cereus* (n=5)**	
BCB01- 02, BCB06, BCB12 – 13	*B. cereus sensu lato*

3.6. Antibiotic-resistance profiles of isolates

Antibiotic susceptibility results for *E. coli* (n=14) and *S. enterica* (n=7) isolates from dairy products revealed that *E. coli* were resistant to sulfonamide (10/14; 71.4%), streptomycin (3/14; 21.43%), sulfamethoxazole-trimethoprim (5/14; 35.71%) and oxytetracycline (7/14; 50%), but all were resistant to tylosin and penicillin. For the *S. enterica* strains, they were all sensitive to streptomycin, sulphamethoxazole-trimethoprim and oxytetracycline, resistant to sulfonamide (6/7; 85.71%), and all resistant to tylosin and penicillin G.

3.7. Techno-functional properties of LAB

The techno-functional properties were assessed on four LAB strains selected based on their technological potential according to literature: *L. lactis* LBB 001, *L. mesenteroides* LBB 007, *Lb. plantarum* LBB 130 and *Lb. fermentum* LBB 275. In addition, their potential antimicrobial activity was assessed on the 21 pathogenic strains (14 *E. coli* and 7 *S. enterica*) isolated from the dairy products.

As shown in Fig. 5.3, 12h of incubation, *L. lactis* LBB 001 displayed a good acidification rate (pH below 5), followed by *L. mesenteroides* LBB 007, which dropped the pH below 5 after 24h of incubation. *Lb. plantarum* LBB 130 and *Lb. fermentum* LBB 275 were the slowest acid producers, requiring more than 24h to decrease the pH below 5 closed to 4.5, which is the maximum acceptable pH value for good fermented milk (Katinan et al., 2012). However, a combination of *L. lactis* LBB 001 with each of the initially slow acidifying strains (LBB 007, LBB

130 and LBB 275) and inoculated into skimmed milk succeeded in lowering the milk pH below 5 after 12h of incubation (Fig. 5.3).

Furthermore, after 24h of incubation, strains LBB001 and LBB 007 allowed for good milk coagulation. Strain LBB 001 showed the fastest coagulation rate, as it was able to coagulate the milk after 12h of incubation. In contrast, LBB 130 and LBB 275 were unable to coagulate the milk within 24h (data not shown). These results revealed that strains of *L. lactis* LBB 001 and *L. mesenteroides* LBB 007 are good candidates for better quality of fermented milk meeting standards (pH below 5). Also, as reported in Table 5.3, both LBB 130 and LBB 275 exhibited good growth at 6.5% NaCl, unlike the other two LAB strains. No proteolytic or lipolytic activities could be detected from the 4 LAB strains.

To ensure the absence of pathogenic potential and infection from a starter, haemolytic activity performed on these LAB showed that none of the examined LAB strains exhibited haemolytic activity, as evidenced by the absence of a clear zone around the bacterial colony. The evaluation of the antimicrobial activity of LAB strains against *E. coli* and *S. enterica* strains showed that LBB 007, LBB 130, and LBB 275 had no inhibitory effect on the tested pathogens. However, a weak inhibitory effect (with an inhibition diameter of 8 mm) of LBB 001 against the majority (71%) of the investigated pathogens was observed (data not shown).

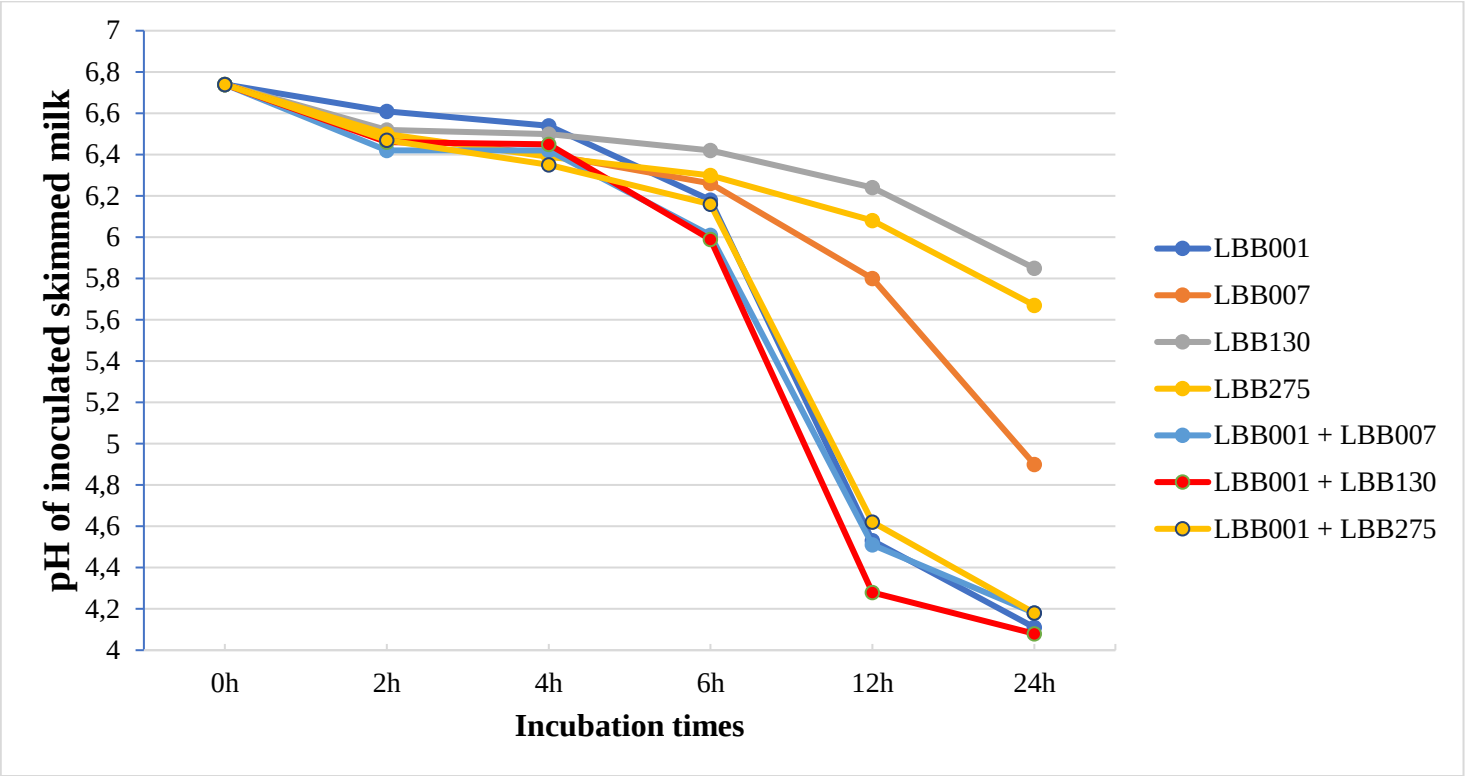


Figure 5.3: Evolution of the pH of skimmed milk inoculated with LAB strains over time.

Table 5.3: Evaluation of growth and enzymatic capacities of the four LAB strains.

	Proteolytic activity*	**Lipolytic activity***	**Growth at different temperatures (°C) ****			**Growth at different concentrations of NaCl (%)****		
			4	30	45	4	6.5	10
LBB 001	-	-	-	++	-	++	-	-
LBB 007	-	-	-	++	-	+	-	-
LBB 130	-	-	+	++	-	++	++	-
LBB 275	-	-	-	++	-	++	++	-

*: "-"indicates that no activity could be detected. **: "++": normal growth, "+": slow growth, "-": no growth. The growth at different NaCl concentrations was tested at 30°C.

4. Discussion

Raw milk and its derivatives have long been perceived as sources of essential nutrients and authentic flavours. However, they harbour various microorganisms that can compromise their safety. Ensuring dairy products safety is vital for human health and economic interests (Peng et al., 2023). Although raw milk typically has low bacterial contamination initially (Martin et al., 2023), handling and processing can introduce pathogenic and beneficial bacteria from various sources (Wu et al., 2018), posing risks of infectious diseases (Olaniran et al., 2022). Our study examined the microbiological quality of raw milk and its derivatives, highlighting significant results with important implications for food safety. The study revealed that the overall microbiological quality of the analyzed products is unsatisfactory. Indeed, regarding hygiene indicator microorganisms, the study showed that the samples have microbial loads exceeding normative limits. The presence of several concerning (potential) pathogens, including *B. cereus, E. coli*, *S. aureus* and *S. enterica* was observed. The detection of such foodborne pathogens in our samples underscores a potential risk to public health. Indeed, these microorganisms are associated with severe foodborne infections and can pose a risk to consumers, ranging from gastrointestinal infections to more severe complications. For instance, *S. enterica* is one of the known pathogens in dairy products, and it is estimated that each year, 1.4 million illnesses are due to *Salmonella* infection, with over 16,000 hospitalizations and 580 deaths in the United States (Mazurek et al., 2004). *E. coli* is another foodborne pathogen present in milk and its products, causing serious illnesses. Some serotypes are capable of causing various diseases in humans,

ranging from mild cases of diarrhoea to severe cases of haemorrhagic colitis and haemolytic uremic syndrome (Paswan et al., 2020). *B. cereus*, a Gram-positive, endospore-forming bacterium, is commonly found in various natural environments and is particularly prevalent in foods, notably dairy products. The major symptoms of food poisoning caused by *B. cereus* typically manifest as either diarrhoea and/or emesis (Liu et al., 2020). *S. aureus* presence in raw milk poses health risks due to toxin production. Their presence highlights the need to implement strict control measures throughout the dairy product supply chain, from production to distribution.

Furthermore, it is worth noting the absence of *L. monocytogenes*, which is an encouraging aspect of our results. Indeed, *L. monocytogenes* is responsible for human listeriosis which is one of the deadliest foodborne diseases with fatality rates as high as 30%. The absence of these pathogens in our samples suggests that certain production or handling practices may be effective in reducing their presence.

We have also observed an abundance of *Candida* yeast and LAB, including dominant species such as *L. lactis*, *Lb. fermentum*, *L. mesenteroides*, and *Lb. plantarum*. LAB counts were high in both raw and fermented milk, with fermentation leading to a notable increase in fermented milk. Their abundance attests natural fermentation processes and contributes to the sensory richness of the finished products. They are also often associated with beneficial effects on human health, including promoting healthy digestion and strengthening the immune system. *L. lactis* has probiotic properties, and some strains have the ability to produce an antimicrobial peptide called nisin, which has

preservation potential against pathogens like *L. monocytogenes*. Its application in dairy products to naturally preserve food contribute to health promotion attributes through probiotic activity would certainly meet the demands of today's consumers (Khemariya et al., 2017, Lim et al., 2020, Na-Kyoung et al., 2015). *Lb. fermentum* has probiotic properties and enhances the immune response, prevents community-acquired upper respiratory and gastrointestinal infections.

The evaluation of techno-functional properties of the LAB strains in our studies revealed that *L. lactis* LBB 001 and *L. mesenteroides* LBB 007 were good candidates for better quality fermented milk meeting standards (pH below 4) as they displayed good acidification speed (pH decrease below 5) and allowed for good milk coagulation. Although these two strains are promising candidates, before their potential use by the food or dairy industry, they must be evaluated regarding their virulence traits. Indeed, some strains of *L. lactis* may harbour a number of unexpressed virulence genes which can confer important pathogenic characteristics (haemolysis) which can pose a risk to consumers (Fusieger et al., 2020). Some strains of *L. mesenteroides* can potentially induce bacteraemia some are known for their ability to produce biogenic amines, formed by the decarboxylation of amino acids that can accumulate in fermented dairy products and cause toxicological effects on consumers (Menegueti et al., 2018). Finally, it is important to note that other LAB are harmful. This is the case *S. lutetiensis* an haemolytic species responsible for infections in both animals and humans, (Gaspar et al., 2018, Chen et al., 2021).

The LAB strains investigated in this study showed no proteolytic or lipolytic activity. Lipolytic activity in different types of curdled milk is

not highly desirable, even though a low level of lipolysis is important for forming the aroma and structure of the final product. Excessive lipolysis can impart a bitter and rancid taste to the curdled milk (Monfredini et al., 2012). Although LAB are not considered highly proteolytic bacteria, their proteolytic system is essential for optimal growth in milk and significantly contributes to the development of certain organoleptic characteristics in various fermented dairy products (Morandi et al., 2013). Furthermore, proteolysis could also contribute to preventing common allergies in children under 3 years old due to poor digestion of milk proteins (de Almeida Júnior et al., 2015). The absence of proteolytic activity is therefore both detrimental and beneficial in the sense that, for the latter aspect, non-proteolysis will stabilize the product over time.

Antibiotic resistance is a serious global health problem, with potentially harmful consequences for animal health, public health and the environment. This study revealed that *E. coli* and *S. enterica* strains isolated from the products studied were resistant to tylosin and penicillin, two antibiotics commonly used by dairy farmers in the study areas (Komagbe et al., 2022). Like the present study, Bagré et al. (2014) observed, in Burkina Faso, penicillin-resistant *E. coli* and *Salmonella* spp. strains isolated from raw and fermented milk. This resistance can be explained by the massive and uncontrolled use of antibiotics in the treatment of bovine pathologies by dairy farmers and could cause antibiotic therapy to fail in the concerned areas. (Dognon et al., 2019). Particular attention should therefore be paid to the antibiotic distribution sector in order to control informal sources and prevent the emergence of resistant strains.

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6. References

de Almeida Júnior, W.L.G., da Silva Ferrari, Í., de Souza, J.V., da Silva, C.D.A., da Costa, M.M., Dias, F.S. (2015) Characterization and evaluation of lactic acid bacteria isolated from goat milk. *Food Control*, 53, 96-103.

Anihouvi, D.G.H., Kpoclou, Y.E., Abdel Massih, M., Iko Afe, O.H., Assogba, M.F., Covo, M., Scippo, M-L., Hounhouigan, J.D., Anihouvi, V., Mahillon, J. (2019) Microbiological characteristics of smoked and smoked-dried fish processed in Benin. *Food Sci. Nutr*. 7, 1821-1827.

Bagré, T.S., Kagambèga, A., Bawa, I.H., Bsadjo Tchamba, G., Dembélé, R., Zongo, C., Savadogo, A. Aggad, H. Traoré, A.S. Barro, N. (2014) Antibiotic susceptibility of *Escherichia coli* and *Salmonella* strains isolated from raw and curds milk consumed in Ouagadougou and Ziniaré, Burkina Faso. *Afr. J. Microbiol. Res.* 8, 1012-1016.

Chen, M.J., Chen, C.C., Huang, Y.C., Tseng, C.C., Hsu, J.T., Lin, Y.F. (2021) Taiwan gastrointestinal disease, *Helicobacter* consortium. The efficacy of *Lactobacillus acidophilus* and *rhamnosus* in the reduction of bacterial load of *Helicobacter pylori* and modification of gut microbiota-a double-blind, placebo-controlled, randomized trial. *Helicobacter* 26, e12857.

Clinical and Laboratory Standards Institute (CLSI) (2020) CLSI document M100. Performances standards for antimicrobial susceptibility testing, 30th ed informational supplement, Wayne, Pa.

Dognon, S.R., Antoine-Moussiaux, N., Douny, C., Gustin, P., Moula, N., Scippo, M.L., Youssao, A.K.I. (2018) The use of antibiotics in cattle in North-East Benin: pharmaceutical inventory and risk practices of cattle breeders. *Trop. Anim. Health Prod*. 50, 1683-1699.

EUCAST (2019) Comité de l'antibiogramme de la Société Française de Microbiologie. Détermination de la sensibilité aux antibiotiques. 144 pp.

European Commission (2005) Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union* 338, 1-26.

FAOSTAT (2022) Organisation des Nations unies pour l'alimentation et l'agriculture Base de données statistiques sur la production agricole. https://www.fao.org/faostat/fr/#data/QCL

Fusieger, A., Perin, L.M., Teixeira, C.G., de Carvalho, A.F., Nero, L.A. (2020) The ability of *Lactococcus lactis* subsp. *lactis* bv.

diacetylactis strains in producing nisin. *Antonie Van Leeuwenhoek*, 113(5), 651-662.

Gaspar, C., Donders, G.G., Palmeira-de-Oliveira, R., Queiroz, J.A., Tomaz, C., Martinez-de-Oliveira, J., Palmeira-de-Oliveira, A. (2018) Bacteriocin production of the probiotic *Lactobacillus acidophilus* KS400. *Amb. Express* 8, 1-8.

Gouissi, F.M., Assongba, H., Assogba, G.M. (2017) Evaluation of the microbiological quality of the cheese-based of cow's milk sold in the markets of Porto-Novo (Benin). *The Int. J. Sci. Tech*., 5, 67-70.

Groupe de la Banque Africaine de Développement (GBAD) (2023) Bénin - Projet d'appui au développement des filières lait et viande et à la promotion des entreprises d'élevage (PRODEFILAV-PEL)- Rapport d'évaluation de projet. Consulté le 10 avril 2023 à l'adresse https://www.afdb.org/fr/documents/benin-projet-dappui-au-developpement-des-filieres-lait-et-viande-et-la-promotion-des-entreprises-delevage-prodefilav-pel-rapport-devaluation-de-projet

Katinan, C.R., Sadat, A.W., Chatigre, K.O., Bohoussou, K.M., Assidjo, N.E. (2012) Assessment of chemical and microbiological quality of home-made fermented milk produced and consumed in Yamoussoukro town, Côte d'Ivoire. *J. Appl. Biosci.* 55, 4020-4027.

Keke, M., Yehouenou, B., de Souza, C., Sohounhloué, D.C.K. (2009) Evaluation of hygienic and nutritional quality of Peulh cheese treated by *Sorghum vulgaris* (L) and *Pimenta racemosa* (Miller) extracts. *Sci. Res.* 10, 29-46.

Ketrouci, L., Dalache, F., Benabdelmoumene, D., Dahou, A.A., Homrani, A. (2021) Technological characterization of lactic acid

bacteria isolated from different sheep's milk. *Asian J. Dairy Food Res.* 40, 239-245.

Khemariya, P., Singh, S., Nath, G., Gulati, A.K. (2017) Probiotic *Lactococcus lactis*: A review. *Turkish J. Agric. Food Sci. Technol.* 5, 556-562.

Komagbe, G.S., Dossou, A., Seko Orou, B. M-T., Sessou, P., Azokpota, P., Youssao, I., Hounhouigan, J., Scippo, M-L., Clinquard, A., Mahillon, J. Farougou, S. (2023) State of the art of breeding, milking, and milk processing for the production of fermented milk and *Wagashi Gassirè* in Benin: Practices favoring the contamination of its dairy products. *Front. Sustain. Food Syst.* 6, 1050592.

Lim, S.M., Lee, N.K., Paik, H.D. (2020) Potential neuroprotective effects of heat-killed *Lactococcus lactis* KC24 using SH-SY5Y cells against oxidative stress induced by hydrogen peroxide. *Food Sci. Biotechnol.* 29, 1735-1740.

Liu, X-Y., Hu, Q., Xu, F., Ding, S-Y., Zhu, K. (2020) Characterization of *Bacillus cereus* in Dairy Products in China. *Toxins* 12, 454.

MAEP (2017) Synthèse sur le Plan Stratégique de Développement du Secteur Agricole (PSDSA) 2025 et le Plan National d'investissements Agricoles et de Sécurité Alimentaire et Nutritionnelle PNIASAN 2017-2021 ainsi que leurs documents connexes. République du Bénin, 21p.

Martin, N.H., Evanowski, R.L., Wiedmann, M. (2023) Invited review: Redefining raw milk quality-Evaluation of raw milk microbiological parameters to ensure high-quality processed dairy products. *J. Dairy Sci*. 106, 1502-1517.

Mazurek, J., Salehi, E., Propes, D., Holt, J.O., Bannerman, T., Nicholson, L.M., Moolenaar, R.L. (2004) A multistate outbreak of *Salmonella enterica* serotype *Typhimurium* infection linked to raw milk consumption-Ohio, 2003. *J. Food Prot.* 67, 2165-2170.

Menegueti, M.G., Gaspar, G.G., Laus, A.M., Basile-Filho, A., Bellissimo-Rodrigues, F., Auxiliadora-Martins, M. (2018) Bacteremia by *Leuconostoc mesenteroides* in an immunocompetent patient with chronic Chagas disease: a case report. *BMC Infect. Dis.* 18, 1-3.

Méthode alternative à la norme ISO 11290-1(2017) validée conformément à la norme ISO 16140-2:2016

Monfredini, L.U.C.A., Settanni, L., Poznanski, E., Cavazza, A., Franciosi, E. (2012) The spatial distribution of bacteria in Grana-cheese during ripening. *Syst. Appl. Microbiol.* 35, 54-63.

Morandi, S., Cremonesi, P., Silvetti, T., Brasca, M. (2013) Technological characterisation, antibiotic susceptibility and antimicrobial activity of wild-type *Leuconostoc* strains isolated from north Italian traditional cheeses. *J. Dairy Res.* 80, 457-466.

Na-Kyoung, L., Kyoung, J.H., Sung-Ho, S., Su, J.E., Si-Kyung, L., Hyun-Dong, P. (2015) Multifunctional effect of probiotic *Lactococcus lactis* KC24 isolated from kimchi. *LWT-Food Sci. Technol.* 64, 1036-1041.

Nami, Y., Vaseghi Bakhshayesh, R., Mohammadzadeh Jalaly, H., Lotfi, H., Eslami, S., Hejazi, M. (2019) Probiotic properties of *Enterococcus* isolated from artisanal dairy products. *Front. Microbiol.* 10, 300.

NF EN ISO 15214, (1998) Microbiologie des aliments - Méthode horizontale pour le dénombrement des bactéries lactiques mésophiles - Technique par comptage des colonies à 30 degrés C

NF EN ISO 16649-1 (2018) Microbiologie alimentaire - Méthode horizontale pour le dénombrement des *Escherichia coli* bêta-glucuronidase positive - Partie 1: Technique de comptage des colonies à 44 °C au moyen de 5-bromo4-chloro-3-indolyl bêta-D-glucuronate.

NF EN ISO 6579-1 (2017) Microbiologie des aliments - Méthode horizontale pour la recherche des *Salmonella* spp. Indice de classement : V08-013. 99. NF EN ISO 10272-1, 2017.

NF EN ISO 6887-5 (2010) Microbiologie des aliments - Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique - Partie 5: Règles spécifiques pour la préparation du lait et des produits laitiers.

NF EN ISO 6888-3 (2003) Microbiologie des aliments - Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (*Staphylococcus aureus* et autres espèces) - Partie 3: recherche et méthode NPP pour les faibles nombres.

NF ISO 21527-1 (2008) Microbiologie des aliments - Méthode horizontale pour le dénombrement des levures et moisissures - Partie 1: Technique par comptage des colonies dans les produits à activité d'eau supérieure à 0,95.

NF ISO 7932 (2004) Amendement 1 (2020) Microbiologie des denrées alimentaires et des aliments pour animaux. Méthode horizontale pour le dénombrement des *Bacillus cereus* présumés. Technique de comptage des colonies à 30 degrés C.

Olaniran, O.B., Iroko, O.T., Famojuro, T.I., Oluwatobi, O.B., Adeleke, O.E. (2022) Microbiological quality assessment of dairy product: detection of extended spectrum beta lactamase genes in bacterial isolates from yoghurt sold in Sagamu Metropolis, Ogun State, Nigeria. *DUJOPAS.* 8, 159-172.

Paswan, R., Park, Y.W. (2020) Survivability of *Salmonella* and *Escherichia coli* O157: H7 pathogens and food safety concerns on commercial powder milk products. *Dairy*, 1, 189-201.

Peng, Z., Li, Y., Yan, L., Yang, S., Yang, D. (2023) Correlation analysis of microbial contamination and alkaline phosphatase activity in raw milk and dairy products. *Int. J. Environ. Res. Public Health* 20, 1825.

Prabhurajeshwar, C., Chandrakanth, R.K. (2017) Probiotic potential of *Lactobacilli* with antagonistic activity against pathogenic strains: An in vitro validation for the production of inhibitory substances. *Biomed. J.* 40, 270-283.

Ribeiro, S.C., Coelho, M.C., Todorov, S.D., Franco, B.D.G.D.M., Dapkevicius, M.L.E., Silva, C.C.G. (2014) Technological properties of bacteriocin-producing lactic acid bacteria isolated from Pico cheese an artisanal cow's milk cheese. *J. Appl. Microbiol*. 116, 573-585.

Sessou, P., Boko, C., Hounmanou, G., Osseni, S.D., Hounkpe, E., Azokpota, P., Youssao, I., Sohounhloue, D., Farougou, S. (2016) Preservation of traditional cheese *Wagashi* using essential oils: impact on microbiological, physico-chemical and sensorial characteristics. *Br. Microbiol. Res. J.* 15, 1-13.

Sessou, P., Farougou, S., Yehouenou, B., Agniwo, B., Alitonou, G.A., Azokpota, P., Youssao, I., Sohounhloue, D. (2013) Biological control of spoilage and pathogens moulds in culture medium and Beninese traditional cheese *Wagashi* by *Syzygium aromaticum* essential oil. *Afr. J. Microbiol. Res*., 7, 2454-2463.

Sessou, P., Keisam, S., Gagara, M., Komagbe, G., Farougou, S., Mahillon, J., Jeyaram, K. (2023) Comparative analyses of the bacterial communities present in the spontaneously fermented milk products of Northeast India and West Africa. *Front. Microbiol.* 14, 1166518.

Shangpliang, H.N.J., Sharma, S., Rai, R. Tamang, J.P. (2017) Some Technological Properties of Lactic Acid Bacteria Isolated from *Dahi* and *Datshi*, naturally fermented milk products of Bhutan. *Front. Microbiol*. 8, 216942.

Sharma, S., Kandasamy, S., Kavitake, D., Shetty, P.H. (2018) Probiotic characterization and antioxidant properties of *Weissella confusa* KR780676, isolated from an Indian fermented food. *LWT* 97, 53-60.

Verruck, S., Balthazar, C.F., Rocha, R.S., Silva, R., Esmerino, E.A., Pimentel, T.C., Freitas, M.Q., Silva, M.C., da Cruz, A.G., Prudencio, E.S. (2019) Dairy foods and positive impact on the consumer's health. *Adv. Food Nutr. Res.* 89, 95-164.

Wu, X., Lu, Y., Xu, H., Lv, M., Hu, D., Lui, Z., Liu, L., Wang, Z., Feng, Y. (2018) Challenges to improve the safety of dairy products in China. *Trends in Food Sci. Technol.* 76, 6-14.

Supplementary data

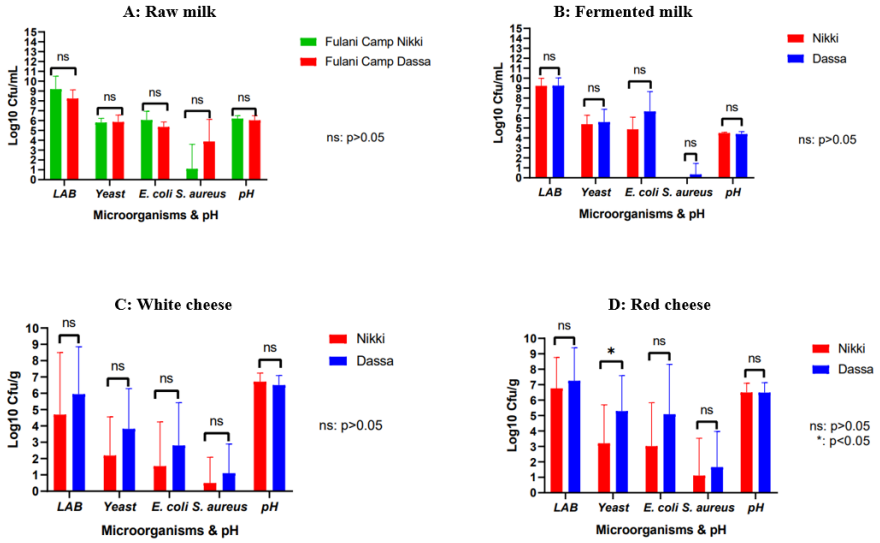

Figure 5.S1: Comparison of the microbial density of different dairy products from Fulani camps in the communes of Nikki and Dassa-Zoumé.

Chapitre 6

6. Diversity and dynamics of bacterial populations in fermented milk (*Fanirè*) and cheese (*Wagashi Gassirè*) from Benin

Following the previous study, a microbiological characterization of raw milk, fermented milk, and *Wagashi Gassirè* was then conducted using a culture-independent approach. This allowed exploration of the diversity and dynamics of cultivable and non-cultivable bacterial populations in the products when stored at room temperature (30°C) without any preservation treatment.

Personal contribution:

I was involved in conceptualizing the study, collecting samples, and conducting metagenomic analyses. I also drafted the original manuscript and handled its revision.

This work is in preparation for publication:

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Chapitre 6

Bacterial diversity and dynamics in *Fanirè* spontaneously fermented milk and *Wagashi Gassirè* cheese during shelf-life in Benin

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Running title: *Fanirè* and *Wagashi Gassirè* bacterial diversity

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Highlights

- This is the first report on bacterial dynamics of spontaneously fermented milk and *Wagashi Gassirè* cheese from Benin.
- Firmicutes and Proteobacteria were the most dominant bacterial phyla present in these dairy products.
- Several potential pathogens such as *Bacillus cereus*, *Clostridium perfringens*, *Klebsiella pneumoniae* and *Streptococcus infantarius* were detected.

Abstract

The present study aimed to investigate the changes in microbial community composition during the production and storage of the fermented milk *Fanirè* and cheese *Wagashi Gassirè*, widely consumed in Benin, to understand the dynamics of their bacterial populations and the sanitary status prevailing during their shelf-life. To this end, high throughput amplicon sequencing of bacterial 16S rRNA gene was used to investigate 114 samples of homemade milk products. The results revealed that Firmicutes/Bacillota (64%) and Proteobacteria/ Pseudomonadota (36%) were the most dominant bacterial phyla present in these dairy products. The milk products from the Nikki and Dassa-Zoumé camps and markets shared bacteria mainly belonging to *Streptococcaceae* (44%), *Enterobacteriaceae* (23%), *Moraxellaceae* (9%), *Lactobacillaceae* (7%) and *Bacillaceae* (5%). At species level, the products were dominated by lactic acid bacteria, mainly *Lactococcus lactis* (21%), *Streptococcus* sp. (18%), *Lactobacillus delbrueckii* (6%), *Streptococcus thermophilus* (2.5%), *Weissella confusa* (2%), *Lactobacillus fermentum* (1%) and *Lactobacillus helveticus* (1%). The bacterial community structure of *Fanirè* and raw milk differed significantly from those of red and white *Wagashi Gassirè* cheeses. Further analysis on bacterial community dynamics of raw milk and *Fanirè* showed no difference between samples collected along shelf-life (24-72h). On the contrary, the bacterial community dynamics of both white and red *Wagashi Gassirè* cheeses showed a significant difference in the bacterial community during shelf-life. Our analysis also detected several potential pathogens, such as *Bacillus cereus*, *Clostridium perfringens, Klebsiella pneumoniae* or *Streptococcus*

infantarius. These findings shed valuable light onto the microbiota diversity and dynamics of these traditional dairy products and should help addressing the safety and spoilage issues related to these important Beninese milk products.

Keywords: *Bacillus cereus*, Dairy products, *Klebsiella pneumoniae*, *Lactobacillus delbrueckii*, *Lactococcus lactis*, Metagenomic, *Streptococcus* sp.

1. Introduction

Milk and manufactured dairy products contain appreciable quantities of essential nutrients and micronutrients and play an irreplaceable role in human diet. However, they also provide an ideal environment for the growth for variety of spoilage and potentially pathogenic microorganisms (Cao et al., 2021). Indeed, milk products often contain *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes* or *Escherichia coli*, which are among the most common pathogens (Asfaw et al., 2023). Other bacteria can reduce milk quality, such as sensory and texture defects. Both pathogenic and spoilage microbiota are often associated with biofilm formation and are present in milk as contaminants (de Souza et al., 2024). Currently, the demand for safe and high-quality food has become the focus of public attention. While milk and dairy products are considered to be one of the healthiest foods with the highest quality, current food recalls and foodborne illness outbreaks have indicated the need for an in-depth investigation (Li et

al., 2018). To ensure delivery of high-quality products with long shelf-life, the dairy industry needs to evaluate milk quality throughout the value chain using genomic technologies and bioinformatics tools for characterization of the total microbiota (Porcellato et al., 2018).

In Benin, raw milk, spontaneous fermented milk *Fanirè* and homemade cheese called *Wagashi Gassirè* produced by Fulani (Peulh) community are most appreciated by consumers (Sessou et al., 2019; 2023). They are often produced in rather unhygienic conditions that affect their sanitary quality and reduce their shelf-life (Komagbe et al, 2023). Moreover, the spontaneous fermentation of *Fanirè* results in highly heterogeneous products (Sun and D'Amico, 2021; Sessou et al., 2023). To improve the qualities and safety of these products, several studies have assessed the yeast and bacterial communities of naturally fermented milk and cheese from Benin and showed that they are dominated by the *Saccharomyces cerevisiae* and *Kluveromyces marxianus* yeasts, and the *Lactobacillaceae* and *Streptococcaceae* bacterial families, including potential pathogens like *Bacillus cereus*, *Clostridium* sp. or *Streptococcus infantarius* (Sessou et al., 2019; 2023). These culture-independent studies took into account small samples of products collected in markets. Nevertheless, studies exploring the dynamics of bacteria communities of these products during their shelf-life and their follow up during production are limited, leading to a significant gap in understanding the sanitary and organoleptic changes in the microbial community composition during the production and storage. Therefore, there is a need to study the dynamic of bacterial communities and safety of these dairy products during their shelf-life (Agyei et al., 2020; de Melo Pereira et al., 2022).

In this study, we aimed to better understand bacterial compositions and dynamics to better assess the safety and quality of homemade milk products such as raw milk, fermented milk *Fanirè* and cheese *Wagashi Gassirè* collected from two communes and different production sites in Benin, using a high-throughput sequencing approach.

2. Materials and methods

2.1. Sampling, homogenisation and metagenomic DNA extraction

In the present study, bacterial communities of raw cow milk (n=6) and follow up of spontaneously fermented milk products called *Fanirè* (n=36), red (stained) cheese (n=36) and white (unstained) cheese (n=36) from two regions, Nikki and Dassa-Zoumé, in Benin was evaluated. In Fulani camps, sampling involved monitoring the entire production process of dairy products, from raw milk to the transformation into fermented milk (*Fanirè*), white cheese, and red cheese (*Wagashi Gassirè*). Sampling took place at the end of the production of each dairy product on Day 0, then on Day 1 (+ 24h) and Day 2 (+ 48h) of their storage duration, excluding raw milk. To avoid any confusion, the initial collection time for raw milk is designated as 0h in this study, while the collection time for fermented milk is designated as 24h, as it is obtained 24h after the collection of raw milk. For the *Wagashi Gassirè* samples, the initial collection time is also designated as 24h, since the cheese is produced after the collection of raw milk. There were 3 samples of raw milk per municipality, each with its derivatives (3 fermented milk, 3 white and 3 red *Wagashi Gassirè* cheeses), originating from 3 different Fulani camps.

The second sampling method involved randomly purchasing dairy products in local markets. Samples were collected independently of the raw milk used in their production at 24, 48, and 72h at room temperature (28-30°C). Details of the sampling are given in Table 6.1.

The samples were transported in ice-cooled boxes and stored in the laboratory at -80°C for subsequent analysis. To assess the bacterial community structure and dynamics of the dairy products, DNA extraction, PCR amplification, and DNA library preparation were conducted following the protocols described by Anihouvi et al. (2021). The DNA samples were stored at -20°C.

Table 6.1. Sampling of the naturally fermented cow milk and cheese *Wagashi Gassirè* collected in Fulani camps and markets at Nikki and Dassa-Zoumé communes in Benin.

Sampling area	Collection site	0h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Nikki	Fulani camp	RM1	FM4	FM5	FM6	WC13	WC14	WC15	RC22	RC23	RC24
		RM2	FM7	FM8	FM9	WC16	WC17	WC18	RC25	RC26	RC27
		RM3	FM10	FM11	FM12	WC19	WC20	WC21	RC28	RC29	RC30
	Market		FM31	FM32	FM33	WC40	WC41	WC42	RC49	RC50	RC51
			FM34	FM35	FM36	WC43	WC44	WC45	RC52	RC53	RC54
			FM37	FM38	FM39	WC46	WC47	WC48	RC55	RC56	RC57
Dassa-Zoumé	Fulani camp	RM58	FM61	FM62	FM63	WC70	WC71	WC72	RC79	RC80	RC81
		RM59	FM64	FM65	FM66	WC73	WC74	WC75	RC82	RC83	RC84
		RM60	FM67	FM68	FM69	WC76	WC77	WC78	RC85	RC86	RC87
	Market		FM88	FM89	FM90	WC97	WC98	WC99	RC106	RC107	RC108
			FM91	FM92	FM93	WC100	WC101	WC102	RC109	RC110	RC111
			FM94	FM95	FM96	WC103	WC104	WC105	RC112	RC113	RC114

FM: fermented milk, RC: red cheese, RM: raw milk, WC: white cheese. 0h refers to sampling made at the time of milking, while 24-72h correspond to samplings performed 24 to 72h later.

2.2. Illumina MiSeq Sequencing and data processing

The DNA samples (Table 6.1) were sequenced in the Illumina MiSeq platform (RTL Genomics, Lubbock, TX, USA) as described by Sessou et al. (2023). The sequence data were processed by PEAR sequence merger, USEARCH clustering and alignment algorithm, and UPARSE algorithm for Operational Taxonomic Unit (OTU) generation at different taxa levels. Further MG-RAST pipeline was used at a 97 % identity threshold against the M5RNA database for the generation of OTU tables at different taxonomic levels.

2.2. Statistical Analysis

The relative abundance data of the bacterial OTUs at the different taxonomic positions were used for statistical analysis. Significant differences in relative abundance (%) of taxa between the study groups were calculated by Student's two-tailed t-test and ANOVA. Principal coordinate analysis (PCoA) was performed using Bray-Curtis dissimilarity (PAST v3.22). The PERMANOVA test with 10,000 permutations was used to observe the significance of the difference in the bacterial taxa in the samples between two communes, sites production and food types and expressed as Bonferroni corrected *p*-value. For calculating the alpha diversity indices (Chao species richness and Shannon diversity index) between different types of products, sites of production and communes, compare_alpha_diversity.py script was used in the QIIME pipeline. The observed differences were visualised as boxplots using BoxPlotR (http://shiny.chemgrid.org/boxplotr/). The Wilcoxon test by using "svDialogs" in the R package (v3.5.2) was

conducted to show the significantly differing bacterial species and expressed as Benjamini-Hochberg (BH) corrected p-value. The hierarchically clustered heat map to show the species level significant difference between food types products, sites production and communes was visualised using "gplots" in R. The OTUs data with a relative abundance of more than 0.1% with a significance of $p<0.001$ was used for the heatmap generation (Sessou et al., 2023).

2.3. Data availability

The sequence data associated with the present work are available on the NCBI Sequence Read Archive (SRA) under the accession numbers PRJNA1105900. https://www.ncbi.nlm.nih.gov/sra/PRJNA1105900

3. Results

The results of this study showed that, in general, Firmicutes/Bacillota and Proteobacteria/Pseudomonadota were the most dominant bacterial phyla in all the dairy products, although their relative abundance varied. For instance, raw cow milk samples collected from Nikki Fulani camps were dominated by Proteobacteria (64%) followed by Firmicutes (36%) while those from Dassa-Zoumé Fulani camps were mainly constituted of Firmicutes/Bacillota (56%) and Proteobacteria/Pseudomonadota (44%). Similarly, the fermented milk (*Fanirè*) samples made from these raw milks displayed 62% of Firmicutes and 38% of Proteobacteria for the Nikki Fulani camps and 68% Firmicutes and 32% Proteobacteria for the Dassa-Zoumé camps (data not shown). Also, unstained *Wagashi Gassirè* cheese collected from Fulani camps in Nikki (Firmicutes: 64% and Proteobacteria: 35%) and Dassa-Zoumé (Firmicutes: 72%;

Proteobacteria: 27%) were dominated by the same phyla. The same observation was made for red-stained cheeses from Fulani camps in Nikki (Firmicutes: 57% and Proteobacteria: 41%) and Dassa-Zoumé (Firmicutes: 56% and Proteobacteria: 43%) (data not shown). In order to get more insights into their bacterial diversity and dynamics, each type of dairy product was further analysed at the bacterial family and genus levels.

3.1. Bacterial diversity and dynamics of fermented milk from Fulani camps and markets

At family level within the Fulani camp samples, studies revealed that the fermented milks were dominated by microbial communities similar to those observed in their parental raw milks (Fig. 6.S1). The raw milk samples from Nikki Fulani camps were mainly composed of *Enterobacteriaceae* (52%), *Streptococcaceae* (34%) and *Moraxellaceae* (10%) whereas those from Dassa-Zoumé Fulani camps were dominated by *Streptococcaceae* (54%), *Enterobacteriaceae* (32%) and *Moraxellaceae* (11%). The fermented milk samples collected from the Nikki and Dassa-Zoumé Fulani camps had practically the same microbial composition except for *Lactobacillaceae* which were more abundant (19%) in Dassa-Zoumé samples than those from Nikki (1%). Also, in most fermented milks, an increase in *Acetobacteraceae* concentration was observed in the 48 and 72h samples (Fig. 6.S1).

More specifically, considering the bacterial genera, fermented milks collected from Nikki Fulani camps were mainly composed of *Lactococcus* (55%), *Klebsiella* (21%), unclassified *Enterobacteriaceae*

(8%) and *Streptococcus* (6%) while those collected from Dassa-Zoumé Fulani camps were dominated by *Streptococcus* (31%), *Klebsiella* (20%), *Lactobacillus* (19%), *Lactococcus* (17%) (Fig. 6.1). In terms of relative abundance of *Lactococcus*, Nikki fermented milk samples were the richest of all the products collected in the camps while those from Dassa-Zoumé were richer in *Streptococcus* and *Lactobacillus*.

At market level, the most dominant genera were *Streptococcus* (30%), *Lactococcus* (26%), *Klebsiella* (21%), *Lactobacillus* (12%) and *Acetobacter* (6%) in samples collected at Nikki and *Klebsiella* (40%), *Lactococcus* (20%), *Streptococcus* (13%), *Enterobacter* (10%), *Escherichia* (4%) and *Acinetobacter* (4%), in samples from the Dassa-Zoumé region. Further analysis of the bacterial community dynamics in fermented milk (Fig. 6.2) showed no significant difference between samples collected along their shelf-lives (24-72h) (p=0.5617, F= 0.8919, PERMANOVA) and their corresponding raw milk (p=0.3756, Bonferroni corrected).

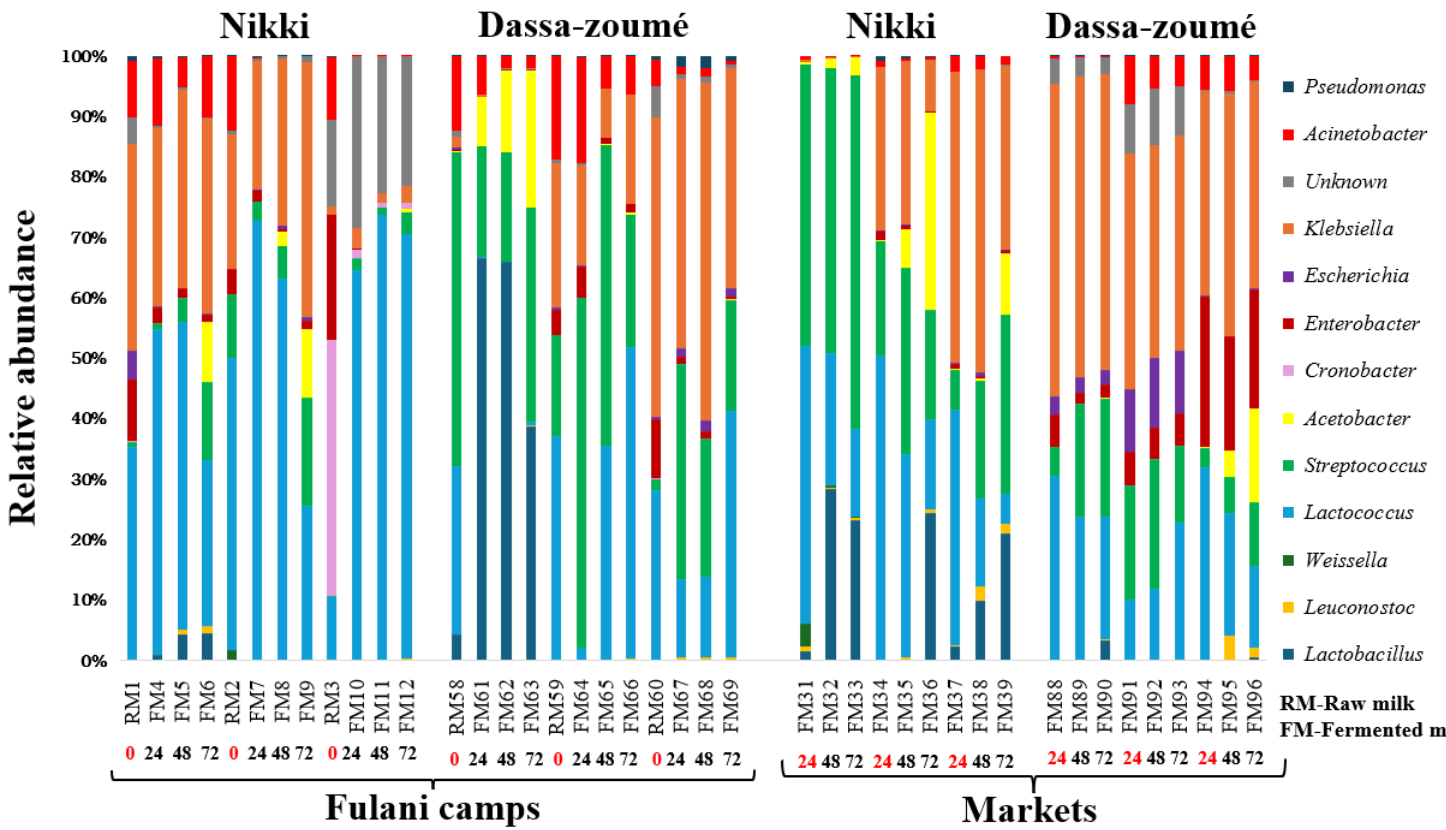


Figure 6.1: Dynamics of bacterial communities compositional of fermented milk, at the genus level. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes.

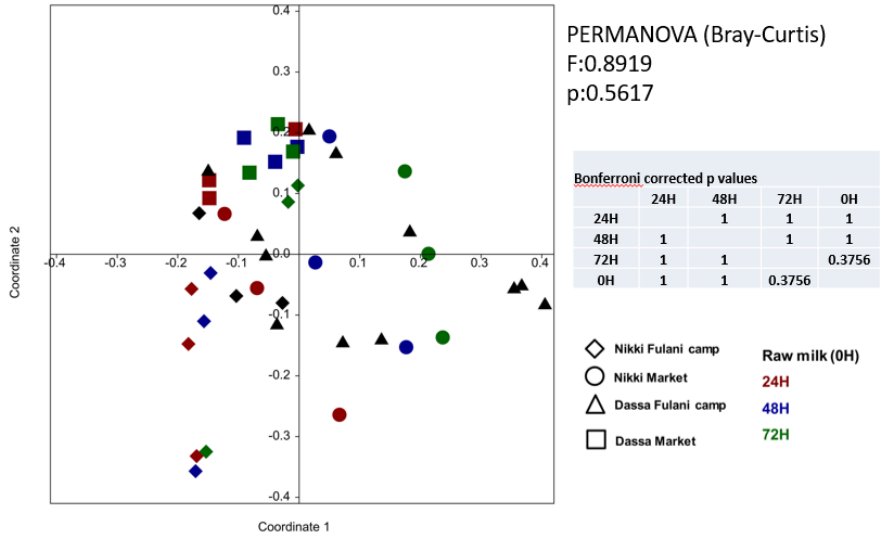


Figure 6.2: Dynamics of bacterial communities compositional of fermented milk. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes. PCoA biplot based on Bray-Curtis dissimilarity of the species-level OTUs shows no significant difference in the overall bacterial community structure over time, among the spontaneously fermented milk of the two communes.

3.2. Bacterial diversity and dynamics of unstained *Wagashi Gassirè* (white cheese) from Fulani camps and markets

Samples from *Wagashi Gassirè* white cheese collected in Nikki Fulani camps were dominated by *Streptococcaceae* (38%), unclassified Enterobacteriales (16%), *Moraxellaceae* (15%), *Enterobacteriaceae* (15%) and *Bacillaceae* (5%) whereas those from Dassa-Zoumé Fulani Camps were mainly composed of *Streptococcaceae* (35%), *Bacillaceae* (27%), *Moraxellaceae* (10%), *Enterobacteriaceae* (10%). The white *Wagashi Gassirè* cheeses from Nikki markets were mainly composed of *Enterobacteriaceae* (31%), *Moraxellaceae* (27%), *Streptococcaceae* (24%) and *Planococaceae* (10%) while those from Dassa-Zoumé markets predominantly contained *Streptococcaceae* (56%), *Lactobacillaceae* (16%), *Planococaceae* (7%), *Leuconostocaceae* (6%), *Bacillaceae* (6%) and *Enterobacteriaceae* (5%). However, the white cheeses collected at the Nikki market had a significantly higher relative abundance in *Enterobacteriaceae* and *Moraxellaceae* than Dassa-Zoumé samples which had a higher relative abundance of *Streptococcaceae*. In addition, *Wagashi Gassirè* from Dassa-Zoumé had significantly more *Lactobacillaceae* than those from Nikki where the relative abundance was close to zero (Fig. 6.S2).

Considering the genus composition of white cheese samples collected in Fulani camps, they were dominated by *Streptococcus* (25%), *Exiguobacterium* (16%), *Acinetobacter* (14%), *Lactococcus* (13%), *Klebsiella* (7%), *Bacillus* (5%), *Lactobacillus* (3%) and *Escherichia* (3%) in Nikki region and *Bacillus* (27%), *Streptococcus* (18%), *Lactococcus* (17%), *Acinetobacter* (11%), *Enterococcus* (7%),

Klebsiella (4%) and *Escherichia* (4%) in the Dassa-Zoumé commune (Fig. 6.3). In most cases, the microbial loads of *Lactococcus*, which were abundant in raw milk, fell considerably in the 24h cheeses before rising again in the 48 and 72h samples (Fig. 6.3).

At market level, studies showed that white cheeses were mainly constituted of *Acinetobacter* (27%), *Klebsiella* (19%), *Lactococcus* (17%), *Kurthia* (10%), *Streptococcus* (6%) and *Escherichia* (5%), for samples collected in Nikki market and *Streptococcus* (41%), *Lactobacillus* (16%), *Lactococcus* (15%), *Kurthia* (7%), *Bacillus* (6%) and *Weissella* (5% for those from Dassa-Zoumé market.

Bacterial community dynamics in white cheese analysis (Fig. 6.4) showed a significant difference in the bacterial community of samples (p=0.001, F= 2.661, PERMANOVA). Indeed, there is significant difference between raw milk bacterial community and white cheese collected at day 1 (p=0.0126, Bonferroni corrected) and day 3 (p=0.0012, Bonferroni corrected).

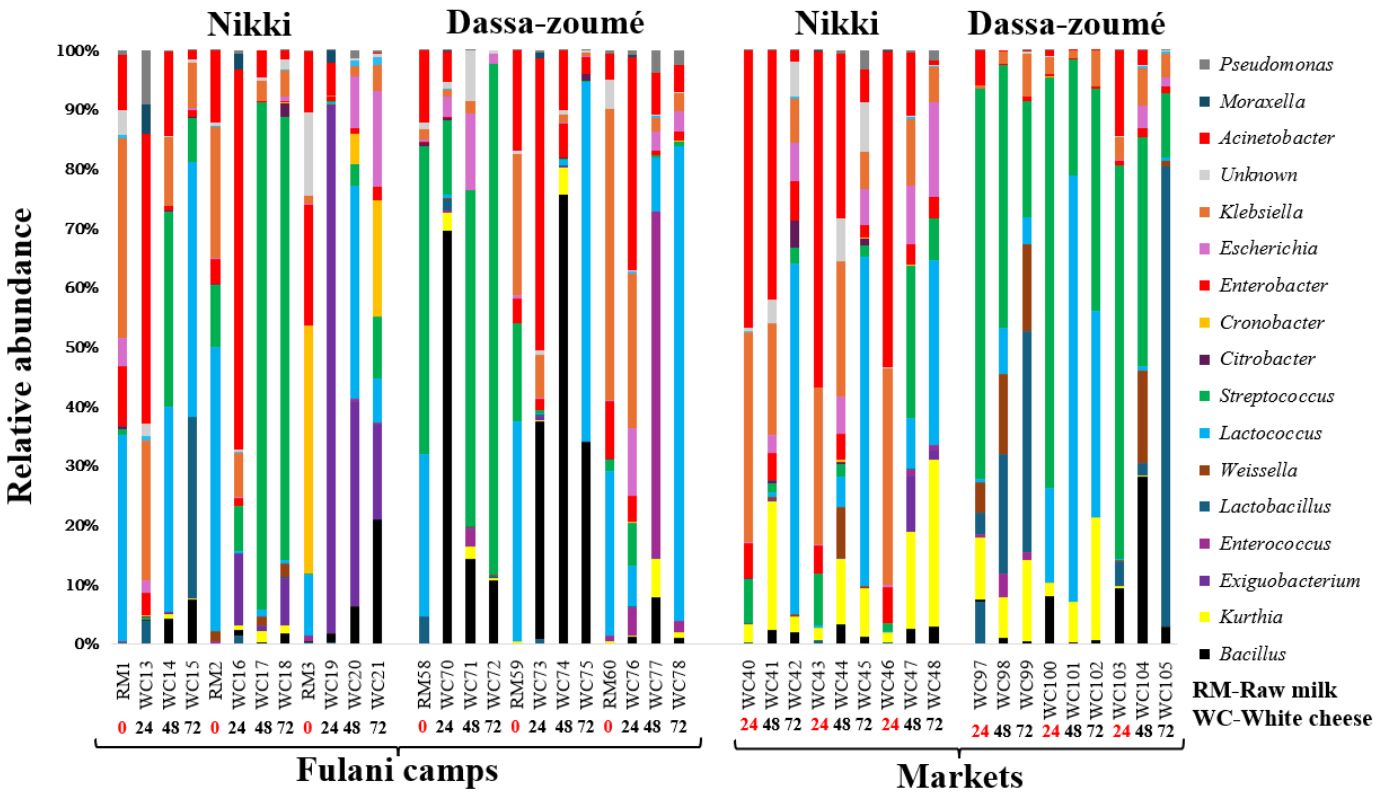


Figure 6.3: Dynamics of bacterial communities compositional of white cheese, at the genus level. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes.

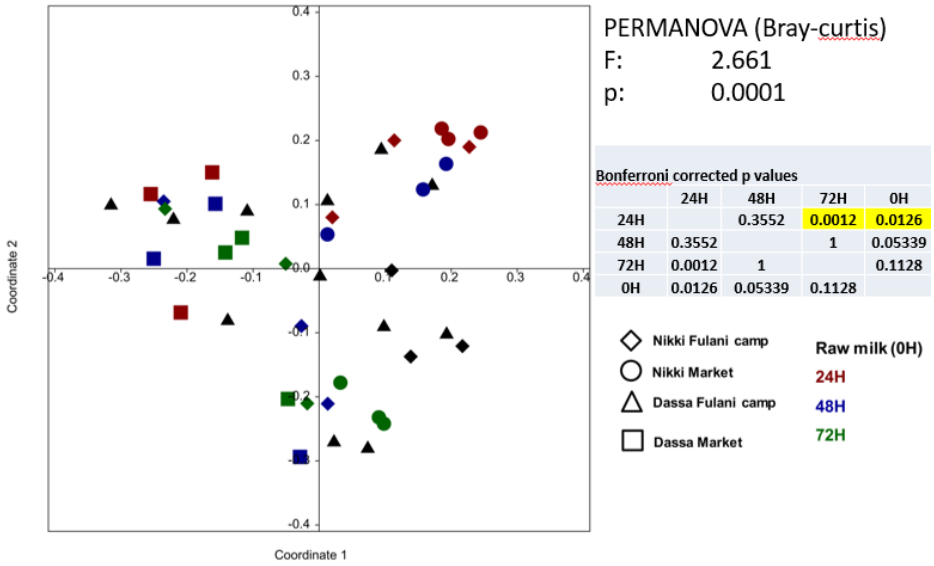


Figure 6.4: Dynamics of bacterial communities of white cheese. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes. PCoA biplot based on Bray-Curtis dissimilarity of the species-level OTUs shows significant difference in the overall bacterial community structure over time (0-72h) between white cheese at 24 and 72h *versus* raw milk of the two communes. The significance of the difference is expressed as a Bonferroni-corrected p-value (p = 0.0001, F = 2.661, PERMANOVA).

3.3. Bacterial diversity and dynamics of stained *Wagashi Gassirè* (red cheese) from Fulani camps and markets

Regarding red cheese from Fulani camps, they were composed mainly of *Streptococcaceae* (48%), *Enterobacteriaceae* (23%), *Moraxellaceae* (8%) for samples collected in Nikki and *Streptococcaceae* (36%), *Enterobacteriaceae* (24%), *Moraxellaceae* (15%), *Bacillaceae* (11%) for Dassa-Zoumé samples. In addition, *Bacillaceae* was undetectable in the raw milk from both communes, in contrast to the red cheese, where a high microbial load of *Bacillaceae* was observed, especially in the Dassa-Zoumé samples. The results also indicated that the relative abundance of *Moraxellaceae* is high in the product at 24h but decreases over time.

The red cheese collected in Nikki markets were dominated by *Streptococcaceae* (36%), *Lactobacillaceae* (33%), *Clostridiaceae* (7%), *Leuconostocaceae* (6%) whereas those collected from Dassa-Zoumé markets were mainly predominant in *Streptococcaceae* (58%), *Enterobacteriaceae* (17%), *Moraxellaceae* (10%) and *Leuconostocaceae* (7%) (Fig. 6.S3).

Considering the genus composition of red samples collected from Fulani camps, the dominant genus were *Lactococcus* (25%), *Streptococcus* (23%), *Pantoea* (11%), *Klebsiella* (8%), *Acinetobacter* (7%) in samples collected in Nikki region and *Streptococcus* (18%), *Lactococcus* (17%), *Acinetobacter* (15%), *Bacillus* (11%), *Escherichia* (7%), *Pantoea* (7%) and *Klebsiella* (6%) in Dassa-Zoumé samples. In most cases, the microbial concentration of *Lactococcus*, which were abundant in the raw milk, decreased considerably in the 24h cheeses

before rising again in the 48 and 72h samples (Fig. 6.5). As shown in Fig. 6.5, samples from red cheese from markets were mainly composed of *Lactobacillus* (33%), *Streptococcus* (22%), *Lactococcus* (13%), *Clostridium* (7%), *Weissella* (5%) at Nikki and *Streptococcus* (49%), *Klebsiella* (14%), *Acinetobacter* (10%), *Lactococcus* (9%), *Weissella* (5%) at Dassa-Zoumé.

Regarding bacterial community dynamics in red cheese (Fig. 6.6), analysis showed significant difference (p=0.0039, F= 2.004, PERMANOVA) in the bacterial community. These differences are shown between raw milk and red cheese collected at day 1 (p=0.003, Bonferroni corrected) and day 3 (p=0.021, Bonferroni corrected).

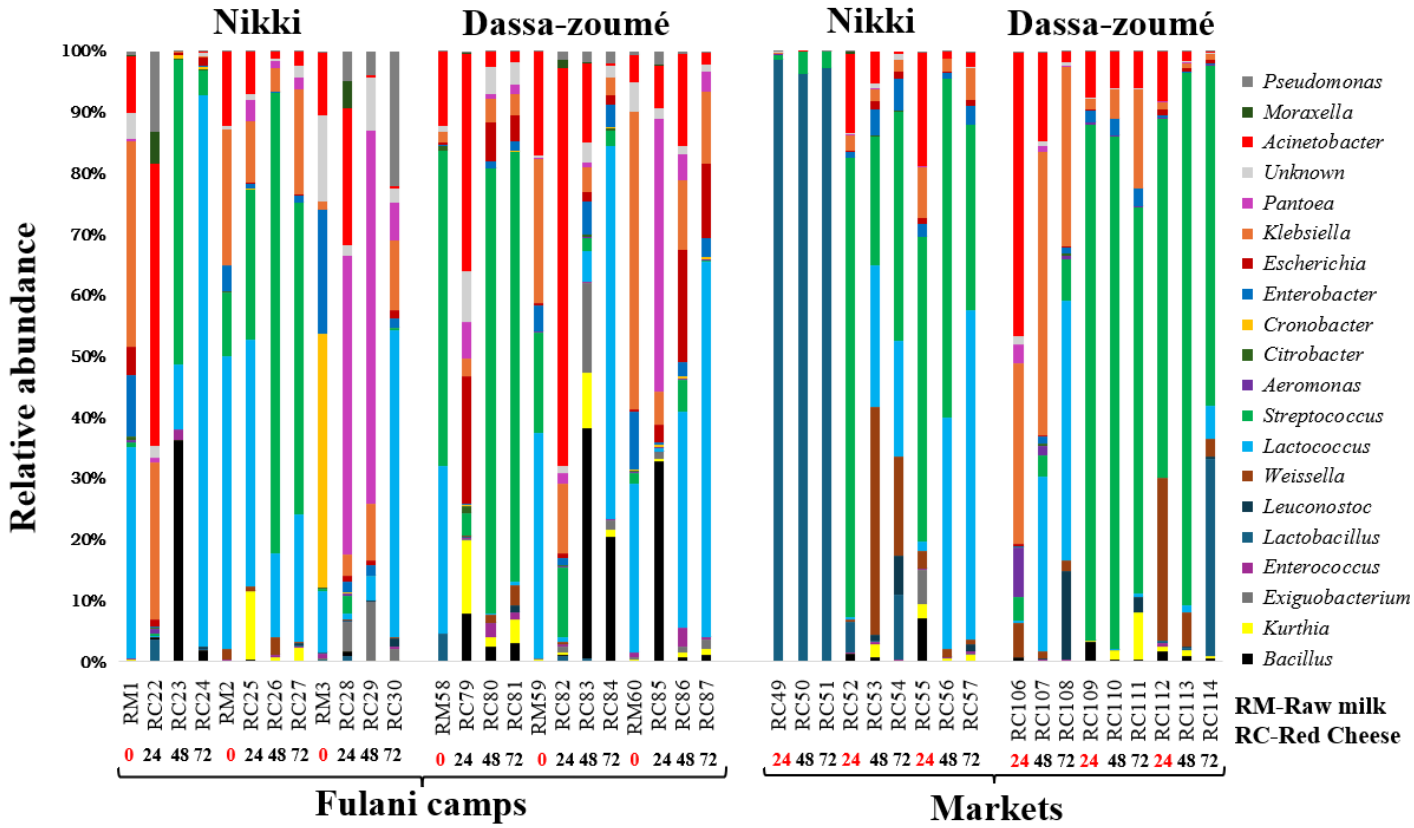


Figure 6.5: Dynamics of bacterial communities compositional of red cheese, at the genus level. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes.

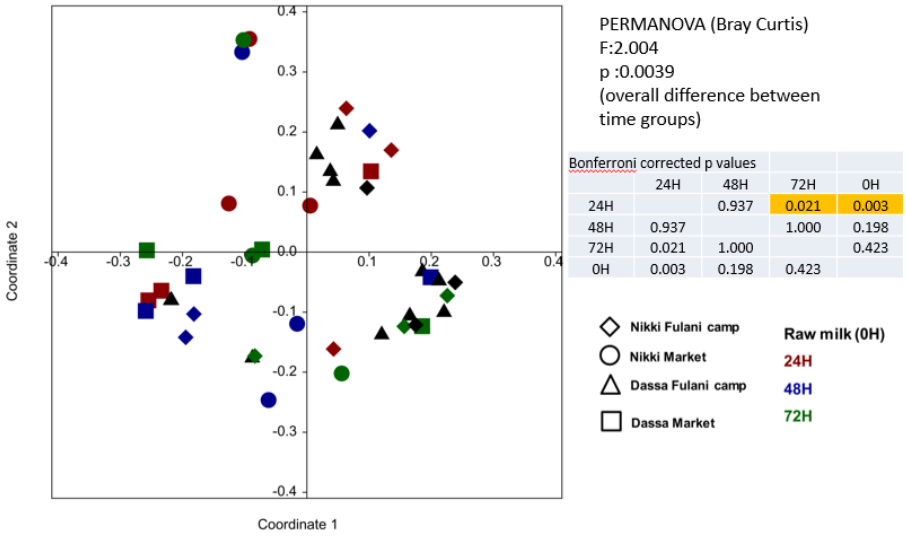


Figure 6.6: Dynamics of bacterial communities compositional of red cheese. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes. PCoA biplot based on Bray-Curtis dissimilarity of the species-level OTUs shows significant difference in the overall bacterial community structure over time (0-72h) between red cheese at 24 and 72h *versus* raw milk of the two communes. The significance of the difference is expressed as a Bonferroni-corrected *p*-value (p=0.0039, F = 2.004, PERMANOVA).

3.4. Global bacterial community dynamics of milk products

The bacterial community structure in these four types of milk products (raw milk, fermented milk, red and white cheese) of Nikki and Dassa-Zoumé significantly differed (p=0.0001, F=3.098, PERMANOVA). Pair-wise ANOVA comparison showed that the bacterial community structure of fermented milk differed significantly with red and white cheese (p=0.001, Bonferroni corrected) and raw milk differed significantly with red and white cheese (p=0.001, Bonferroni corrected). (Fig. 6.7).

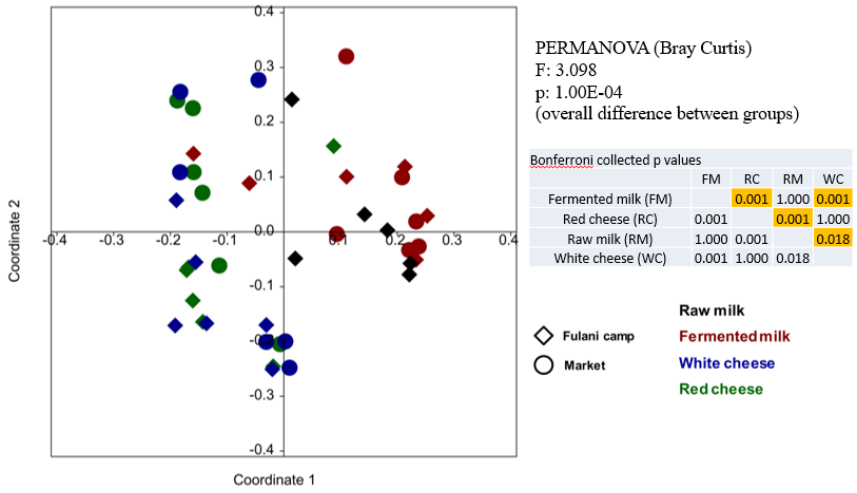


Figure 6.7: Dynamics of bacterial communities compositional of four types of milk products. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes. PCoA biplot based on Bray-Curtis dissimilarity of the species-level OTUs shows significant difference in the overall bacterial community structure between red cheese, raw milk and fermented milk and between white cheese, fermented milk and raw milk of the two communes. The

significance of the difference is expressed as a Bonferroni-corrected p-value (p=0.0001, F = 3.098, PERMANOVA).

These studies revealed that *Enterobacteriaceae* were more dominant in raw milk than fermented milk and cheese samples collected in Fulani camps whereas *Bacillaceae* are highly dominant in cheese samples and very negligible proportion in raw milk and fermented milk. The white cheese samples had the less relative abundance in *Enterobacteriaceae* but this family did not differ significantly in all products (Fig. 6.8).

Of all the samples collected at the market, the red cheeses from Nikki were the richest in *Lactobacillaceae* and the red cheeses from Dassa-Zoumé had a negligible relative abundance. In terms of the relative abundance of *Moraxellaceae* in the samples, the white *Wagashi Gassirè* samples from Nikki were the richest, followed by the red cheeses collected in Dassa-Zoumé, with the lowest relative abundances obtained for fermented milk from Nikki and white *Wagashi Gassirè* from Dassa-Zoumé (Fig. 6.9).

At the genus level, considering the relative abundance of *Lactococcus*, Nikki fermented milk was the richest of all the products collected in the camps. The relative abundance of *Lactococcus* in stained cheeses from this area is almost the same as in white cheeses. No significant difference (p> 0.05) was observed between the *Lactococcus* microbial content of cheese samples collected in Nikki and all the products collected in the camps in Dassa-Zoumé. About the relative abundance of *Streptococcus* and *Klebsiella*, no significant difference was observed between the products from the two communes, except for the relative abundance of *Streptococcus* in the red and white *Wagashi Gassirè*

samples in Dassa-Zoumé, where that of the red cheeses was significantly higher (Fig. 6.10).

For the market, the relative abundance of *Klebsiella* was significantly higher in the fermented milk of Dassa-Zoumé than in the white cheese of Dassa-Zoumé and red cheeses from Nikki. In terms of *Acinetobacter*, the white *Wagashi Gassirè* samples from Nikki were significantly richer, while the fermented milk Nikki and the white cheese from Dassa-Zoumé were the least concentrated (Fig. 6.11).

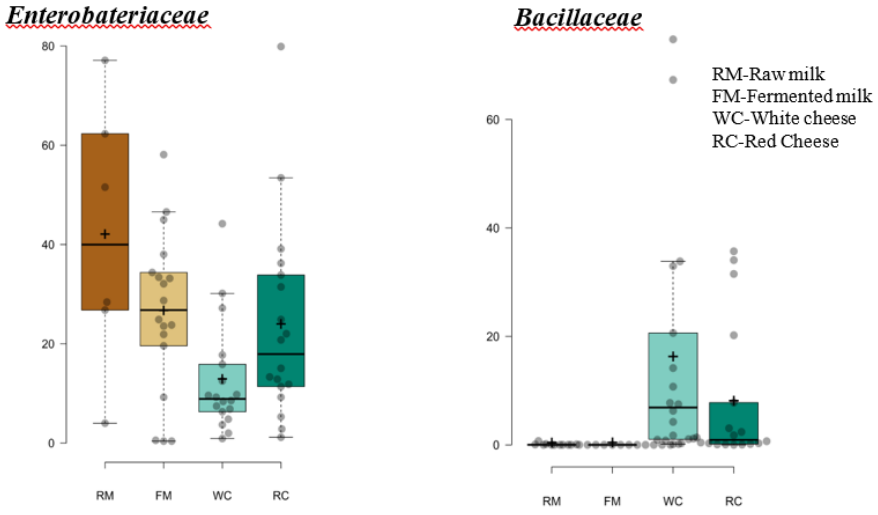
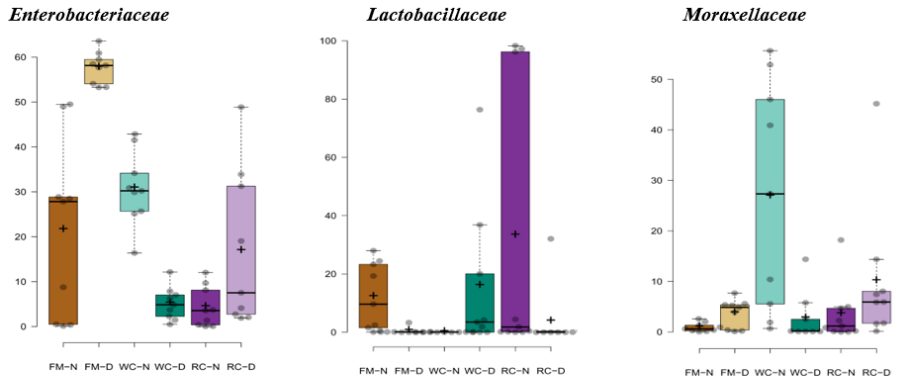
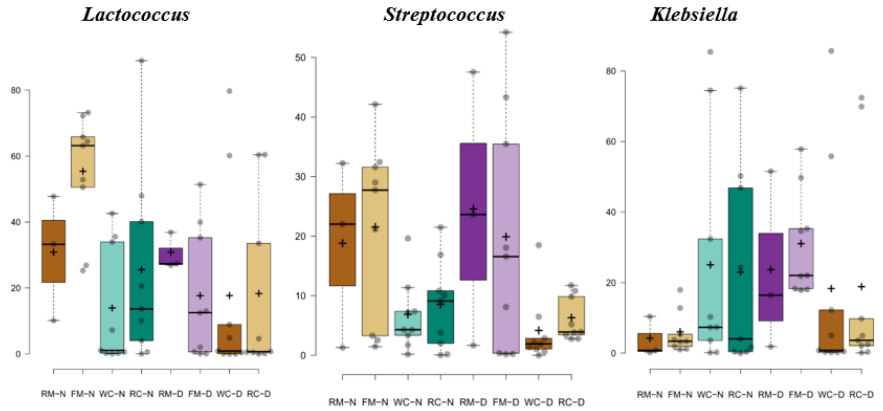


Figure 6.8: Boxplot showing difference in relative abundance of *Enterobacteriaceae* and *Bacillaceae* in camp dairy products.



FM-N Fermented milk- Nikki, FM-D Fermented milk -Dassa-Zoumé, WC-N White cheese-Nikki, WC-D White cheese- Dassa-Zoumé, RC-P Red Cheese-Nikki, RC-D Red Cheese- Dassa-Zoumé

Figure 6.9: Boxplot showing difference in relative abundance of *Enterobacteriaceae*, *Lactobacillaceae* and *Moraxellaceae* in market dairy products.



RM-N Raw milk-Nikki, FM-N Fermented milk- Nikki, WC-N White cheese-Nikki, RC-N Red Cheese-Nikki, RM-D Raw milk-Dassa-Zoumé, FM-D Fermented milk -Dassa-Zoumé, WC-D White cheese-Dassa-Zoumé, RC-D Red Cheese-Dassa-Zoumé.

Figure 6.10: Boxplot showing difference in relative abundances of *Lactococcus*, *Streptococcus* and *Klebsiella* in dairy products from Fulani camps

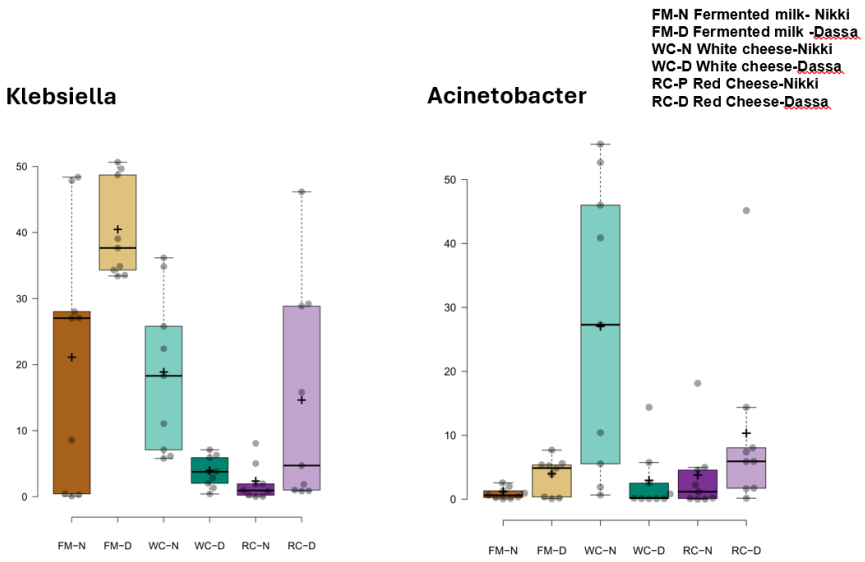


Figure 6.11: Boxplot showing difference in relative abundances of *Klebsiella* and *Acinetobacter* in dairy products from market

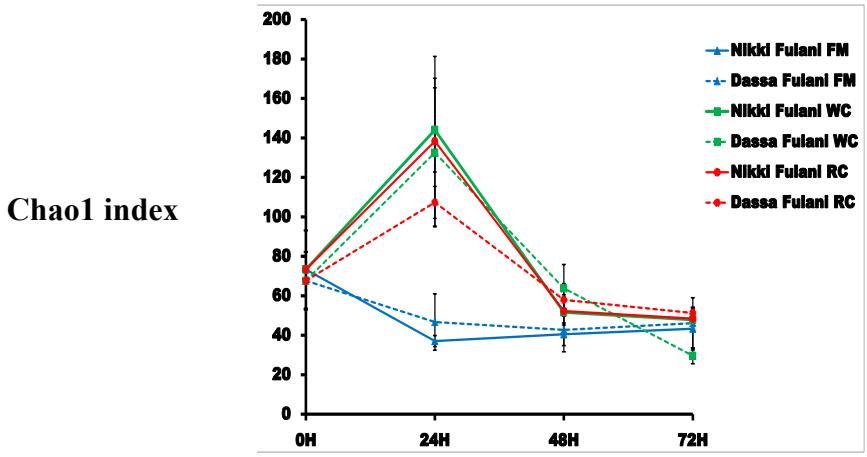
3.5. Differential abundance of bacterial taxa across different types of food, regions, production sites, and over time

The comparison of microbiome of different food types studies showed that the bacteria taxa significantly differed across food types (raw milk, fermented milk, white cheese and red cheese), regions (Nikki × Dassa), production sites (Fulani camps × markets) and time (0 *vs* 24 *vs* 48 *vs* 72h). Regarding food types, Operational Taxonomic Units (OTUs) of *L. lactis, Vagococcus* sp., *Klebsiella* sp., *Acetobacter lovaniensis, Exigobacterium* sp., *Bradyrhizobium* sp., *Acetobacter* sp., *Bacillus* sp., *Acetobacter cibinongensis, P. dispersa, B. cereus* and *K. gibsonii* significantly differed between food types. Concerning regions, the results showed that OTUs of *S. infantarius* significantly differed between regions, and also production. The results regarding time of analysis of the products revealed that OTUs of *Leuconostoc pseudomesenteroides, Bradyrhizobium* sp., *Moraxella* sp., *Acinetobacter* sp., unclassified *Cyanobacteria, Brevundimonas* sp., unclassified *Sphingomonadaceae* and *Sphingomonas* sp. differed across time.

To evaluate the species richness and diversity of samples analysed, we calculated Chao1 index which is an indicator of species richness and diversity. Results showed that samples are rich in species and that their diversity is higher in 24h samples collected in Fulani camps compared to 0h of collection and decreased in 48 and 72h of samples collection. Fermented milk collected in Fulani camps were less rich in species than the others milk products studied. In other words, the bacterial species richness in the samples collected in the camps increases initially and

then decreases over time, except for fermented milk samples where this richness decreases over time (Fig. 6.12A). The bacterial richness and diversity of products collected from markets decreases over time (Fig. 6.12B).

A: Evolution of the bacterial diversity in dairy products, at the Fulani camps (0-72h)



B: Evolution of the bacterial diversity in dairy products, at the market (0-48h)

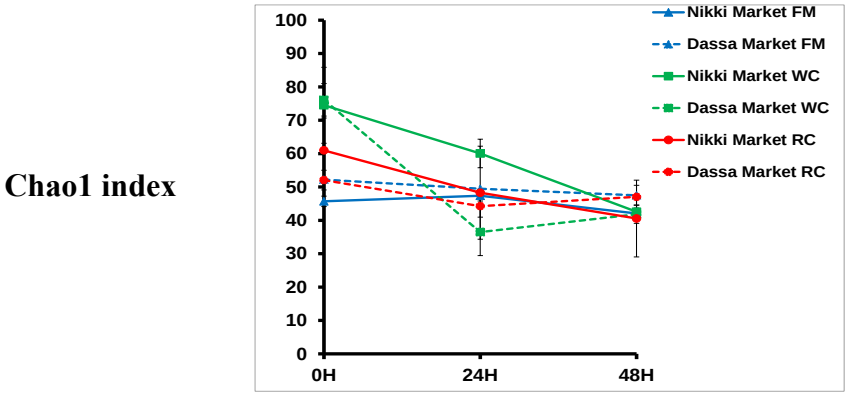


Figure 6.12: Evolution of the bacterial diversity in dairy products based on the Chao 1 index.

4. Discussion

To gain deeper insights into the bacterial diversity and dynamics of spontaneously fermented milk and homemade cheese production in two communes in Benin, we conducted high-throughput screening of these products using Illumnia Miseq. This study revealed that these products were mainly composed of Bacillota (Firmicutes) and Pseudomonadota (Proteobacteria) comprising, in decrease order, *Streptococcaceae*, *Enterobacteriaceae*, *Lactobacillaceae*, *Bacillaceae*, *Planococcaceae*, *Leuconostococaceae* and *Acetobacteraceae*. Samples analysed contained beneficial LAB (*L. lactis*, *Streptococcus* sp., *Lactobacillus delbrueckii*, *Weissella confusa*, *Lactobacillus fermentum*) and acetic acid bacteria that are of great industrial interest (Kohlmann et al., 2016). LAB are used in the production of many fermented food products and belongs to *Streptococcaceae* and *Lactobacillaceae* families. They play important roles in ensuring the protection and safety of dairy products through production of antimicrobial agents including lactic acid, diacetyl, hydrogen peroxide, and bacteriocins (Yerlikaya, 2019). Particularly, *L. lactis,* one of the most important starter bacteria in dairy technology, including cheese, butter, cream, and fermented milks (Rahmeh et al., 2019; Yerlikaya, 2019). It has been recognized as generally regarded as safe (GRAS) by United States Food and Drug Administration (FDA). Its antimicrobial metabolites, particularly nisin, are in use for the control of spoilage bacteria and foodborne pathogens in food (Khemariya et al., 2017; Liu et al., 2020). The bacteriocinogenic and probiotic status of the *L. lactis* strains isolated from these natural dairy products should be done before their potential use as starter culture in fermented milk.

L. delbrueckii is also a predominant and widely found LAB species in homemade dairy products (Song et al., 2016). It is used as starter, together with *Streptococcus thermophilus*, in the production of dairy products like yogurt (Dan et al., 2017; 2019). Although the genus *Streptococcus* includes pathogenic microorganisms like *S. infantarius*, or *Streptococcus agalactiae*, a pathogen involved in neonatal sepsis and meningitis in humans and mastitis in animals, it harbours useful species such as *S. thermophilus* currently approved for use in dairy fermentations by EFSA as safe (Jans et al., 2017).

The dairy products we investigated exhibited a notable abundance of *Enterobacteriaceae*, a diverse group of Gram-negative rods commonly found in both human and animal intestinal tracts. Some *Enterobacteriaceae* species possess the ability to ferment lactose, producing acid and gas in dairy products like cheese (Mladenović et al., 2021). This family encompasses various genera, including *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Serratia*, and *Proteus*, which can infiltrate milk through multiple pathways, including faecal contamination, bedding, insufficiently sanitized teats, and contaminated equipment (Fierer et al., 2017). Certain members of this family have been identified as potential opportunistic pathogens, harboring virulence genes coding for Shiga-like toxins or adherence and effacement factor (Jian-li et al., 2017). Moreover, some *Enterobacteriaceae* bacteria can induce adverse effects, compromising the product quality and rendering it unsuitable for sale or consumption. Their detection serves as a reliable indicator of faecal contamination and raises concerns about the potential presence of associated enteric pathogens (Mladenović et al., 2021). The presence of elevated

concentration *Enterobacteriaceae* in these dairy products suggests lapses in manufacturing, inadequate pasteurization, and/or post-pasteurization contamination, highlighting the need for stricter hygiene measures within the dairy industry (Sobeih et al., 2020). Considering the prevalence of *Enterobacteriaceae* in these dairy products and their consumption in the study areas, this poses a significant health risk to consumers. In fact, consumption of raw milk and its derivatives has been linked to numerous outbreaks of foodborne illnesses, often associated with pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. (Cancino-Padilla et al., 2017; Sobeih et al., 2020).

The study also highlighted the dominance and significance of the *Moraxellaceae* family, particularly *Acinetobacter*, which is present in various environments (*e.g.* soil, water, sewage), but also in dairy products, (*e.g.* fresh meat, chicken carcasses), often contributing to their spoilage. The prevalence of *Acinetobacter* in raw milk, where it can exist in high numbers, can lead to the production of levan, a fructose polymers acting as capsular polysaccharides. Accumulation of this polymer can result in the ropy spoilage of milk or the formation of slime on the surface of soft cheese or curds (Yang, 2014).

The analysis of the samples also showed the presence of several potential pathogens in all the dairy products samples: *A. baumannii*, *Acetobacter indonesiensis*, *B. cereus*, *Clostridium perfringens*, *Cronobacter sakazakii*, *K. pneumoniae* and *S. infantarius*. Several earlier reports showed the presence of these pathogens in milk products. Indeed, Sessou et al. (2023) identified in the same milk products *B.*

cereus and *S. infantarius*, with high relative abundance in some *Wagashi Gassirè* cheese samples in their studies. *B. cereus*, isolated in various foods like starchy foods, rice, vegetables, bread, dairy, meat products, and ready-to-eat foods, is a highly resistant food poisoning bacterium and a significant food safety concern (Rahnama et al., 2023).

C. perfringens, a major cause of severe food poisoning, has been identified in raw milk and milk products in Egypt (Elariny et al., 2021; Bhattacharya et al., 2020). *C. sakazakii*, contaminating dairy products, causes high mortality in premature newborns, with neonatal meningitis and septicaemia mortality rates at 15% and 25% respectively (Parra-Flores et al., 2023). *K. pneumoniae*, a common zoonotic pathogen in hospitals and communities, detected in milk from dairy cows, mainly causes pneumonia, liver abscess, urinary diseases, toxaemia, septicaemia, and other infections (Li et al., 2023). *A. baumannii*, a major multidrug-resistant microorganism in hospitals, causes infections in the blood, urinary tract, lungs, and wounds (Nocera et al., 2021). Our analysis using Illumina amplicon sequencing revealed both beneficial bacteria and foodborne pathogens, varying across products, highlighting food safety issues and suggesting strategies to combat these pathogens.

Comparing the microbiota of our raw milk to that studied by Ribeiro Júnior et al. (2018) in refrigerated raw milk from Brazil, we remark that they harbour similar dominant bacteria (*L. lactis*, *Enterobacter* sp.). Most of earlier studies reported such dominance of *Streptococcaceae* mainly *L. lactis* in naturally fermented milk products (Von Gastrow et al., 2020; Jans et al., 2017; Moonga et al., 2020; Shangpliang et al.,

2018; Groenenboom et al., 2020). Results obtained in our studies are like those obtained by Sessou et al. (2023) regarding microbial diversity of fermented milk and uncoloured and coloured cheese samples collected in Benin.

The study revealed that dairy products possess remarkable bacterial diversity, with microorganisms known to impart a variety of odours and characteristics, as well as potentially volatile secondary metabolites that could influence their quality during storage. There is a need to develop strategies to improve safety and the preservation of these products and enhance their health and nutritional benefits.

7. Acknowledgements

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8. References

Anihouvi, D.G.H., Henriet, O., Kpoclou, Y.E., Scippo, M.L., Hounhouigan, D.J., Anihouvi, V.B., Mahillon, J. (2021) Bacterial diversity of smoked and smoked-dried fish from West Africa: a metagenomic approach. *J. Food Process. Preserv*. 45, e15919.

Agyei, D., Owusu-Kwarteng, J., Akabanda, F., Akomea-Frempong, S., (2020) Indigenous African fermented dairy products: Processing technology, microbiology and health benefits. *Crit. Rev. Food Sci. Nutr*. 60, 991-1006.

Asfaw, T., Genetu, D., Shenkute, D., Shenkutie, T.T., Amare, Y.E., Habteweld, H.A., Yitayew, B. (2023) Pathogenic Bacteria and Their Antibiotic Resistance Patterns in Milk, Yoghurt and Milk Contact Surfaces in Debre Berhan Town, Ethiopia. *Infect. Drug Resist.* 16, 4297-4309.

Bhattacharya, A., Shantikumar, S., Beaufoy, D., Allman, A., Fenelon, D., Reynolds, K., Normington, A., Afza, M., Todkill, D. (2020) Outbreak of *Clostridium perfringens* food poisoning linked to leeks in cheese sauce: an unusual source. *Epidemiol. Infect.* 148, e43. doi:10.1017/S095026882000031X

Cancino-Padilla, N., Fellenberg, M.A., Franco, W., Ibáñez, R.A., Pérez, E.V. B. (2017) Foodborne bacteria in dairy products: Detection by molecular techniques. *Cien. Inv. Agr*. 44, 215-229. doi: 10.7764/rcia.v44i3.1811

Cao, H., Yan, Y., Wang, L., Dong, L., Pang, X., Tang, S., Li, A., Xiang, A., Zhang, L., Zheng, B. (2021) High-Throughput Sequencing Reveals Bacterial Diversity in Raw Milk production environment and production chain in Tangshan city of China. *Food Sci. Anim. Resour*. 41, 452-467.

Dan, T., Ren, W., Liu, Y., Tian, J., Chen, H., Li, T., Liu, W. (2019) Volatile flavor compounds profile and fermentation characteristics of milk fermented by *Lactobacillus delbrueckii subsp. bulgaricus*. *Front. Microbiol.* 10, 2183.

Dan, T., Wang, D., Wu, S., Jin, R., Ren, W., Sun, T. (2017) Profiles of volatile flavor compounds in milk fermented with different proportional combinations of L*actobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. *Molecules* 22, 1633.

de Melo Pereira, G.V., de Carvalho Neto, D.P., Maske, B.L., De Dea Lindner, J., Vale, A.S., Favero, G.R., Viesser, J., de Carvalho, J.C., Góes-Neto, A. Soccol, C.R. (2022) An updated review on bacterial community composition of traditional fermented milk products: what next-generation sequencing has revealed so far? *Crit. Rev. Food Sci. Nutr*. 62, 1870-1889.

de Souza, M.T.P., Fagnani, R., Alegro, L.C.A., de Santana, E.H.W. (2024) Non-starter lactic acid bacteria and citrate fermenting bacteria in milk supply chain: Are they easily controlled? *Int. Dairy J.* 149, 105839.

Elariny, E.Y.T., Ahmed, H.A., Khatab, A.A.H., Mohamed, R.E. (2021) Genotyping, antibiotic resistance and biofilm formation ability of *Clostridium perfringens* isolated from raw milk, dairy products and human consumers. *J. Anim. Health Prod*. 9, 34-41.

Fierer, J., Looney, D., Pechère, J.C. (2017) Nature and pathogenicity of micro-organisms. In: Cohen, J., Powderly, W.G., Opal, S.M. (eds) Infectious Diseases, E-Book 4th edition. *Elsevier Ltd Health Sciences*. pp. 4-25. doi: 10.1016/B978-0-7020-6285-8.00002-2

Jans, C., Meile, L., Kaindi, D.W.M., Kogi-Makau, W., Lamuka, P., Renault, P., Kreikemeyer, B., Lacroix, C., Hattendorf, J., Zinsstag, J., Schelling, E., Fokou, F., Bonfoh, B. (2017) African fermented dairy products-overview of predominant technologically important microorganisms focusing on African *Streptococcus infantarius* variants and potential future applications for enhanced food safety and security. *Int. J. Food Microbiol*. 250, 27-36.

Jian-li, W., Yuan-yuan, S., Shou-yu, G., Fei-fei, D., Jia-yu, Y., Xue-hua, W., Yong-feng, Z., Shi-jin, J., Zhi-jing, X. (2017) Serotype and

virulence genes of *Klebsiella pneumoniae* isolated from mink and its pathogenesis in mice and mink. *Sci Rep* 7, 1-7.

Khemariya, P., Singh, S., Nath, G., Gulati, A. K. (2017) Probiotic *Lactococcus lactis*: A review. *Turkish J. Agric. Food Sci. Technol.* 5, 556-562.

Kohlmann, R., Barenberg, K., Anders, A., Gatermann, S.G. (2016) *Acetobacter indonesiensis* bacteremia in child with metachromatic leukodystrophy. *Emerg. Infect. Dis.* 22, 1681. doi: 10.3201/eid2209.160566

Komagbe, G.S., Dossou, A., Seko Orou, B.M.T., Sessou, P., Azokpota, P., Youssao, I., Hounhouigan, J., Scippo, M.L., Clinquart, A., Mahillon, J., Farougou, S. (2023) State of the art of breeding, milking, and milk processing for the production of curdled milk and *Wagashi Gassirè* in Benin: Practices favoring the contamination of its dairy products. *Front. Sustain. Food Syst.* 6, 1050592.

Li, L., Ma, J., Cheng, P., Li, M., Yu, Z., Song, X., Yu, Z., Sun, H., Zhang, W., Wang, Z. (2023) Roles of two-component regulatory systems in *Klebsiella pneumoniae*: Regulation of virulence, antibiotic resistance, and stress responses. *Microbiol. Res*. 272, 127374.

Li, W., Wu, S., Fu, P., Liu, J., Han, H., Bai, L., Pei, X., Li, N., Liu, X., Guo, Y. (2018) National molecular tracing network for foodborne disease surveillance in China. *Food Control* 88, 28-32.

Liu, J., Meng, F., Du, Y., Nelson, E., Zhao, G., Zhu, H., Caiyin, Q., Zhang, Z., Qiao, J. (2020) Co-production of nisin and γ-aminobutyric acid by engineered *Lactococcus lactis* for potential application in food preservation. *Front. Microbiol*. 11, 49.

Mladenović, K.G., Grujović, M., Kiš, M., Furmeg, S., Tkalec, V.J., Kocić-Tanackov, S.D. (2021) *Enterobacteriaceae* in food safety with an emphasis on raw milk and meat. *Appl. Microbiol. Biotechnol.* 105, 8615-8627.

Moonga, H.B., Schoustra, S.E., Van den Heuvel, J., Linnemann, A.R., Samad, M.S., Shindano, J., Smid, E.J. (2020) Composition and diversity of natural bacterial communities in mabisi, a traditionally fermented milk. *Front. Microbiol*. 11, 1816.

Nocera, F.P., Attili, A.R., De Martino, L. (2021) *Acinetobacter baumannii*: Its clinical significance in human and veterinary medicine. *Pathogens* 10, 127.

Parra-Flores, J., Flores-Soto, F., Flores-Balboa, C., Alarcón-Lavín, M. P., Cabal-Rosel, A., Daza-Prieto, B., Springer, B., Cruz-Córdova, A., Leiva-Caro, J., Forsythe, S., Ruppitsch, W. (2023) Characterization of *Cronobacter sakazakii* and *Cronobacter malonaticus* strains isolated from powdered dairy products intended for consumption by adults and older adults. *Microorganisms* 11, 2841.

Porcellato, D., Aspholm, M., Skeie, S.B., Monshaugen, M., Brendehaug, J., Mellegård, H. (2018) Microbial diversity of consumption milk during processing and storage. *Int. J. Food. Microbiol.* 266, 21-30.

Rahmeh, R., Akbar, A., Kishk, M., Al-Onaizi, T., Al-Azmi, A., Al-Shatti, A., Shajan, A., Al-Mutairi, S., Akbar, B. (2019) Distribution and antimicrobial activity of lactic acid bacteria from raw camel milk. *New Microbes New Infect*. 30, 100560.

Rahnama, H., Azari, R., Yousefi, M.H., Berizi, E., Mazloomi, S.M., Hosseinzadeh, S., Derakhshan, Z., Ferrante, M., Conti, G.O. (2023) A systematic review and meta-analysis of the prevalence of *Bacillus cereus* in foods. *Food Control* 143, 109250.

Ribeiro Júnior, J.C., de Oliveira, A.M., Silva, F. de G., Tamanini, R., de Oliveira, A.L.M., Beloti, V. (2018) The main spoilage-related psychrotrophic bacteria in refrigerated raw milk. *J. Dairy Sci.* 101, 75-83.

Sessou, P., Keisam, S., Tuikhar, N., Gagara, M., Farougou, S., Jeyaram, K., (2019) High-throughput Illumina Miseq amplicon sequencing of yeast communities associated with indigenous dairy products from republics of Benin and Niger. *Front. Microbiol.* 10, 1-12.

Sessou, P., Keisam, S., Gagara, M., Komagbe, G., Farougou, S., Mahillon, J., Jeyaram, K. (2023) Comparative analyses of the bacterial communities present in the spontaneously fermented milk products of Northeast India and West Africa. *Front. Microbiol.* 14, 1166518.

Shangpliang, H.N.J., Rai, R., Keisam, S., Jeyaram, K., Tamang, J.P., (2018) Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing. *Sci. Rep.* 8, 1532.

Sobeih, A., AL-Hawary, I., Khalifa, E., Ebied, N. (2020) Prevalence of *Enterobacteriaceae* in raw milk and some dairy products. *Kafrelsheikh Vet. Med. J.* 18 9-13.

Song, Y., Sun, Z., Guo, C., Wu, Y., Liu, W., Yu, J., Menghe, B., Yang, R., Zhang, H. (2016) Genetic diversity and population structure of *Lactobacillus delbrueckii* subspecies *bulgaricus* isolated from naturally fermented dairy foods. *Sci. Rep*. 6, 22704.

Sun, L., D'Amico, D.J. (2021) Composition, succession, and source tracking of microbial communities throughout the traditional production of a Farmstead cheese. *mSystems* 6, 1-18.

Von Gastrow, L., Madec, M. N., Chuat, V., Lubac, S., Morinière, C., Lé, S., Santoni, S., Sicard, D., Valence, F. (2020) Microbial diversity associated with Gwell, a traditional French mesophilic fermented milk inoculated with a natural starter. *Microorganisms* 8, 982.

Yang, X. (2014) *Moraxellaceae*. In: Batt, C.A., Tortorello, M.L. (eds) Encyclopedia of food microbiology (Second Edition), *Academic Press* pp. 826-833.

Yerlikaya, O. (2019) Probiotic potential and biochemical and technological properties of *Lactococcus lactis ssp. lactis* strains isolated from raw milk and kefir grains. *J. Dairy Sci.* 102, 124-134.

Supplementary data

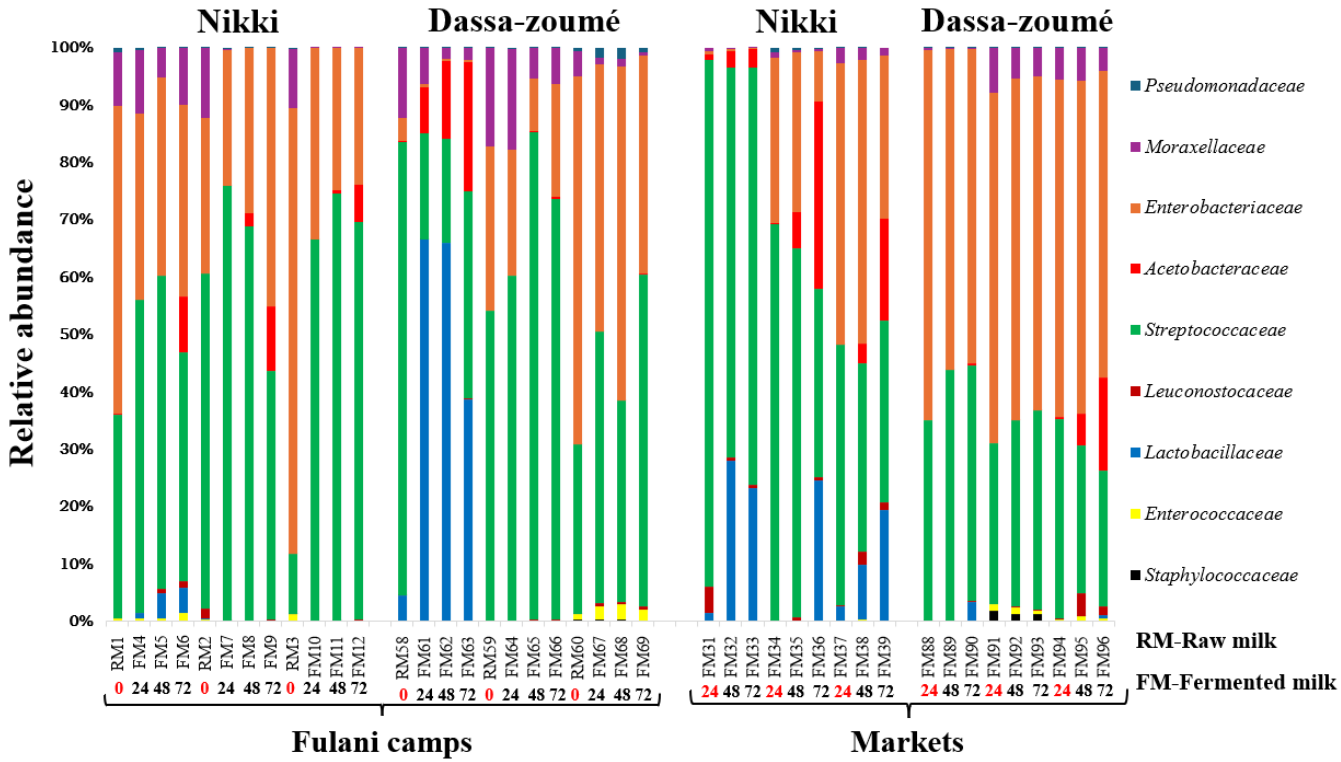

Figure 6.S1: Dynamics of bacterial communities compositional of fermented milk, at the family level. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes.

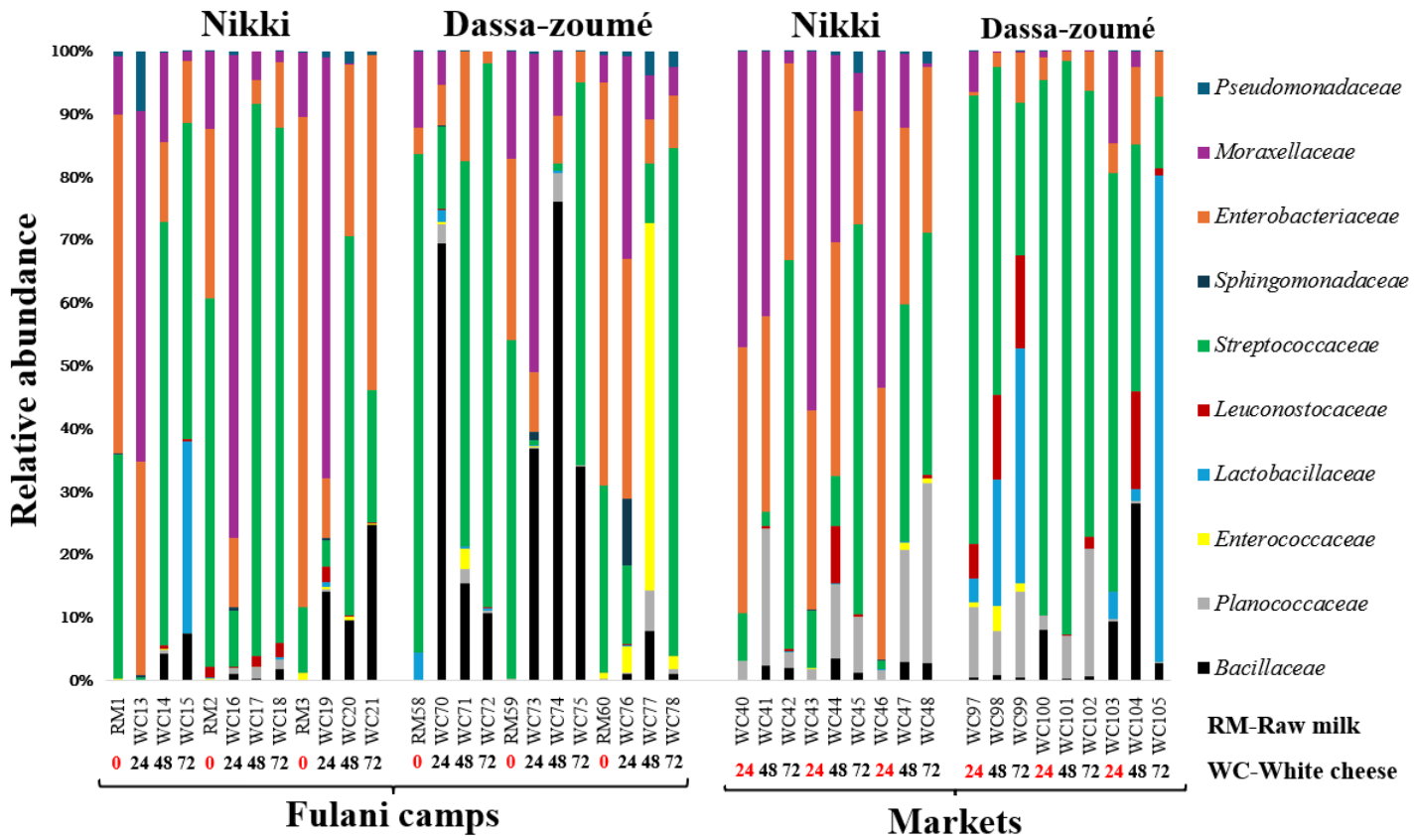

Figure 6.S2: Dynamics of bacterial communities compositional of white cheese, at the family level. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes.

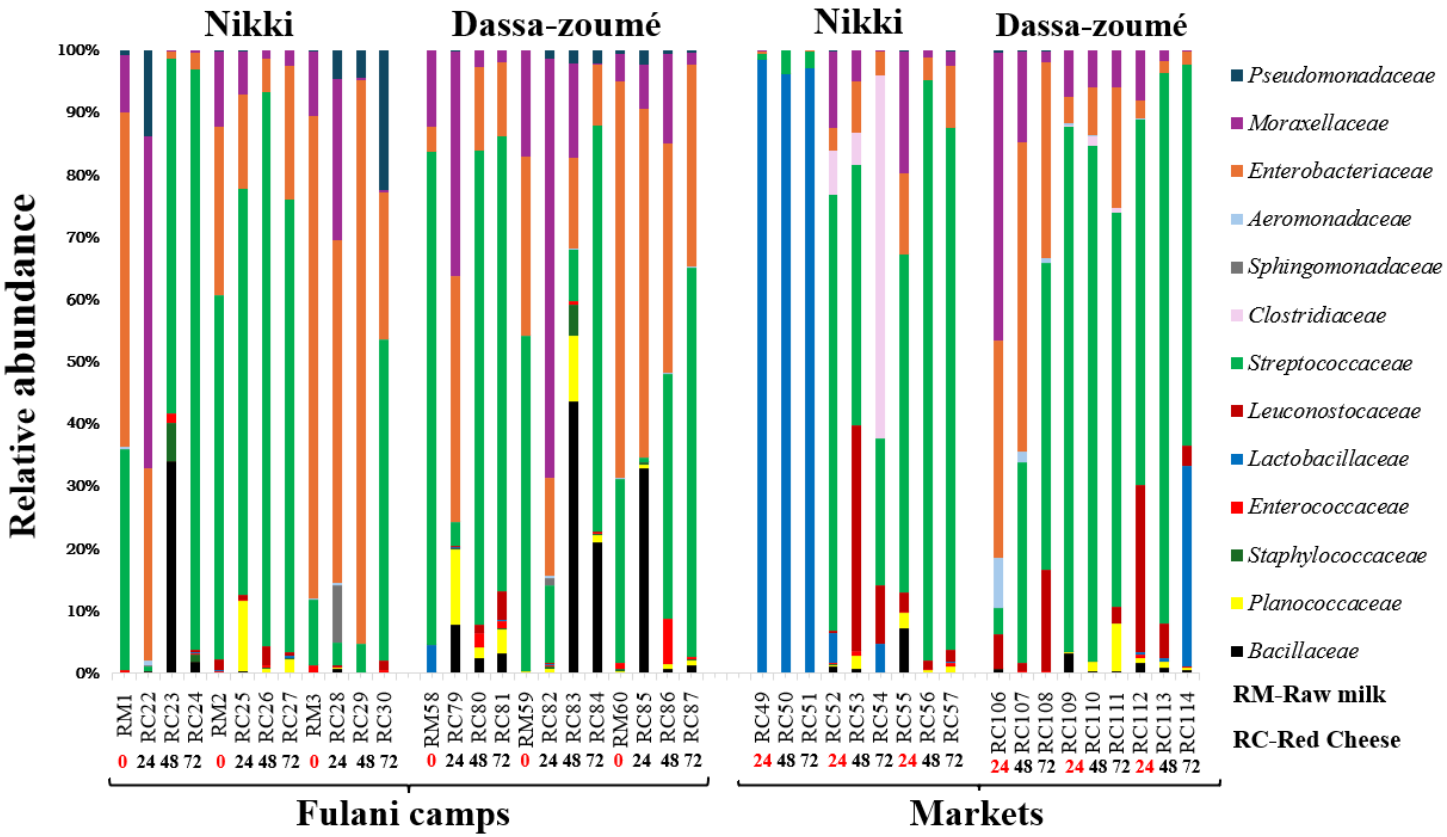

Figure 6.S3: Dynamics of bacterial communities compositional of red cheese, at the family level. Samples originated from Fulani camps and markets of the Nikki and Dassa-Zoumé communes.

Chapitre 7

7. Effect of heat treatment and packaging on *Wagashi Gassirè* and controlled fermentation on *Fanirè* safety

Suggestions for improving fermented milk and *Wagashi Gassirè* were made following the unsatisfactory sanitary quality of the products reported in the previous sections, as well as the production constraints identified during the survey of stakeholders, notably the difficulty of preservation. These suggestions were then tested under laboratory conditions, and microbiological analyses of the improved products were conducted using both dependent- and independent-culture approaches.

Personal contribution:

I was involved in conceptualizing the study, producing samples, and performing microbiological analyses. I also drafted the original manuscript and handled its revision.

This work is in preparation for publication:

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Chapitre 7

Effect of heat treatment and packaging on *Wagashi Gassirè* and controlled fermentation on *Fanirè* safety

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Abstract

This study aims to assess the effect of heat treatment and packaging on the sanitary quality of *Wagashi Gassirè* cheese, as well as the effect of controlled fermentation on the safety of the fermented milk *Fanirè*. For this purpose, *Wagashi Gassirè* samples underwent heat treatment at 100°C for 20 min and then packaged in polyethylene bags in two different ways, simple packaging and vacuum packaging. *Fanirè* samples were subjected to fermentation using various starter cultures, including *Lactococcus lactis* alone through direct and backslopping methods and a combination of *L. lactis* with *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, and *Lactobacillus fermentum*. The controlled milk fermentation lasted for 24h at room temperature (30°C), with follow-up conducted 48h after production. Samples of *Wagashi Gassirè* were also monitored over three days, and analyses were conducted on the first day of production and after three days of storage. Microbiological characteristics of the products were evaluated using both culture-dependent and independent methods, providing comprehensive insights into the bacterial composition and safety of improved *Wagashi Gassirè* and *Fanirè*. The results indicated that the fermented milk produced with the *L. lactis* strain alone as starter resulted in a *Fanirè* of suitable microbiological quality regardless of the analysis time when considering the culture-dependent technique. As for the products obtained by backslopping and the combination of lactic acid bacteria (LAB), the results showed that the product quality improves with storage. Regarding the *Wagashi Gassirè* samples, double cooking, with or without vacuum packaging, helped preserve the sanitary quality of the cheeses throughout the monitoring period.

The in-depth study of the quality of these products using Illumnia Miseq revealed the significant presence of *Bacillus* spp. and *Acinetobacter* spp. in the packaged cheeses and *Pseudomona*s spp. and *Bacillus* spp. in the fermented milk *Fanirè*, which can affect their sanitary and commercial quality. Technologies should still be developed to better control these germs.

Keywords: Dairy products, controlled fermentation, cheese, fermented milk, food safety.

1. Introduction

Wagashi Gassirè and *Fanirè* are two homemade dairy products that play a significant role in the local diets in Benin. However, concerns regarding their safety have arisen due to inadequate production and storage practices, leading to potential contamination with harmful bacteria such as *Salmonella* spp. and *Escherichia coli* (Sessou et al., 2023). The traditional production methods of *Wagashi Gassirè* and *Fanirè* lack sufficient measures to control bacterial growth and prevent contamination (Sessou et al., 2019). Indeed, poor handling and storage practices further increase the risk of microbial contamination, posing a threat to consumer health. Ensuring the sanitary quality of these products is crucial to safeguard public health and uphold consumer trust. Implementing stringent food safety measures during production, storage, and distribution is imperative to address these concerns. Emphasizing hygiene practices among food handlers, maintaining

proper storage temperatures, utilizing appropriate packaging materials, and regular monitoring for microbial presence are essential steps in ensuring the safety of *Wagashi Gassirè* and *Fanirè*. Several techniques have been used to better preserve cheese, but these methods have remained confined to laboratories and have not considered fresh products stored at room temperature. This study aims to fill this gap by exploring the impact of thermal treatments and packaging of *Wagashi Gassirè* stored at 30°C, as well as fermented milk obtained through controlled fermentation. The objective is to identify the most promising approach that producers can use to improve their products in terms of sanitary quality and preservation.

2. Materials and methods

To evaluate the technological improvements, the *Wagashi Gassirè* samples underwent three types of treatment after production, before being stored at room temperature (30°C): *i*) packaging *Wagashi Gassirè* in food packaging (simple sealing, T1), *ii*) packaging *Wagashi Gassirè*, then cooking it at 100°C for 20 min (simple packaging+ cooking, T2), and *iii*) vacuum packing *Wagashi Gassirè* and cooking it at 100°C for 20 min (vacuum packaging + cooking, T3). Analyses were conducted at the end of production (Day 0) and after 72h (Day 3).

For the fermented milk, technological tests were carried out on pasteurised milk with *Lactococcus lactis* (both directly and through backslopping) and with a combination of the four strains (*L. lactis* LBB 001, *Leuconostoc mesenteroides* LBB 007, *Lactobacillus plantarum* LBB 130, *Lactobacillus fermentum* LBB 275). Microbial suspensions with turbidity set at 2 McFarland (*ca.* 6×10^8 CFU/mL) were inoculated

at 1% into pasteurized milk. The different products obtained were analyzed on the day of production (24h after inoculation of the strains) and 48h after production. Like the milk fermented with LAB, the non-inoculated control sample (pasteurised milk) was also monitored over time.

For the different products obtained, standard microbiological analyses were performed, including the enumeration of *Enterobacteriaceae* (ISO 21528-2:2017; with VRBG), *E. coli* (ISO 16649-1:2018; with TBX), *Listeria monocytogenes* (alternative method for the enumeration of *L. monocytogenes* using Rapid'L mono, validated in accordance with ISO 16140-2:2016) *Staphylococcus aureus* (ISO 6888-3:2003; with Baird-Parker + RPF), and the detection of *Salmonella* spp. (ISO 6579-1:2017; with XLD and Rapid *Salmonella*) for the fermented milk and *Wagashi Gassirè* samples. Additionally, an evaluation of the bacterial diversity of these products was conducted by Illumina MiSeq sequencing of their 16S rRNA gene amplicons (Sessou et al., 2023).

3. Results and discussion

This study focused on the effect of controlled fermentation on the sanitary quality of fermented milk and the effect of heat treatments and packaging on the safety of traditional cheese produced in Benin. These effects were assessed based on microbiological controls of these products using the culture-dependent technique and, more thoroughly, using culture-independent assessment of the bacterial dynamics via 16S rRNA amplicon sequencing (Illumina MiSeq).

3.1. Quality of dairy products using culture-dependent assessment methods

As shown in Table 7.1, fermented milk produced with the *L. lactis* strain LBB 001 alone as a starter culture maintained good microbiological quality according to EC regulation 2073/2005, regardless of the analysis time, when using the culture-dependent technique. Interestingly, products obtained through backslopping and with a combination of lactic acid bacteria (LAB) showed improved quality with longer storage duration, likely due to the production of significant amounts of antimicrobial substances, and/or acidification, by the LAB during storage. Of note, the naturally fermented milk (control sample) made with pasteurized milk did not comply with the above regulation.
For the *Wagashi Gassirè* samples, double cooking combined with or without vacuum packaging effectively preserved the sanitary quality of the cheeses throughout the monitoring period. The *Wagashi Gassirè* sample sealed without undergoing a second cooking, does not meet these acceptability criteria by the third day of storage with enterobacteria serving as the discriminating parameter. The persistence of these enterobacteria could be due to the inefficiency of the thermal treatment in eliminating residual enterobacteria or, more likely, to post-production contamination. They can produce gas in cheese *Wagashi* (Dash et al., 2022). Furthermore, no *Salmonella* spp. could be detected in the samples, and the concentrations of *E. coli*, *L. monocytogenes* and *S. aureus* were below the detection limit of 10 CFU/g.

Table 7.1: Microbiological quality of fermented milk (*Fanirè*) and *Wagashi Gassirè* samples after treatments.

Treatments	Samples	Microorganisms (CFU/g or mL)					Status
		E. coli	**ETB**	***L. mono***	**(*) *Salmonella* spp.**	***S. aureus***	
Control sample	FM11 (0h)	<10	1.9×10^6	<10	ND	<10	Non-compliant
	FM28 (72h)	<10	8.4×10^7	<10	ND	<10	Non-compliant
Backslopping	FM12 (0h)	<10	1.9×10^3	<10	ND	<10	Non-compliant
	FM30 (72h)	<10	<10	<10	ND	<10	Compliant
***L. lactis* LBB 001**	FM13 (0h)	<10	<10	<10	ND	<10	Compliant
	FM29 (72h)	<10	<10	<10	ND	<10	Compliant
4 LAB	FM14 (0h)	<10	6.9×10^4	<10	ND	<10	Non-compliant
	FM31 (72h)	<10	<10	<10	ND	<10	Compliant
	Criteria*	NA	10	10^2	Abs in 25 mL	10^2-10^3	
Simple packaging	WG2 (0h)	<10	5.5×10^2	<10	ND	<10	Compliant
	WG5 (72h)	<10	2.8×10^6	<10	ND	<10	Non-compliant
Packaging+ cooking	WG3 (0h)	<10	<10	<10	ND	<10	Compliant
	WG6 (72h)	<10	<10	<10	ND	<10	Compliant
Vacuum+ cooking	WG4 (0h)	<10	<10	<10	ND	<10	Compliant
	WG7 (72h)	<10	3×10^1	<10	ND	<10	Compliant
	Criteria*	10^2-10^3	10^4-10^5	10^2	Absence in 25 g	NA	

(*) *Salmonella* spp.: research in 25ml/g, FM: Fermented Milk, WG: *Wagashi Gassirè***,** NA, not applicable, ND: not detected in 25g or mL, ETB: *Enterobacteriaceae*, *L. mono: L. monocytogenes*, * EC regulation 2073/2005.

3.2. Culture-independent assessment of the microbial diversity and dynamics of the dairy products

The culture-independent analysis showed that the raw milk used for the production of fermented milk was predominantly composed of Proteobacteria (92%), followed by Bacteroidetes (6%) and Firmicutes (1%), while the corresponding fermented milk contained Firmicutes (92-99%) for the control, Firmicutes (97-99%) for the milk fermented by backslopping, and Firmicutes (24-30%) and Proteobacteria (70-76%) for the fermented milk made with strains tested (Fig. 7.S1A). As shown in Fig. 7.S1B, the raw milk used for the production of fermented milks was rich in *Flavobacteriaceae*, *Aeromonaceae*, *Enterobacteriaceae* and *Moraxellaceae*. After pasteurization and natural fermentation, the fermented milk consisted mainly of *Bacillaceae*, *Paenibacilliaceae* and *Enterobacteriaceae* on the first day of production, with the appearance of *Clostridiaceae* and *Peptostreptococcaceae* on the third day of analysis. Fermented milks obtained by backslopping were mainly composed of *Streptococcaceae* and *Bacillaceae*, while those obtained with *L. lactis* contained *Bacillaceae*, *Streptococcaceae* and *Pseudomonaceae*, (which practically disappeared after 72h of storage). Fermented milks with bacterial combinations contained decreasing proportions of *Pseudomonaceae*, *Bacillaceae*, *Streptococcaceae* and *Enterobacteriaceae*.

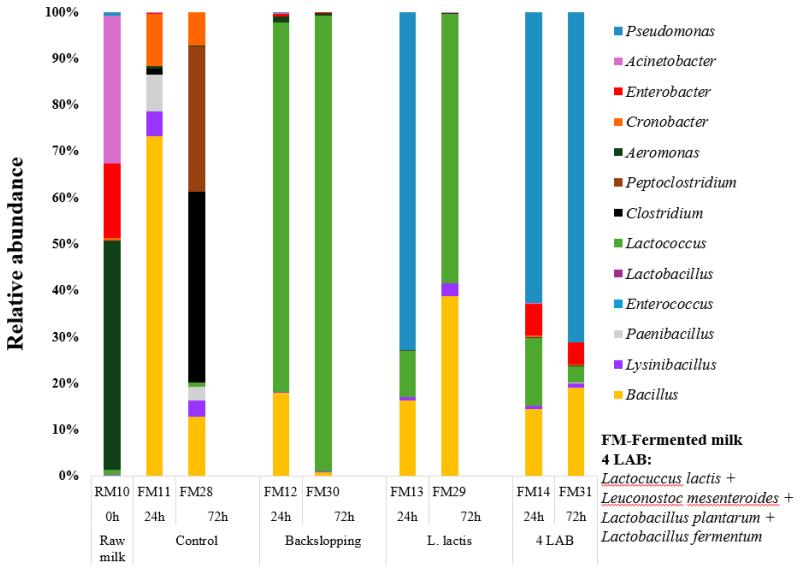
More specifically, at the genus level (Fig. 7.1A), the fermented milk was produced from raw milk composed of 44% *Aeromonas*, 29% *Acinetobacter*, and 14% *Enterobacter* as the main genera. This milk

pasteurized and fermented naturally contained species of the *Bacillus* (72%), *Cronobacter* (11%), *Paenibacillus* (8%), and *Lysinibacillus* (8%) dominant genera. This last fermented milk tolerates the significant multiplication of undesirable microorganisms such as *Bacillus cereus* (11.35-67%), *Clostridium bifermentans* (31%), *Clostridium butyricum* (24%), *Clostridium perfringens* (12%), *Cronobacter sakazakii* (7-11%), *Bacillus* sp. (4.6%), *Paenibacillus* sp. (6%), and *Lysinibacillus* (5%) (Fig. 7.1B). It presents a risk of food poisoning due to the presence of pathogenic microorganisms. Indeed, some strains of *B. cereus* can cause diarrhoea and/or vomiting and may eventually lead to fatal consequences (Ibrahim et al., 2022, Drobniewski, 1993). *C. perfringens* is another foodborne pathogen associated with acute gastrointestinal infections ranging from diarrhoea to necrotizing enterocolitis and myonecrosis in humans. This pathogen possesses an arsenal of toxins responsible for disease pathogenesis and its spores can survive normal cooking temperatures (Mehdizadeh Gohari et al., 2021). *C. sakazakii* is a species isolated from a wide variety of foods. This bacterium is an opportunistic pathogen associated with life-threatening infections in neonates, with reported mortality rates of 50-80% (Healy et al., 2010).

Regarding fermented milk obtained with *L. lactis* strains and with the mixture of the 4 LAB strains, they were composed of 73% and 63% *Pseudomonas*, 16% and 14% *Bacillus*, and 10% and 15% *Lactococcus*, respectively, on the first day of production. Additionally, unlike the milk fermented with *L. lactis*, the one produced with the mixture of the 4 LAB contained 10% *Enterobacter*. By day two, there was a significant growth of *Lactococcus* (58%), *Bacillus* (39%), and *Lysinibacillus* (3%) with disappearance of *Pseudomonas* (<1%) in the

samples fermented with *L. lactis* only (Fig. 7.1A). As for the milk fermented with the 4 strains, the microbial composition was similar to that on first day. In summary, controlled fermentation of milk with *L. lactis* allowed the reduction to insignificant levels of *Pseudomonas* spp. *L. lactis* thus exhibited antimicrobial action against *Pseudomonas*. This was also demonstrated by Nakamura et al. (2015), who showed that *L. lactis* is capable of preventing the growth of *Pseudomonas aeruginosa*. Similarly, Cho et al. (2020) demonstrated that *L. lactis* in co-culture had an antimicrobial effect on a strain of *P. aeruginosa*. Surprisingly, the association of *L. lactis* with the other LAB strains did not achieve the control of *Pseudomonas* spp. This might be due to the antagonistic action of the combined strains with *L. lactis*. Finally, the starter bacteria tested alone or in combination could not reduce the relative abundance of *B. cereus*, as opposed to the backslopping slot.

A: Composition of *Fanirè* at the genus level



B: Composition of *Fanirè* at the species level

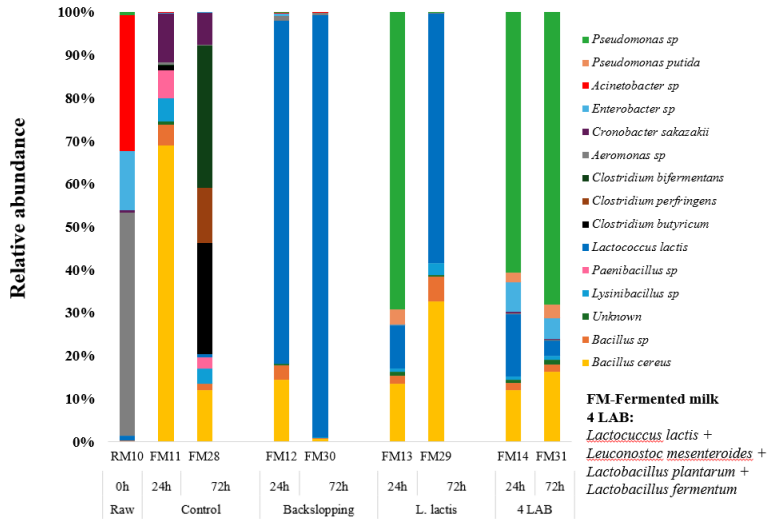
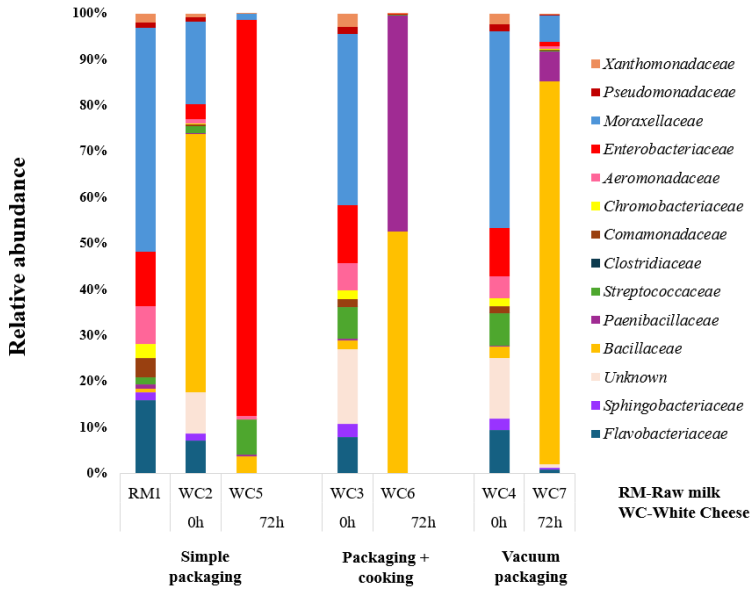


Figure 7.1: Dynamics of bacterial communities compositional of *Fanirè*.

Concerning the treated *Wagashi Gassirè* cheeses, the dominant families in the raw milk used for their preparation were *Moraxellaceae* (45%), *Flavobacteriaceae* (14%) and *Enterobacteriaceae* (11%) (Fig. 7.2A). The transformation of this milk into *Wagashi Gassirè* drastically reduced the abundance of these spoilage flora but favoured the predominance of *Bacillaceae* in the samples. Storing this cheese for three days allowed the growth of *Enterobacteriaceae*, which became predominant in this product, followed by *Streptococcaceae*. A second cooking of this cheese, vacuum-packed or not, and stored for three days drastically reduced the previous spoilage flora while favoring the significant growth of *Bacillaceae* consisting of species from the *Bacillus* (25-82%), *Lysinibacillus* and *Paenibacillus* genera (Fig. 7.2B). Extending the storage of *Wagashi Gassirè*, whether vacuum-packed or not and double-cooked, managed to control the majority of encountered spoilage strains except *Bacillaceae*. The persistence and growth of Bacilli in this product, especially in the packaged products, indicates the adaptability of these normally aerobic strains to limited oxygen conditions (Nakano et al., 1997). To better preserve the quality of this cheese and prevent its spoilage by these spoilage bacteria, particularly *Bacillus* spp. strains, it is important to develop strategies such as reducing the moisture content of the products by drying coupled with the addition of 3% salt in *Wagashi Gassirè* cheese; this should reduce the presence of *B. cereus* (Raevuori and Gnigeorgis, 1975; Rukure and Bester, 2001).

A: Composition of *Wagashi Gassirè* at the family level



B: Composition of *Wagashi Gassirè* at the genus level.

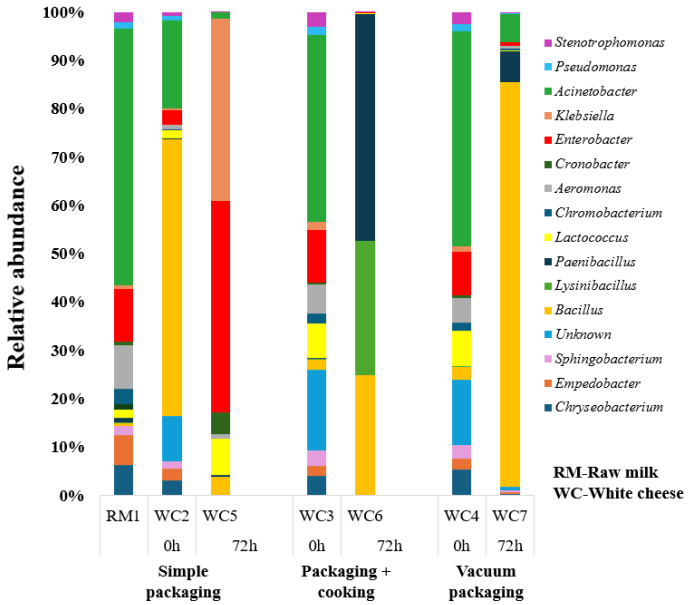


Figure 7.2: Dynamics of bacterial communities compositional of *Wagashi Gassirè.*

4. Conclusion

To improve the quality of *Wagashi Gassirè* and traditional fermented milk produced in Benin, a study on the effects of heat treatment, packaging, and controlled milk fermentation for obtaining fermented milk was conducted. The results indicate that the explored methods for preserving *Wagashi Gassirè* relatively ensure its sanitary quality, despite the persistence of spoilage microorganisms, predominantly *Bacillaceae*. Regarding the effect of controlled fermentation, the study reveals that using a strain of *L. lactis*, an indigenous microorganism of these traditional products, as a single starter allows for the production of fermented milk with improved sanitary quality. Further studies on the control of *Bacillaceae* in *Wagashi Gassirè* and the organoleptic quality of fermented milk improved by the promising *L. lactis* strain are essential.

5. Acknowledgements

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6. References

Cho, D.Y., Skinner, D., Lim, D.J., Mclemore, J.G., Koch, C.G., Zhang, S., Swords, W.E., Hunter, R., Crossman, D.K., Crowley, M.R., Grayson, J.W., Rowe, S.M., Woodworth, B.A. (2020) The impact of *Lactococcus lactis* (probiotic nasal rinse) co-culture on growth of patient-derived strains of *Pseudomonas aeruginosa*. *Int. Forum Allergy Rhinol.* 10, 444-449.

Dash, K.K., Fayaz, U., Dar, A.H., Shams, R., Manzoor, S., Sundarsingh, A., Deka, P., Khan, S.A. (2022) A comprehensive review on heat treatments and related impact on the quality and microbial safety of milk and milk-based products. *Food Chem. Adv*. 1, 100041.

Drobniewski, F.A. (1993) *Bacillus cereus* and related species. *Clin. Microbiol. Rev*. 6, 324-338.

Healy, B., Cooney, S., O'Brien, S., Iversen, C., Whyte, P., Nally, J., Callanan, J.J., Fanning, S. (2010) *Cronobacter* (*Enterobacter sakazakii*): an opportunistic foodborne pathogen. *Foodborne Pathog. Dis*. 7, 339-350.

Ibrahim, A.S., Hafiz, N.M., Saad, M.F. (2022) Prevalence of *Bacillus cereus* in dairy powders focusing on its toxigenic genes and antimicrobial resistance. *Arch. Microbiol*. 204, 339.

Mehdizadeh Gohari, I., Navarro, M.A., Li, J., Shrestha, A., Uzal, F., McClane, B.A. (2021) Pathogenicity and virulence of *Clostridium perfringens*. *Virulence* 12, 723-753.

Nakamura, S., Morimoto, Y.V., Kudo, S. (2015) A lactose fermentation product produced by *Lactococcus lactis subsp. lactis*, acetate,

inhibits the motility of flagellated pathogenic bacteria. *Microbiol*. 161, 701-707.

Nakano, M.M., Dailly, Y.P., Zuber, P., Clark, D.P. (1997) Characterization of anaerobic fermentative growth of *Bacillus subtilis*: identification of fermentation end products and genes required for growth. *J. Bacteriol.* 179, 6749-6755.

Raevuori, M., Genigeorgis, C. (1975) Effect of pH and sodium chloride on growth of *Bacillus cereus* in laboratory media and certain foods. *Appl. Microbiol.* 29, 68-73.

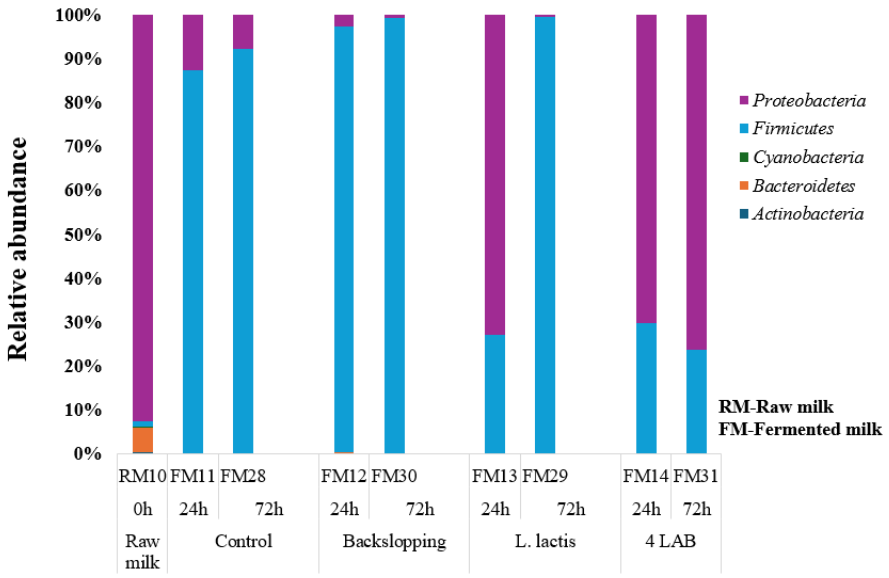
Rukure, G., Bester, B.H. (2001) Survival and growth of *Bacillus cereus* during Gouda cheese manufacturing. *Food Control*. 12, 31-36.

Sessou, P., Keisam, S., Tuikhar, N., Gagara, M., Farougou, S., Jeyaram, K. (2019) High-Throughput Illumina MiSeq amplicon sequencing of yeast communities associated with indigenous dairy products from Republics of Benin and Niger. *Front. Microbiol*. 10, 594.

Sessou, P., Keisam, S., Gagara, M., Komagbe, G., Farougou, S., Mahillon, J., Jeyaram, K. (2023) Comparative analyses of the bacterial communities present in the spontaneously fermented milk products of Northeast India and West Africa. *Front. Microbiol*. 14, 1166518.

Supplementary data

A: Composition of *Fanirè* at the phylum level.



B: Composition of *Fanirè* at the family level.

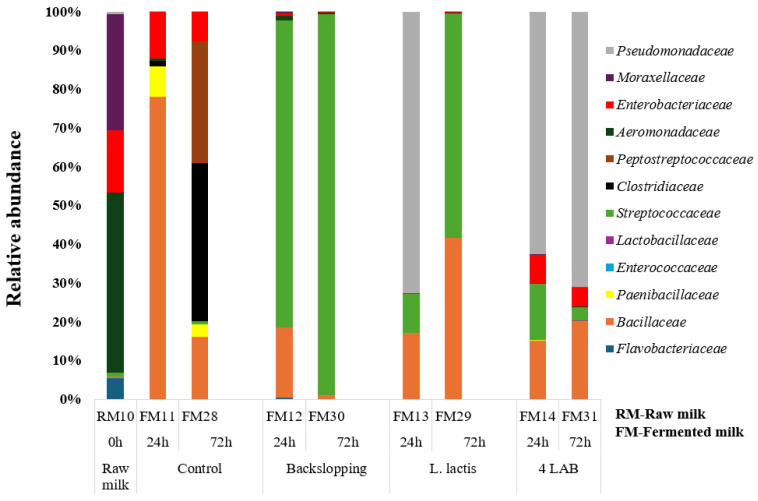


Figure 7.S1: Dynamics of bacterial communities compositional of *Fanirè*.

III. DISCUSSION, CONCLUSION ET PERSPECTIVES

Chapitre 8

8. Discussion générale

Dans ce chapitre, les résultats obtenus dans le cadre de cette thèse sont discutés et mis en perspective. Cette discussion est structurée en trois sections en fonction de l'approche méthodologique adoptée dans cette thèse. La première consistait à identifier auprès des acteurs de la filière lait les pratiques susceptibles de compromettre la qualité des produits cibles. Ensuite, une caractérisation microbiologique des laits crus, laits caillés et des *Wagashi Gassirè* a été réalisée, suivie d'une proposition d'améliorations desdits produits. Enfin, les améliorations proposées ont été testées en laboratoire et la qualité microbiologique des produits améliorés a aussi été évaluée.

8.1. Pratiques d'hygiène en rapport avec la traite et la transformation du lait en laits caillés et *Wagashi Gassirè* et facteurs favorisant leurs contaminations

Le lait et ses dérivés laits caillés et *Wagashi Gassirè* constituent des produits laitiers très appréciés au Bénin et dans la région ouest africaine en raison de leur grande valeur nutritionnelle et de leur disponibilité (Sessou et al., 2019). Produits de façon traditionnelle dans des conditions sanitaires souvent insuffisantes sans contrôle officiel de conformité aux normes réglementaires internationales, en l'absence de celles nationales, leur consommation est susceptible de mettre la santé publique en danger, bien que ce risque n'ait jamais été pleinement

estimé sur une base scientifique, en raison du manque de données sur les modes de consommation, de données épidémiologiques et de mise en place de programmes de surveillance appropriés (Adeyeye, 2017). Afin de bénéficier des éléments nutritifs dont il regorge, le lait cru de vache et ses dérivés dont le lait caillé et le *Wagashi Gassirè* sont consommés par la population béninoise qui s'expose à des risques sanitaires qui pourraient en découler.

La présente étude s'est penchée sur les différentes facettes de la chaîne laitière au Bénin, identifiant les acteurs clés et les facteurs influençant la qualité sanitaire du lait et de ses dérivés dans les communes de Nikki et de Dassa-Zoumé. Pour ce faire, une enquête auprès des éleveurs laitiers, des producteurs et vendeurs de lait caillé et de *Wagashi Gassirè* et des consommateurs a été réalisée. Les données ont porté sur divers aspects, tels que les caractéristiques sociodémographiques des répondants, les pratiques d'élevage, les stratégies de traitement des maladies animales, les méthodes de traite, de traitement et de stockage du lait et des produits laitiers, les méthodes de conservation ainsi que les pratiques de commercialisation.

Les résultats issus de cette étude ont révélé que l'élevage laitier est principalement pratiqué par des hommes non instruits du groupe Fulani. Ce manque d'éducation pose des défis pour la mise en place de bonnes pratiques d'élevage et d'hygiène, notamment dans la traite. Le niveau d'éducation des parties prenantes est crucial pour assurer le respect des normes sanitaires et le bon déroulement des opérations agricoles (Gökdai et al., 2020). Les maladies bovines les plus courantes incluent la fièvre aphteuse, la pasteurellose, la dermatophilose, la trypanosomiase et la péripneumonie contagieuse bovine.

La prévalence de ces maladies dans les troupeaux bovins peut entraîner des pertes économiques importantes en termes de production laitière et de santé animale (Wittwer, 2024). Elles représentent donc une menace importante pour la sécurité alimentaire et le développement économique du Bénin. De plus, ces maladies peuvent affecter la qualité du lait et de la viande, ayant ainsi un impact sur la santé des consommateurs. Il est préoccupant de constater que les éleveurs diagnostiquaient et traitaient souvent eux-mêmes ces pathologies sans la supervision d'un vétérinaire, utilisant notamment de manière abusive les antibiotiques et sans respect des délais d'attente. Cela peut conduire à la présence de résidus d'antibiotiques dans les produits laitiers, posant un risque pour la santé publique (Chiesa et al., 2020). De plus, le non-respect des instructions de dosage et des périodes d'attente après le traitement contribue à cette problématique.

Concernant la traite, nous avons observé que celle-ci est généralement manuelle et que le lavage des mains avant la traite n'est pas toujours pratiqué, et l'utilisation de matériaux inappropriés tels que les calebasses favorise la contamination du lait par des micro-organismes environnementaux. De plus, un faible pourcentage d'éleveurs met en œuvre les bonnes pratiques d'hygiène recommandées, ce qui augmente le risque de contamination croisée des produits laitiers (Nyokabi et al., 2021; Deddefo et al., 2023). Par ailleurs, la production de *Wagashi Gassirè* se déroule dans des conditions d'hygiène insuffisantes, exposant ainsi les produits laitiers à une contamination environnementale (Nyokabi et al., 2021).

Face à ces constats, des interventions ciblées sont nécessaires pour sensibiliser les acteurs de la filière laitière aux bonnes pratiques d'élevage et d'hygiène. L'adoption de pratiques d'élevage et d'hygiène adéquates est essentielle pour garantir la qualité et la sécurité des produits laitiers, tout en préservant la santé des consommateurs et le bien-être animal (Ledo et al., 2021). Le renforcement des contrôles de qualité et de la réglementation dans la filière laitière est crucial pour garantir la sécurité des produits laitiers destinés à la consommation humaine.

8.2. Caractérisations microbiologiques du lait caillé et des fromages *Wagashi Gassirè* produits au Bénin

Les *Fanirè* et *Wagashi Gassirè* du Bénin, sont des piliers de l'alimentation locale. Toutefois, en raison de leur richesse en éléments nutritifs, ils sont sujets à une prolifération microbienne, mettant en péril leur sécurité et leur qualité. L'étude a scruté la qualité microbiologique de ces produits laitiers traditionnels de Dassa-Zoumé et de Nikki au Bénin, ainsi que la résistance aux antibiotiques des souches d'*E. coli* et de *S. enterica* isolées. Parallèlement, elle a exploré certaines caractéristiques techno-fonctionnelles des principales bactéries lactiques présentes dans les échantillons de lait fermenté et procédé au screening des levures dans ces produits.

S'agissant de la qualité des produits, l'étude a mis en évidence une qualité microbiologique globalement insatisfaisante des produits analysés avec des niveaux de microorganismes indicateurs d'hygiène dépassant les normes établies au niveau européen et la présence de plusieurs agents pathogènes antibiorésistants préoccupants tels que

S. enterica et *E. coli*. La contamination bactérienne de ces produits par des microorganismes pathogènes peut être causée par divers facteurs, tels que le processus de traite, les ustensiles utilisés, l'environnement, ainsi que le personnel. De plus, ils peuvent être contaminés lors d'un stockage et d'un transport non hygiéniques. La présence de bactéries entériques, y compris *E. coli*, dans ces produits alimentaires est un indicateur fiable de la contamination fécale et un bioindicateur de la résistance aux antimicrobiens (Ashraf et al., 2023). En outre, la détection de souches pathogènes antibiorésistantes souligne un risque potentiel pour la santé publique, ces microorganismes étant associés à des infections alimentaires graves allant de troubles gastro-intestinaux à des complications plus sévères (Oyedeji et al., 2023). Ils peuvent causer des maladies graves allant de la diarrhée à des syndromes potentiellement mortels. *Salmonella*, par exemple, est responsable de diarrhées chez 2,8 milliards de personnes par an (Lando et al., 2023). La présence de ces microorganismes dans ces produits est préoccupante en raison de leur capacité à se multiplier et/ou produire des toxines (Oyedeji et al., 2023). Ces résultats soulignent la nécessité de renforcer les mesures de contrôle tout au long de la chaîne de production du lait et de sa transformation en ces produits laitiers afin de garantir la sécurité des consommateurs.

Par rapport aux bactéries lactiques et levures présentes dans les produits investigués, l'étude a révélé la présence de levures potentiellement pathogènes à dominance *Candida* et des bactéries lactiques ayant des applications en industrie alimentaire dont l'une des plus dominantes, *L. lactis*, possède des propriétés acidifiante et coagulante intéressantes pouvant être exploitées en tant que culture starter pour l'amélioration

du lait caillé. En raison du grand potentiel des bactéries lactiques en tant que probiotiques pour l'homme et les animaux et de leurs effets bénéfiques pour la santé (Lee et al., 2021; Sharma et al., 2023), l'étude recommande la consommation de ces produits laitiers améliorés du point de vue qualité sanitaire.

8.3. Diversité microbienne du lait caillé et *Wagashi Gassirè* produits au Bénin

Dans le but d'investiguer les changements dans la composition de la communauté microbienne pendant la production et le stockage du lait fermenté *Fanirè* et du fromage *Wagashi Gassirè*, largement consommés au Bénin, et d'apprécier leur statut sanitaire prévalant pendant leur durée de conservation, nous avons procédé au séquençage à haut débit des amplicons du gène de l'ARNr 16S bactérien des échantillons.

Les résultats ont révélé la prédominance des Firmicutes/Bacillota et des Proteobacteria/Pseudomonadota dans ces produits laitiers. Ces produits laitiers collectés dans des camps et marchés des deux communes, Nikki et Dassa-Zoumé, partageaient principalement et de façon décroissante, des bactéries appartenant aux *Streptococcaceae, Enterobacteriaceae, Moraxellaceae, Lactobacillaceae*, *Bacillaceae, Planococcaceae*, *Leuconostococaceae* et *Acetobacteraceae*. Les produits étaient dominés par des bactéries lactiques, principalement *L. lactis*, et des bactéries acétiques d'un grand intérêt industriel. Les bactéries lactiques sont essentielles pour assurer la protection et la sécurité des produits laitiers grâce à la production d'agents antimicrobiens (Sharma et al., 2023). L'étude a cependant révélé une présence notable

d'*Enterobacteriaceae*, une famille diversifiée de bacilles à Gram-négatif que l'on retrouve fréquemment dans les intestins humains et animaux. Ces bactéries d'origine fécale peuvent contaminer le fromage par le lait à cause de déficiences hygiéniques à la ferme, lors de la traite ou de la manipulation du lait, ou pendant la fabrication du fromage et l'utilisation d'eau contaminée. Certains membres de cette famille ont été identifiés comme des pathogènes potentiels et leur présence soulève des inquiétudes quant à la qualité des produits laitiers et à la sécurité alimentaire (Mladenović et al, 2021). En effet, les genres bactériens, comme *Salmonella*, *Shigella* et *Escherichia*, peuvent conduire à des toxi-infections et sont des agents pathogènes potentiels du fromage (Centeno et Carballo, 2023). De plus, certaines entérobactéries ont la capacité de produire des amines biogènes dans les produits laitiers et peuvent ainsi altérer la qualité et la sécurité des aliments (Decadt et De Vuyst, 2023).

Les entérobactéries fermentant le lactose (coliformes) peuvent aussi conduire à des fermentations indésirables, entraînant un gonflement précoce du fromage (Tabla et al., 2016; 2018). De plus, la présence de certaines entérobactéries peut être associée à la production d'aldéhydes, de composés sulfurés et aromatiques dans les produits laitiers, et notamment dans les échantillons de *Wagashi Gassirè*. Ces composés jouent un rôle essentiel dans le développement des arômes et des saveurs des fromages. En effet, à faibles teneurs les aldéhydes comme l'acétaldéhyde, le 2-méthyl propanal et le 3-méthyl butanal contribuent à des arômes fruités et floraux, tandis que les composés sulfurés peuvent ajouter des notes de sulfure et de soufre (altération du produit).

Les cétones, les alcools et les acides, contribuent également à la complexité des saveurs, offrant des notes variées telles que le beurre, le fruité et l'acidité (Morales et al., 2004; Natrella et al., 2020).
L'étude a, par ailleurs, mis en évidence la dominance significative de la famille *Moraxellaceae*, en particulier *Acinetobacter* spp., dans les produits examinés. L'altération des produits laitiers par les *Moraxellaceae* peut se manifester sous forme de changements de texture et de goût, via une dégradation des protéines et des graisses, et la production de composés volatils indésirables, contribuant ainsi à des odeurs ou des arômes anormaux. En raison de leur capacité à altérer les produits laitiers, il est important de surveiller et de contrôler la présence des *Moraxellaceae* dans les installations laitières et les produits finis. Des pratiques d'hygiène rigoureuses, telles que le nettoyage et la désinfection fréquents des équipements et des surfaces de travail, ainsi que le maintien de bonnes pratiques de manipulation, peuvent aider à réduire le risque d'altération des produits laitiers par les *Moraxellaceae* (Saad et al., 2018).

Des variations dans les profils bactériens selon les produits et régions de production des produits laitiers ont été observées, ainsi que des changements significatifs dans la dynamique bactérienne pendant la conservation du lait fermenté et des fromages. En outre, plusieurs agents pathogènes potentiels dans ces produits ont été identifiés. Leur présence souligne l'importance de mettre en œuvre des stratégies visant à lutter contre ces pathogènes et à améliorer la sécurité alimentaire des produits laitiers traditionnels. Ces découvertes fournissent donc des informations précieuses sur la diversité microbienne et la dynamique bactérienne des produits laitiers traditionnels béninois et peuvent

contribuer à l'amélioration de leur sécurité alimentaire et de leur durée de conservation.

8.4. Proposition d'amélioration de la qualité microbiologique du lait caillé et du *Wagashi Gassirè*

Cette étude a exploré l'effet de la fermentation contrôlée sur la qualité sanitaire du lait caillé *Fanirè* et l'impact des traitements thermiques et de l'emballage sur la sécurité du fromage traditionnel *Wagashi Gassirè* au Bénin. Les analyses ont été effectuées en utilisant des méthodes de culture-dépendante et de culture-indépendante (séquençage de l'amplicon 16S rRNA). Les résultats obtenus ont montré que le lait fermenté avec *L. lactis* seul maintenait une bonne qualité microbiologique conformément aux critères microbiologiques du règlement CE 2073/2005, quel que soit le moment de l'analyse. En revanche, le lait caillé obtenu par ensemencement avec une combinaison de bactéries lactiques a affiché une amélioration de sa qualité avec la durée de stockage. Le lait fermenté obtenu naturellement avec du lait pasteurisé ne respectait pas cette réglementation. Pour les échantillons de *Wagashi Gassirè*, la double cuisson, avec ou sans emballage sous vide, maintenait efficacement la qualité sanitaire des fromages pendant toute la période de surveillance. En revanche, les échantillons sans deuxième cuisson ne respectaient pas les critères de qualité au troisième jour de stockage, en raison de la présence d'entérobactéries, ce qui pourrait être dû à une contamination post-production (Mladenović et al., 2021).

L'analyse des résultats de la culture-indépendante a révélé que le lait cru utilisé pour la production de lait fermenté était principalement composé de Proteobacteria/Pseudomonadota (très abondants), suivi de Bacteroidetes (abondants) et de Bacillota (faibles). Le lait fermenté issu de ce lait cru se composait principalement de Bacillota (dominants) pour le contrôle et pour le lait fermenté par ensemencement, tandis que les produits laitiers à fermentation contrôlée contenaient des Bacillota (modérés) et Pseudomonadota (très abondants). Le lait cru pour la production de lait fermenté était riche en microorganismes d'altération, notamment les *Flavobacteriaceae*, *Aeromonaceae*, *Enterobacteriaceae* et *Moraxellaceae*, couramment retrouvés dans le lait cru et ayant un impact significatif sur la qualité et la sécurité du fromage (Parente et al., 2020). En effet, les *Flavobacteriaceae* et *Moraxellaceae* (*Acinetobacter* et *Moraxella*) bien qu'elles ne soient généralement pas pathogènes, peuvent contribuer à la détérioration en causant des arômes indésirables et des changements de texture.

La présence de ces bactéries dans le lait cru utilisé pour la production de fromage *Wagashi Gassirè* indique une contamination et des pratiques d'hygiène inadéquates. Leur impact se traduit par la détérioration du fromage, les risques pour la santé, notamment de la part des *Enterobacteriaceae* et des *Aeromonaceae*, ainsi que la dégradation de sa qualité (Karanth et al., 2023). La transformation de ce lait cru en *Wagashi Gassirè* a considérablement réduit l'abondance de la flore de détérioration, mais a favorisé la prédominance dans les échantillons des *Bacillaceae* dont *Bacillus et Paenibacillus,* deux genres de bactéries sporulantes aérobies qui posent des préoccupations particulières en raison de leur capacité à survivre à la pasteurisation et à se développer

dans le lait pasteurisé à longue durée de conservation (Porcellato et al., 2018; 2019). La persistance et la croissance de *Bacillus* dans le fromage, en particulier dans les produits emballés, indiquent l'adaptabilité de ces souches normalement aérobies aux conditions de faible teneur en oxygène (Nakano et al., 1997). Pour mieux préserver la qualité de ce fromage et prévenir sa détérioration par ces bactéries, notamment les souches de *Bacillus,* il est important de développer des stratégies telles que la réduction de la teneur en humidité des produits par séchage, associée à l'ajout de sel dans le fromage *Wagashi Gassirè* (Rukure et Bester, 2001).

Chapitre 9

9. Conclusion et perspectives

Dans le paysage alimentaire du Bénin, le lait caillé et le fromage *Wagashi Gassirè* occupent une place de choix, tant sur le plan nutritionnel que culturel. Ces produits laitiers traditionnels sont non seulement des éléments incontournables de l'alimentation locale, mais aussi des témoins de la richesse gastronomique et culturelle du pays. Cependant, malgré leur importance, ces produits sont souvent sujets à des problèmes de contamination microbienne, compromettant ainsi leur qualité et leur sécurité. Dans cette optique, notre étude s'est concentrée sur l'analyse des pratiques d'hygiène adoptées par les acteurs de la chaîne de production laitière dans les communes de Nikki et Dassa-Zoumé au Bénin afin d'identifier les facteurs favorisant la contamination du lait cru, du lait caillé et du fromage *Wagashi Gassirè* et de proposer des stratégies d'amélioration de leur qualité et de leur sécurité.

À travers une enquête transversale auprès de 401 acteurs de la filière laitière, trois types d'éleveurs laitiers et trois groupes de producteurs de *Wagashi Gassirè* ont été identifiés, chacun caractérisé par des pratiques d'hygiène spécifiques. Ces résultats mettent en évidence l'importance cruciale des pratiques d'hygiène dans la préservation de la qualité sanitaire des produits laitiers et qui doivent être améliorées dans les zones prospectées.

Parallèlement, pour apprécier l'impact des pratiques d'hygiène sur la chaine de transformation du lait en ces deux dérivés majoritaires, notre étude s'est également penchée sur l'analyse microbiologique approfondie de ses produits laitiers traditionnels du Bénin, mettant en lumière la complexité des communautés bactériennes présentes dans le fromage *Wagashi Gassirè* et le lait caillé *Fanirè*. Ces analyses ont révélé la présence de potentielles menaces pour la santé publique, soulignant ainsi l'urgence d'interventions visant à améliorer la sécurité alimentaire.

Enfin, pour l'amélioration de la qualité de ces produits, nous avons évalué l'effet du traitement thermique et de l'emballage sur la qualité sanitaire du fromage *Wagashi Gassirè*, ainsi que de la fermentation contrôlée sur la sécurité du lait caillé *Fanirè*. Ces recherches offrent un aperçu précieux des interventions technologiques potentielles pour préserver la qualité microbiologique des produits laitiers traditionnels du Bénin.

En conclusion, cette étude a contribué à une meilleure compréhension des défis liés à la sécurité et à la qualité des produits laitiers traditionnels au Bénin, ainsi qu'à proposer des stratégies d'amélioration visant à garantir leur sécurité alimentaire et leur valeur culturelle. Pour ce faire, il s'avère nécessaire de poursuivre les travaux en approfondissant les propriétés techno-fonctionnelles de *L. lactis* investiguée dans la présente étude à travers la recherche de ces propriétés probiotiques et à sa caractérisation moléculaire complète. D'autres méthodes de conservation devront être testées (*e.g.* ajout de sel pour limiter la croissance des bactéries) et les acteurs de la chaine de transformation

du lait devront être mieux formés à l'hygiène alimentaire et les éleveurs aux pratiques d'hygiène en élevage.

Références bibliographiques

Abdoukarim, I.Y., Dahouda, M., Attakpa, E.Y., Koutinhouin, G.B., Ahounou, G.S., Toleba, S.S., Balogoun, B.S. (2013) Diversité des systèmes d'élevages de bovins de race bovine Borgou dans la zone soudanienne du Bénin. *Int. J. Biol. Chem. Sci*. 7, 125-146.

Abril, G.A., Villa, G.T., Barros-Velázquez, J., Cañas, B., Sánchez-Pérez, A., Calo-Mata, P., Carrera, M. (2020) *Staphylococcus aureus* exotoxins and their detection in the dairy industry and mastitis. *Toxins* 12, 2-18.

Adeniyi, B.A., Adetoye, A., Ayeni, F.A. (2015) Antibacterial activities of lactic acid bacteria isolated from cow faeces against potential enteric pathogens. *Afr. Health Sci.* 15, 888-895.

Adeyeye, S.A.O. (2017). Safety issues in traditional West African foods: A Critical Review. *J. Culinary Sci. Technol.* 15, 101-125.

Agyei, D., Owusu-Kwarteng, J., Akabanda, F., Akomea-Frempong, S. (2020) Indigenous African fermented dairy products: Processing technology, microbiology and health benefits. *Crit. Rev. Food Sci. Nutr.* 60, 991-1006.

Ahmadi, E. (2019) Potential public health risk due to consumption of contaminated bovine milk with aflatoxin M1 and *Coxiella burnetii* in the West of Iran. *Int. J. Dairy Technol.* 73, 479-485.

Ahmed, A.I.M.A., Faye, B., Blanchard, L., Bakheit, S.A. (2010) Assessment of quality of camel milk and gariss, north Kordofan State Sudan. *Res. J. Anim. Vet. Sci.* 5, 18-22.

Aïssi, V.M., Soumanou, M.M., Bankolè, H., Toukourou, F., de Souza, C.A. (2009) Evaluation of hygienic and mycological quality of local cheese marketed in Benin. *Aust. J. Basic Appl. Sci.* 3, 2397-2404.

Akogou, F.U.G., Den Besten, H.M.W., Polycarpe Kayodé, A.P., Fogliano, V., Linnemann, A.R. (2018) Antimicrobial evaluation of red, phytoalexin-rich sorghum food biocolorant. *PLoS One* 13, 1-15.

Aldrete-Tapia, A., Escobar-Ramírez, M.C., Tamplin, M.L., Hernández-Iturriaga, M. (2014) High-throughput sequencing of microbial communities in Poro cheese, an artisanal Mexican cheese. *Food Microbiol.* 44, 136-141.

Alegría, Á., Szczesny, P., Mayo, B., Bardowski, J., Kowalczyk, M. (2012) Biodiversity in *Oscypek*, a traditional Polish cheese, determined by culture-dependent and-independent approaches. *Appl. Environ. Microbiol.* 78, 1890-1898.

Allard, G., Ryan, F.J., Jeffery, I.B., Claesson, M.J. (2015) SPINGO: a rapid species-classifier for microbial amplicon sequences. *BMC bioinformatics* 16, 1-8.

Ampe, F., Omar, B.N., Moizan, C., Wacher, C., Guyot, J.P. (1999) Polyphasic study of the spatial distribution of microorganisms in Mexican pozol, a fermented maise dough, demonstrates the need for cultivation-independent methods to investigate traditional fermentations. *Appl. Environ. Microbiol.* 65, 5464-5473.

Amit, S.K., Uddin, M.M., Rahman, R., Islam, S.R., Khan, M.S. (2017) A review on mechanisms and commercial aspects of food preservation and processing. *Agric. Food Secur.* 6, 51.

Ameer, S., Aslam, S., Saeed, M. (2019) Preservation of milk and dairy products by using biopreservatives. *Middle East J. Appl. Sci. Technol.* 2, 72-79.

Anihouvi, D.G.H., Henriet, O., Kpoclou, Y.E., Scippo, M.L., Hounhouigan, D.J., Anihouvi, V.B., Mahillon, J. (2021) Bacterial diversity of smoked and smoked-dried fish from West Africa: A metagenomic approach. *J. Food Process. Preserv*. 45, 1-12.

Anihouvi, D.G.H., Kpoclou, Y.E., Abdel Massih, M., Iko Afe, O.H., Assogba, M.F., Covo, M., Scippo, M-L., Hounhouigan, J.D., Anihouvi, V., Mahillon, J. (2019) Microbiological characteristics of smoked and smoked-dried fish processed in Benin. *Food Sci. Nutr.* 7, 1821-1827.

Antunes, I.C., Bexiga, R., Pinto, C., Roseiro, L.C., Quaresma, M.A.G. (2022) Cow's milk in human nutrition and the emergence of plant-based milk alternatives. *Foods* 12, 99.

Apaliya, M.T., Osae, R., Kwaw, E., Mahunu, G.K., Osei-Kwarteng, M., Hardi, I.M. (2022) Omics in traditional fermented foods and beverages. In: Sulieman, A.M.E, Mariod, A.A. (eds) African fermented food products. New trend, *Springer cham* pp. 551-563.

Arslan, S., Eyi, A., Özdemir, F. (2011) Spoilage potentials and antimicrobial resistance of *Pseudomonas* spp. isolated from cheeses. *J. Dairy Sci.* 94, 5851-5856.

Asare, P.T., Zurfluh, K., Greppi, A., Lynch, D., Schwab, C., Stephan, R., Lacroix, C. (2020) Reuterin demonstrates potent antimicrobial activity against a broad panel of human and poultry meat *Campylobacter* spp. isolates. *Microorganisms* 8, 384.

Asfaw, T., Genetu, D., Shenkute, D., Shenkutie, T.T., Amare, Y.E., Habteweld, H.A., Yitayew, B. (2023) Pathogenic Bacteria and Their Antibiotic Resistance Patterns in Milk, Yoghurt and Milk Contact Surfaces in Debre Berhan Town, Ethiopia. *Infect. Drug Resist*. 16, 4297-4309.

Ashraf, D., Ombarak, R.A., Samir, A., Abdel-Salam, A.B. (2023) Characterization of multidrug-resistant potential pathogens isolated from milk and some dairy products in Egypt. *J. Adv. Vet. Anim. Res*. 10, 275-283.

Aworh, O.C., Egounléty, M., (1986) Preliminary evaluation of effects of gas packaging on organoleptic and microbiological quality of *warankasi*. *Niger. Food J.* 1, 75-79.

Ayivi, R.D., Gyawali, R., Krastanov, A., Aljaloud, S.O., Worku, M., Tahergorabi, R., da Silva, C.R., Ibrahim, S.A. (2020) Lactic acid bacteria: Food safety and human health applications. *Dairy* 1, 202-232.

Bagré, T.S., Kagambèga, A., Bawa, I.H., Bsadjo Tchamba, G., Dembélé, R., Zongo, C., Savadogo, A. Aggad, H. Traoré, A.S. Barro, N. (2014) Antibiotic susceptibility of *Escherichia coli* and *Salmonella* strains isolated from raw and curds milk consumed in Ouagadougou and Ziniaré, Burkina Faso. *Afr. J. Microbiol. Res.* 8, 1012-1016.

Barooah, M., Bora, S.S., Goswami, G. (2020) Ethnic fermented foods and beverages of Assam. In: Tamang, J.P. (eds) Ethnic fermented foods and beverages of India: Science history and culture. *Springer Nature Singapore Pte Ltd* 152, 85-104.

Barreto, Í.M.L.G., Rangel, A.H.D.N., Urbano, S.A., Bezerra, J.D.S., Oliveira, C.A.D.A. (2019) Equine milk and its potential use in the human diet. *Food Sci. Technol.* 39, 1-7.

Barłowska, J., Szwajkowska, M., Litwińczuk, Z., Król, J. (2011) Nutritional value and technological suitability of milk from various animal species used for dairy production. *Compr. Rev. Food Sci. Food Safety* 10, 291-302.

Benin, S., Wood, S., Nin-Pratt, A. (2016) Introduction. In: Benin S. (eds) Agricultural productivity in Africa. Trends, patterns and determinants. *Int. Food Policy Res. Inst*. pp. 26-47.

Berhe, T., Ipsen, R., Seifu, E., Kurtu, M.Y., Fugl, A., Hansen, E.B. (2019) Metagenomic analysis of bacterial community composition in *Dhanaan*: Ethiopian traditional fermented camel milk. *FEMS Microbiol. Lett.* 366, 1-6.

Bhatt, V.D., Ahir, V.B., Koringa, P.G., Jakhesara, S.J., Rank, D.N., Nauriyal, D.S., Joshi, C.G. (2012) Milk microbiome signatures of subclinical mastitis-affected cattle analysed by shotgun sequencing. *J. Appl. Microbiol.* 112, 639-650.

Bhattacharya, A., Shantikumar, S., Beaufoy, D., Allman, A., Fenelon, D., Reynolds, K., Normington, A., Afza, M., Todkill, D. (2020) Outbreak of *Clostridium perfringens* food poisoning linked to leeks in cheese sauce: an unusual source. *Epidemiol. Infect.* 148, e43.

Bintsis, T. (2018) Lactic acid bacteria: their applications in foods. *J. Bacteriol. Mycol.* 6, 89-94.

Boko, C., Adje, Y., Atindogbe, G., Hounmanou, Y.M., Dossa, F., Sessou, P., Farougou, S. (2016) Evaluation of the production

technologies and the microbial and physico-chemical qualities of curdled milk produced in Benin. *J. Appl. Biosci.* 104, 10019-10033.

Bokulich, N.A., Amiranashvili, L., Chitchyan, K., Ghazanchyan, N., Darbinyan, K., Gagelidze, N., Sadunishvili, T., Goginyan, V., Kvesitadze, G., Torok, T., Mills, D.A. (2015) Microbial biogeography of the transnational fermented milk M*atsoni*. *Food Microbiol*. 50, 12-19.

Bokulich, N.A., Mills, D.A. (2013) Facility-specific "house" microbiome drives microbial landscapes of artisan cheesemaking plants. *Appl. Environ. Microbiol.* 79, 5214-5223.

Cancino-Padilla, N., Fellenberg, M.A., Franco, W., Ibáñez, R.A., Pérez, E.V.B. (2017) Foodborne bacteria in dairy products: Detection by molecular techniques. *Cien. Inv. Agr*. 44, 215-229. doi: 10.7764/rcia.v44i3.1811.

Cao, H., Yan, Y., Wang, L., Dong, L., Pang, X., Tang, S., Li, A., Xiang, A., Zhang, L., Zheng, B. (2021) High-Throughput Sequencing Reveals Bacterial Diversity in Raw Milk production environment and production chain in Tangshan city of China. *Food Sci. Anim. Resour*. 41, 452-467.

Capodifoglio, E., Vidal, A.M.C., Lima, J.A.S., Bortoletto, F., D'Abreu, L.F., Gonçalves, A.C.S., Netto, A.S. (2016) Lipolytic and proteolytic activity of *Pseudomonas* spp. isolated during milking and storage of refrigerated raw milk. *J. Dairy Sci.* 99, 5214-5223.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R. (2012) Ultra-high-

throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1-5.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. U S A* 108, 4516-4522.

Cardinali, G., Corte, L., Robert, V. (2017) Next generation sequencing: problems and opportunities for next generation studies of microbial communities in food and food industry. *Curr. Opin. Food Sci.*17, 62-67.

Carminati, D., Bonvini, B., Rossetti, L., Zago, M., Tidona, F., Giraffa, G. (2019) Investigation on the presence of blue pigment-producing *Pseudomonas* strains along a production line of fresh mozzarella cheese. *Food Contr.* 100, 321-328.

Carraro, L., Maifreni, M., Bartolomeoli, I., Martino, M.E., Novelli, E., Frigo, F., Marino, M., Cardazzo, B. (2011) Comparison of culture-dependent and-independent methods for bacterial community monitoring during Montasio cheese manufacturing. *Res. Microbiol.* 162, 231-239.

Centeno, J.A., Carballo, J. (2023) Current Advances in Cheese Microbiology. *Foods* 12, 2577.

Chabi Toko, R., Adegbidi, A., Lebailly, P. (2015) Valorisation des produits laitiers dans les ménages Peulh du Nord-Est du Bénin. *Int. J. Biol. Chem. Sci.* 9, 2716-2726.

Chandan, R.C. (2015) Role of milk and dairy foods in nutrition and health. In: Chandan, R.C., Kilara, A., Shah, N.P. (eds) Dairy

processing and quality assurance: an overview. *John Wiley & Sons, Ltd*. pp. 428-466.

Chen, M.J., Chen, C.C., Huang, Y.C., Tseng, C.C., Hsu, J.T., Lin, Y.F. (2021) Taiwan gastrointestinal disease, *Helicobacter* consortium. The efficacy of *Lactobacillus acidophilus* and *rhamnosus* in the reduction of bacterial load of *Helicobacter pylori* and modification of gut microbiota-a double-blind, placebo-controlled, randomized trial. *Helicobacter* 26, e12857.

Chiesa, L.M., DeCastelli, L., Nobile, M., Martucci, F., Mosconi, G., Fontana, M., Castrica, M., Arioli, F., Panseri, S. (2020) Analysis of antibiotic residues in raw bovine milk and their impact toward food safety and on milk starter cultures in cheese-making process. *LWT-Food Sci. Technol.* 131, 109783.

Cho, D.Y., Skinner, D., Lim, D.J., Mclemore, J.G., Koch, C.G., Zhang, S., Swords, W.E., Hunter, R., Crossman, D.K., Crowley, M.R., Grayson, J.W., Rowe, S.M., Woodworth, B.A. (2020) The impact of *Lactococcus lactis* (probiotic nasal rinse) co-culture on growth of patient-derived strains of *Pseudomonas aeruginosa*. *Int. Forum Allergy Rhinol.* 10, 444-449.

Clarridge, J.E. (2004) Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin. Microbiol. Rev.* 17, 840-862.

CLSI (2020) Clinical and Laboratory Standards Institute. CLSI document M100. Performances standards for antimicrobial susceptibility testing, 30th ed informational supplement, Wayne, Pa.

Commission Européenne (2005) Règlement (CE) n°2073/2005 de la commission du 15 novembre 2005 concernant les critères microbiologiques applicables aux denrées alimentaires. *J. O. U. E.* L 338, 1-30.

Commission Européenne (2007) Règlement (CE) n°1441/2007 modifiant le règlement (CE) n°2073/2005 concernant les critères microbiologiques applicables aux denrées alimentaires. *J. O. U. E.* L 322, 12-29.

Cornillon, P.A., Guyader, A., Husson, F., Jégou, N., Josse J., Klutchnikoff, N., Matzner-Lober, E., Rouvière, L., Thieurmel, B., (2018) R pour la statistique et la science des données. *Saic. Rennes*, 420p.

Costa, C., Lucera, A., Lacivita, V., Saccotelli, M.A., Conte, A., Del Nobile, M.A. (2016) Packaging optimisation for portioned Canestrato di Moliterno cheese. *Int. J. Dairy Technol*. 69, 401-409.

Dahouda, M., Mama Yero Boubacar, A., Dossa, L.H., Dotche, O.I., Ahounou, G.S., Kiki, P., Youssao, A.K.I. (2019) Stratégies d'alimentation et gestion des ressources alimentaires dans les élevages bovins des Communes de Nikki, Kalalé et N'Dali au Nord Est Bénin. *Rev. Int. Sci. Appl*. 2, 48-70.

Dalmasso, A., Soto del Rio, M.D., Civera, T., Pattono, D., Cardazzo, B., Bottero, M.T. (2016) Characterization of microbiota in Plaisentif cheese by high-throughput sequencing. *LWT - Food Sci. Technol*. 69, 490-496.

Dan, T., Ren, W., Liu, Y., Tian, J., Chen, H., Li, T., Liu, W. (2019) Volatile flavor compounds profile and fermentation characteristics

of milk fermented by *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Front. Microbiol*. 10, 2183.

Dan, T., Wang, D., Wu, S., Jin, R., Ren, W., Sun, T. (2017) Profiles of volatile flavor compounds in milk fermented with different proportional combinations of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. *Molecules* 22, 1633.

Dash, K.K., Fayaz, U., Dar, A.H., Shams, R., Manzoor, S., Sundarsingh, A., Deka, P., Khan, S.A. (2022) A comprehensive review on heat treatments and related impact on the quality and microbial safety of milk and milk-based products. *Food Chem. Adv*. 1, 100041.

de Almeida Júnior, W.L.G., da Silva Ferrari, Í., de Souza, J.V., da Silva, C.D.A., da Costa, M.M., Dias, F.S. (2015) Characterization and evaluation of lactic acid bacteria isolated from goat milk. *Food Control*, 53, 96-103.

Decadt, H., De Vuyst, L. (2023) Insights into the microbiota and defects of present-day Gouda cheese productions. *Curr. Opin. Food Sci.* 52, 101044.

Deddefo, A., Mamo, G., Asfaw, M., Amenu, K. (2023) Factors affecting the microbiological quality and contamination of farm bulk milk by *Staphylococcus aureus* in dairy farms in Asella, Ethiopia. *BMC Microbiol*. 23, 65.

De Mandal, S., Singh, S.S., Muthukumaran, R.B., Thanzami, K., Kumar, V., Kumar, N.S. (2018) Metagenomic analysis and the functional profiles of traditional fermented pork fat 'sa-um' of Northeast India. *AMB Express* 8, 1-11.

de Melo Pereira, G.V., de Carvalho Neto, D.P., Maske, B.L., De Dea Lindner, J., Vale, A.S., Favero, G.R., Viesser, J., de Carvalho, J.C., Góes-Neto, A., Soccol, C.R. (2022) An updated review on bacterial community composition of traditional fermented milk products: what next-generation sequencing has revealed so far? *Crit. Rev. Food Sci. Nutr*. 62, 1870-1889.

de Souza, M.T.P., Fagnani, R., Alegro, L.C.A., de Santana, E.H.W. (2024) Non-starter lactic acid bacteria and citrate fermenting bacteria in milk supply chain: Are they easily controlled? *Int. Dairy J.* 149, 105839.

Deka, P., Mehetre, G.T., Lalnunmawii, E., Upadhyaya, K., Singh, G., Hashem, A., Al-Arjani, A.B.F., Abd_Allah, E.F., Singh, B.P. (2021) Metagenomic analysis of bacterial diversity in traditional fermented foods reveals food-specific dominance of specific bacterial taxa. *Ferment.* 7, 1-17.

Delcenserie, V., Taminiau, B., Delhalle, L., Nezer, C., Doyen, P., Crevecoeur, S., Roussey, D., Korsak, N., Daube, G. (2014) Microbiota characterisation of a Belgian protected designation of origin cheese, Herve cheese, using metagenomic analysis. *J. Dairy Sci.* 97, 6046-6056.

Dertli, E., Çon, A.H. (2017) Microbial diversity of traditional kefir grains and their role on kefir aroma. *LWT* 85, 151-157.

Dewan, S., Tamang, J.P. (2007) Dominant lactic acid bacteria and their technological properties isolated from the Himalayan ethnic fermented milk products. *Antonie van Leeuwenhoek* 92, 343-352.

Dierick, K., Van Coillie, E., Swiecicka, I., Meyfroidt, G., Devlieger, H., Meulemans, A., Hoedemaekers, G., Fourie, L., Heyndrickx, M.,

Mahillon, J. (2005) Fatal family outbreak of *Bacillus cereus* associated food poisoning. *J. Clin. Microbiol*. 43, 4277-4279.

Djobo, O., Sina, H., Kombienou, D.P., Djenontin, M.W., Ahyi, V., Youssao, I.A.K., Adjanohoun, A., Rendueles, M., Baba-Moussa, L. (2021) Uses and microbiological quality of fresh cow's milk sold in three markets in South Benin. *Afr. J. Food Sci.* 15, 345-352.

Dognon, S.R. (2018) Évaluation de l'impact de l'utilisation de molécules bioactives en agriculture et en élevage sur la qualité sanitaire des produits carnés: cas des résidus d'antibiotiques et de pesticides dans la viande bovine consommée au Nord-Est du Bénin. Thèse de doctorat en Sciences Vétérinaires de la Faculté de Médecine Vétérinaire de l'Université de Liège, Belgique, 245p.

Dognon, S.R., Antoine-Moussiaux, N., Douny, C., Gustin, P., Moula, N., Scippo, M.L., and Youssao, A.K.I. (2018) The use of antibiotics in cattle in North-East Benin: pharmaceutical inventory and risk practices of cattle breeders. *Trop. Anim. Health Prod*. 50, 1683-1699.

Dossou, J., Atchouké, G.D., Dabadé, D.S., Azokpota, P., Montcho, J. K. (2016) Évaluation comparative de la qualité nutritionnelle et sanitaire du lait de différentes races de vaches de quelques zones d'élevage du Bénin. *Eur. Sci. J*. 12, 141-159.

Dossou, J., Hounzangbe-Adote, S., Soulé, A., (2006) Production et transformation du lait frais en fromage en fromage Peulh au Bénin. Rapport de guide de bonnes pratiques. Université d'Abomey-Calavi, Abomey-Calavi, Bénin. pp. 1-33.

Dossou, J., Montcho, J.K., Londji, S., Atchouké, G.D.L., Odjo, S. (2016) Procédé amélioré de conservation et de stabilisation du

fromage Peulh par l'effet combiné du traitement thermique et du conditionnement sous vide. *Eur. Sci. J*. 12, 189-209.

Dossou, W.A., Seko Orou Baké M.T., Komagbe G., Sessou P., Youssao A.K.I., Farougou S., Hounhouigan D.J., Mahillon J., Mongbo R., Poncelet M., Madode Y.E., Douny C., Scippo M-L., Clinquart A., Azokpota P. (2022) Processing and preservation methods of *Wagashi Gassirè*, a traditional cheese produced in Benin, *Heliyon*, 8, e10605.

Drobniewski, F.A. (1993) *Bacillus cereus* and related species. *Clin. Microbiol. Rev*. 6, 324-338.

Duru, I.C., Laine, P., Andreevskaya, M., Paulin, L., Kananen, S., Tynkkynen, S., Auvinen, P., Smolander, O.P. (2018) Metagenomic and metatranscriptomic analysis of the microbial community in Swiss-type Maasdam cheese during ripening. *Int. J. Food Microbiol.* 281, 10-22.

Elariny, E.Y.T., Ahmed, H.A., Khatab, A.A.H., Mohamed, R.E. (2021) Genotyping, antibiotic resistance and biofilm formation ability of *Clostridium perfringens* isolated from raw milk, dairy products and human consumers. *J. Anim. Health Prod.* 9, 34-41.

Elshaghabee, F.M. (2023) Probiotics in dairy foods: Advantages and disadvantages. *Egypt. J. Agric. Res.* 74, 1-18.

El-Zamkan, M.A., Hameed, K.G.A. (2016) Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in raw milk and some dairy products. *Vet. World* 9, 1147-1151.

Ercolini, D. (2013) High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. *Appl. Environ. Microbiol.* 79, 3148-3155.

EUCAST (2019) Comité de l'antibiogramme de la Société Française de Microbiologie. Détermination de la sensibilité aux antibiotiques, p.144.

European Commission (2005) Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union* 338, 1-26.

Fagbemigun, O., Cho, G.S., Rösch, N., Brinks, E., Schrader, K., Bockelmann, W., Ogountoyinbo, F.A., Franz, C.M.A.P. (2021) Isolation and characterisation of potential starter cultures from the Nigerian fermented milk product nono. *Microorganisms* 9, 640.

FAO (2009) Base de données FAOLEX. https://www.fao.org/faolex/results/details/fr/c/LEX-FAOC146460/

FAO (2009) Base de données FAOLEX. https://www.fao.org/faolex/results/details/fr/c/LEX-FAOC146457/

FAO. Global food losses and food waste: Extent, causes and prevention. Rome. 2011. http://www.fao.org/docrep/014/mb060e/mb060e00.pdf

FAO (2020) Deuxième partie: Plan National d'investissements Agricoles et de Sécurité Alimentaire et Nutritionnelle de seconde génération (PNIASAN, 2017-2021). Consulté le 14 Mai 2020 à l'adresse suivante: https://www.fao.org/faolex/results/details/fr/c/LEX-FAOC184003/

FAO (2021) Sécurité sanitaire et qualité des aliments. https://www.fao.org/foodsafety/background/fr/

FAOSTAT (2022) Organisation des Nations unies pour l'alimentation et l'agriculture Base de données statistiques sur la production agricole. https://www.fao.org/faostat/fr/#data/QCL

Farougou, S., Sessou, P., Yehouenou, B., Dossa, F. (2012) Microbiological quality of raw milk processed from cows raised under extensive system in the Republic of Benin. *Res. J. Microbiol.* 7, 337.

Fayad, N., Kallassy Awad, M., Mahillon, J. (2019) Diversity of *Bacillus cereus sensu lato* mobilome. *BMC Genom.* 20, 436.

Fellows, P.J. (2017) 6 - Food biotechnology. In: Fourth, E. (eds) Food science, technology and nutrition. *Woodhead Publishing* pp. 387-430.

Fierer, J., Looney, D., Pechère, J.C. (2017) Nature and pathogenicity of micro-organisms. In: Cohen, J., Powderly, W.G., Opal, S.M. (eds) Infectious Diseases, E-Book 4th edition. *Elsevier Ltd Health Sciences*. pp. 4-25.

Foroutan, A., Guo, A.C., Vazquez-Fresno, R., Lipfert, M., Zhang, L., Zheng, J., Badran, H., Budinski, Z., Ametaj, B.N., Wishart, D.S. (2019) Chemical composition of commercial cow's milk. *J. Agric. Food Chem.* 67, 4897-4914.

Fox, P.F. (2003) The major constituents of milk. In: Smit, G. (eds) Dairy processing, improving quality. *Woodhead Publishing Limited* pp. 5-38.

Fuka, M.M., Wallisch, S., Engel, M., Welzl, G., Havranek, J, Schloter, M. (2013) Dynamics of bacterial communities during the ripening process of different croatian cheese types derived from raw ewe's milk cheeses. *PLoS ONE* 8, e80734.

Fusieger, A., Perin, L.M., Teixeira, C.G., de Carvalho, A.F., Nero, L.A. (2020) The ability of *Lactococcus lactis* subsp. *lactis* bv.

diacetylactis strains in producing nisin. *Antonie Van Leeuwenhoek*, 113, 651-662.

Gagara, M.H., Sessou, P., Dossa, F., Ahounou, S., Azokpota, P., Youssao, I., Gouro, A.S., Farougou, S. (2019) A study on the indigenous methods of processing milk in Niger. *Curr. Agric. Res. J.* 7, 213-223.

Garabal, J.I., Rodríguez-Alonso, P., Franco, D., Centeno, J.A. (2010) Chemical and biochemical study of industrially produced San Simón da Costa smoked semi-hard cow's milk cheeses: effects of storage under vacuum and different modified atmospheres. *J. Dairy Sci*. 93, 1868-1881.

Garnier, L., Valence, F., Mounier, J. (2017) Diversity and control of spoilage fungi in dairy products: An update. *Microorganisms* 5, 42.

Gaspar, C., Donders, G.G., Palmeira-de-Oliveira, R., Queiroz, J.A., Tomaz, C., Martinez-de-Oliveira, J., Palmeira-de-Oliveira, A. (2018) Bacteriocin production of the probiotic *Lactobacillus acidophilus* KS400. *Amb Express* 8, 1-8.

Gemechu, T. (2015) Review on lactic acid bacteria function in milk fermentation and preservation. *Afr. J. Food Sci*. 9, 170-175.

Gérard, A., El-Hajjaji, S., Burteau, S., Fall, P.A., Pirard, B., Taminiau, B., Daube, G., Sindic, M. (2021) Study of the microbial diversity of a panel of Belgian artisanal cheeses associated with challenge studies for *Listeria monocytogenes*. *Food Microbiol*. 100, 103861.

Ghosh, T., Beniwal, A., Semwal, A., Navani, N.K. (2019) Mechanistic insights into probiotic properties of lactic acid bacteria associated with ethnic fermented dairy products. *Front. Microbiol.* 10, 502.

Girma, A., Aemiro, A. (2021) Antibacterial activity of lactic acid bacteria isolated from fermented Ethiopian traditional dairy products against food spoilage and pathogenic bacterial strains. *J. Food Qual.* 2021, 1-10.

Gökdai, A., Sakarya, E., Contiero, B., Gottardo, F. (2020) Milking characteristics, hygiene, and management practices in Saanen goat farms: a case of Canakkale Province, Turkey. *Italian J. Animl. Sci*. 19, 213-221.

Gonzales-Barron, U., Cadavez, V., Guillier, L., Sanaa, M. (2023) A critical review of risk assessment models for *Listeria monocytogenes* in dairy products. *Foods* 12, 4436.

Gouissi, F.M., Assongba, H., Assogba, G.M. (2017) Evaluation of the microbiological quality of the cheese-based of cow's milk sold in the markets of Porto-Novo (Benin). *The Int. J. Sci. Tech*., 5, 67-70.

Gouvernement du Bénin (2022) Performances agricoles au Bénin : Des progressions en 2020 et de nouvelles ambitions. Accessed online 4 July 2022 at https://www.gouv.bj/actualite/1436/performances-agricoles-benin-progressions-2020-nouvelles-ambitions/

Gouvernement du Bénin (2022) Programme d'Actions du Gouvernement (PAG 2021-2022): présentation générale, 62p. Accessed on 17 July 2022 at file:///C:/Users/home/Downloads/presentation_pag-2021-2026-seance-appropriation-06-01-2021.pdf%20(5).pdf

Gran, H.M.M.A., Wethlesen, A., Narvhus, J.A., (2002) Smallholder dairy processing in Zimbabwe: the production of fermented milk products with particular emphasis on sanitation and microbiological quality. *Food Contr.* 13, 161-168.

Greenfield, H.J. (2010) The secondary products revolution: the past, the present and the future. *World Archaeol.* 42, 29-54.

Groenenboom, A.E., Shindano, J., Cheepa, N., Smid, E.J., Schoustra, S.E. (2020) Microbial population dynamics during traditional production of *Mabisi*, a spontaneous fermented milk product from Zambia: a field trial. *World J. Microbiol. Biotechnol*. 36, 1-14.

Groupes de la Banque Africaine de Développement (GBAD) (2022) Bénin-Projet d'appui aux filières lait et viande (PAFILAV). Accessed online 15 July 2022 at https://projectsportal.afdb.org/dataportal/VProject/show/P-BJ-AB0-005?lang=fr

Groupe de la Banque Africaine de Développement (GBAD) (2023) Bénin - Projet d'appui au développement des filières lait et viande et à la promotion des entreprises d'élevage (PRODEFILAV-PEL) -Rapport d'évaluation de projet. Consulté le 10 avril 2023 à l'adresse https://www.afdb.org/fr/documents/benin-projet-dappui-au-developpement-des-filieres-lait-et-viande-et-la-promotion-des-entreprises-delevage-prodefilav-pel-rapport-devaluation-de-projet

Hammer, Ø., Harper, D.A.T. (2008) Paleontological data analysis. *John Wiley & Sons Ltd*. 128p.

Hassan, G.M., Meshref, A.M.S., El-Newery, H.A., Mohamed, A.S. (2021) Prevalence of *Escherichia coli* in milk and some dairy products in Beni-Suef governorate, Egypt. *J. Vet. Med. Res.* 27, 161-167.

Healy, B., Cooney, S., O'Brien, S., Iversen, C., Whyte, P., Nally, J., Callanan, J.J., Fanning, S. (2010) *Cronobacter* (*Enterobacter*

sakazakii): an opportunistic foodborne pathogen. *Foodborne Pathog. Dis*. 7, 339-350.

Hendler, R., Zhang, Y. (2018) Probiotics in the treatment of colorectal cancer. *Medicines* 5, 1-14.

Hong, J.Y., Lee, N.K., Yi, S.H., Hong, S.P., Paik, H.D. (2019) Physicochemical features and microbial community of milk kefir using a potential probiotic *Saccharomyces cerevisiae* KU200284. *J. Dairy Sci.* 102, 10845-10849.

Hotchkiss, J.H., Werner, B.G., Lee, E.Y. (2006) Addition of carbon dioxide to dairy products to improve quality: A comprehensive review. *Compr. Rev. Food Sci. Food Saf.* 5, 158-168.

Husson, F., Lê, S., Pagès, J. (2017) Exploratory multivariate analysis by Example Using R, *CRC Press: New York* 15, 77p.

Ibrahim, S.A., Ayivi, R.D., Zimmerman, T., Siddiqui, S.A., Altemimi, A.B., Fidan, H., Esatbeyoglu, T., Bakhshayesh, R.V. (2021) Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. *Foods* 10, 3131.

ICMR Report (2017) Indian Council of Medical Research, Public Health Foundation of India, and Institute for Health Metrics and Evaluation. India: Health of the Nation's States - The India State-Level Disease Burden Initiative. New Delhi: ICMR, PHFI, and IHME.

Idland, L., Bø-Granquist, E.G., Aspholm, M., Lindbäck, T. (2022) The ability of Shiga toxin-producing *Escherichia coli* to grow in raw cow's milk stored at low temperatures. *Foods* 11, 3411.

Jalilzadeh, A., Tunçtürk, Y., Hesari, J. (2015) Extension shelf life of cheese: A review. *Int. J. Dairy Sci*. 10, 44-60.

Jans, C., Meile, L., Kaindi, D.W.M., Kogi-Makau, W., Lamuka, P., Renault, P., Kreikemeyer, B., Lacroix, C., Hattendorf, J. Zinsstag, J. Schelling, E., Fokou, G. Bonfoh, B. (2017) African fermented dairy products-overview of predominant technologically important microorganisms focusing on African *Streptococcus infantarius* variants and potential future applications for enhanced food safety and security. *Int. J. Food Microbiol.* 250, 27-36.

Jayashree, S., Pushpanathan, M., Rajendhran, J., Gunasekaran, P. (2013) Microbial diversity and phylogeny analysis of buttermilk, a fermented milk product, employing 16S rRNA-Based pyrosequencing. *Food Biotechnol*. 27, 213-221.

Jian-li, W., Yuan-yuan, S., Shou-yu, G., Fei-fei, D., Jia-yu, Y., Xue-hua, W., Yong-feng, Z., Shi-jin, J., Zhi-jing, X. (2017) Serotype and virulence genes of *Klebsiella pneumoniae* isolated from mink and its pathogenesis in mice and mink. *Sci Rep* 7, 1-7.

Jiang, Y.H., Ying, J.P., Xin, W.G., Yang, L.Y., Li, X.Z., Zhang, Q.L. (2022) Antibacterial activity and action target of phenyllactic acid against *Staphylococcus aureus* and its application in skim milk and cheese. *J. Dairy Sci.* 105, 9463-9475.

Joishy, T.K., Dehingia, M., Khan, M.R. (2019) Bacterial diversity and metabolite profiles of curd prepared by natural fermentation of raw milk and back sloping of boiled milk. *World J. Microbiol. Biotechnol*. 35, 1-12.

Kamilari, E., Anagnostopoulos, D.A., Papademas, P., Kamilaris, A., Tsaltas, D. (2020) Characterizing Halloumi cheese's bacterial communities through metagenomic analysis. *LWT* 126, 109298.

Kamilari, E., Tsaltas, D., Stanton, C., Ross, R.P. (2022) Metataxonomic mapping of the microbial diversity of Irish and Eastern Mediterranean cheeses. *Foods* 11, 2483.

Karanth, S., Feng, S., Patra, D., Pradhan, A.K. (2023) Linking microbial contamination to food spoilage and food waste: the role of smart packaging, spoilage risk assessments, and date labeling. *Front. Microbiol*.14, 1198124.

Katinan, C.R., Sadat, A.W., Chatigre, K.O., Bohoussou, K.M., Assidjo, N.E. (2012) Assessment of chemical and microbiological quality of home-made fermented milk produced and consumed in Yamoussoukro town, Côte d'Ivoire. *J. Appl. Biosci.* 55, 4020-4027.

Kaur, H., Kaur, G., Ali, S.A. (2022) Dairy based probiotic fermented functional foods: An update on their health-promoting properties. *Ferment.* 8, 425.

Kebede, A., Viljoen, B.C., Gadaga, T.H., Narvhus, J.A., Lourens-Hattingh, A. (2007) The effect of container type on the growth of yeast and lactic acid bacteria during production of *Sethemi*, South African spontaneously fermented milk. *Food Res. Int.* 40, 33-38.

Kees, M., (1996) Le fromage Peulh: facile à produire et bien apprécié, une technologie à vulgariser. Rapport de recherche GTZ, Université Eschborn, RFA. 25p.

Keisam, S., Romi, W., Ahmed, G., Jeyaram, K. (2016) Quantifying the biases in metagenome mining for realistic assessment of microbial ecology of naturally fermented foods. *Sci. Rep*. 6, 155.

Keisam, S., Tuikhar, N., Ahmed, G., Jeyaram, K. (2019) Toxigenic and pathogenic potential of enteric bacterial pathogens prevalent in the

traditional fermented foods marketed in the Northeast region of India. *Int. J. Food Microbiol.* 296, 21-30.

Kèkè, M., Yèhouénou, B., de Souza, C., Sohounhloué, D.C.K. (2009) Evaluation of hygienic and nutritional quality of Peulh cheese treated by *Sorghum vulgaris* (L) and *Pimenta racemosa* (Miller) extracts. *Sci. Study Res*. 10, 29-46.

Ketrouci, L., Dalache, F., Benabdelmoumene, D., Dahou, A.A., Homrani, A. (2021) Technological characterization of lactic acid bacteria isolated from different sheep's milk. *Asian J. Dairy Food Res.* 40, 239-245.

Khalid, K. (2011) An overview of lactic acid bacteria. *Int J. Biosci.* 1, 1-13.

Khasapane, N.G., Khumalo, Z.T.H., Kwenda, S., Nkhebenyane, S.J., Thekisoe, O. (2023) Characterisation of milk microbiota from subclinical mastitis and apparently healthy dairy cattle in free state province, South Africa. *Vet. Sci.* 10, 616.

Khemariya, P., Singh, S., Nath, G., Gulati, A.K. (2017) Probiotic *Lactococcus lactis*: A review. *Turkish J. Agric. Food Sci. Technol.* 5, 556-562.

Kohlmann, R., Barenberg, K., Anders, A., Gatermann, S.G. (2016) *Acetobacter indonesiensis* bacteremia in child with metachromatic leukodystrophy. *Emerg. Infect. Dis*. 22, 1681.

Komagbe, G.S., Dossou, A., Seko Orou, B.M.T., Sessou, P., Azokpota, P., Youssao, I., Hounhouigan, J., Scippo, M.L., Clinquart, A., Mahillon, J., Farougou, S. (2023) State of the art of breeding, milking, and milk processing for the production of curdled milk and

Wagashi Gassirè in Benin: Practices favoring the contamination of its dairy products. *Front. Sustain*. *Food Syst*. 6, 1050592.

Kpodékon, T.M., Ogni, C.A., Dassou, H., Dougnon, T.J., Boko, C., Koutinhouin, G.B., Goussanou, J.S., Akoegninou, A., Youssao, I. (2015) Dominant viral pathologies in the extensive and semi-intensive animal breeding and their treatment mode in ethno veterinary medicine in Benin. *Vet. World* 8, 1424-1434.

Lado, B.H., Yousef, A.E. (2002) Alternative food-preservation technologies: Efficacy and mechanisms. *Microbes Infect.* 4, 433-440.

Lambrini, K., Aikaterini, F., Konstantinos, K., Christos, I., Ioanna, P. V., Areti, T. (2021) Milk nutritional composition and its role in human health. *J. Pharm. Pharmacol.* 9, 8-13.

Landi, N., Ragucci, S., Di Maro, A. (2021) Amino acid composition of milk from cow, sheep and goat raised in Ailano and Valle Agricola, two localities of 'Alto Casertano' (Campania Region). *Foods* 10, 2431.

Lando, V., Valduga, N.Z., Moroni, L.S. (2023) Functional characterization of *Lactobacilli* strains with antimicrobial activity against *Salmonella* spp. and cell viability in fermented dairy product. *Biocatal. Agric. Biotechnol.* 47, 102605.

Ledo, J., Hettinga, K.A., Bijman, J., Kussaga, J., Luning, P.A. (2021) A tailored food safety and hygiene training approach for dairy farmers in an emerging dairy chain. *Food Control.* 124, 107918.

Lee, S.J., Jeon, H.S., Yoo, J.Y., Kim, J.H. (2021) Some important metabolites produced by lactic acid bacteria originated from Kimchi. *Foods* 10, 2148.

LeMay-Nedjelski, L., Copeland, J., Wang, P.W., Butcher, J., Unger, S., Stintzi, A., O'Connor, D.L. (2018) Methods and Strategies to Examine the Human Breastmilk Microbiome. In: Beiko, R., Hsiao, W., Parkinson, J. (eds) Microbiome Analysis. Methods in Molecular Biology. *Humana Press* pp. 63-86.

Leone, C., Thippareddi, H., Ndiaye, C., Niang, I., Diallo, Y., Singh, M. (2022) Safety and quality of milk and milk products in Senegal: A Review. *Foods* 11, 3479.

Li, L., Ma, J., Cheng, P., Li, M., Yu, Z., Song, X., Yu, Z., Sun, H., Zhang, W., Wang, Z. (2023) Roles of two-component regulatory systems in *Klebsiella pneumoniae*: Regulation of virulence, antibiotic resistance, and stress responses. *Microbiol. Res.* 272, 127374.

Li, W., Wu, S., Fu, P., Liu, J., Han, H., Bai, L., Pei, X., Li, N., Liu, X., Guo, Y. (2018) National molecular tracing network for foodborne disease surveillance in China. *Food Control* 88, 28-32.

Liang, T., Xie, X., Zhang, J., Ding, Y., Wu, Q. (2021) Bacterial community and composition of different traditional fermented dairy products in China, South Africa, and Sri Lanka by high-throughput sequencing of 16S rRNA genes. *LWT-Food Sci. Technol.* 144, 111209.

Lim, S.M., Lee, N.K., Paik, H.D. (2020) Potential neuroprotective effects of heat-killed *Lactococcus lactis* KC24 using SH-SY5Y cells against oxidative stress induced by hydrogen peroxide. *Food Sci. Biotechnol.* 29, 1735-1740.

Lin, C.M., Moon, S.S., Doyle, M.P., Mcwatters, K.H. (2002) Inactivation of *Escherichia coli* O157: H7, *Salmonella enterica*

serotype *Enteritidis*, and *Listeria monocytogenes* on lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide with mild heat. *J. Food Prot*. 65, 1215-1220.

Liu, J., Meng, F., Du, Y., Nelson, E., Zhao, G., Zhu, H., Caiyin, Q., Zhang, Z., Qiao, J. (2020) Co-production of nisin and γ-aminobutyric acid by engineered *Lactococcus lactis* for potential application in food preservation. *Front. Microbiol*. 11, 49.

Liu, S.N., Han, Y., Zhou, Z.J. (2011) Lactic acid bacteria in traditional fermented Chinese foods. *Food Res. Int.* 44, 643-651.

Liu, X-Y., Hu, Q., Xu, F., Ding, S-Y., Zhu, K. (2020) Characterization of *Bacillus cereus* in Dairy Products in China. *Toxins* 12, 454.

Longenberger, A.H., Palumbo, A.J., Chu, A.K., Moll, M.E., Weltman, A., Ostroff, S.M. (2013) *Campylobacter jejuni* infections associated with unpasteurized milk - Multiple states, 2012. *Clin. Infect. Dis.* 57, 263-266.

Lu, M., Wang, N.S. (2017) Spoilage of milk and dairy products. In: Bevilacqua, A., Corbo, M.R, Sinigaglia, M. (eds) The microbiological quality of food. *Woodhead Publishing* pp. 151-178.

Lusk, T.S., Ottesen, A.R., White, J.R., Allard, M.W., Brown, E.W., Kase, J.A. (2012) Characterization of microflora in Latin-style cheeses by Next Generation Sequencing technology. *BMC Microbiol.* 12, 1-10.

Machado, S.G., da Silva, F.L., Bazzolli, D.M., Heyndrickx, M., Costa, P.M.D.A., Vanetti, M.C.D. (2015) *Pseudomonas* spp. and *Serratia liquefaciens* as predominant spoilers in cold raw milk. *J. Food Sci.* 80, 1842-1849.

MAEP (2017) Synthèse sur le Plan Stratégique de Développement du Secteur Agricole (PSDSA) 2025 et le Plan National d'investissements Agricoles et de Sécurité Alimentaire et Nutritionnelle PNIASAN 2017-2021 ainsi que leurs documents connexes. République du Bénin, 21p.

Mallappa, R.H., Balasubramaniam, C., Nataraj, B.H., Ramesh, C., Kadyan, S., Pradhan, D., Muniyappa, S.K., Grover, S. (2021) Microbial diversity and functionality of traditional fermented milk products of India: Current scenario and future perspectives. *Int. Dairy J*. 114, 104941.

Martin, N.H., Evanowski, R.L., Wiedmann, M. (2023) Invited review: Redefining raw milk quality-Evaluation of raw milk microbiological parameters to ensure high-quality processed dairy products. *J. Dairy Sci*. 106, 1502-1517.

Martin, N.H., Torres-Frenzel, P., Wiedmann, M. (2021) Invited review: Controlling dairy product spoilage to reduce food loss and waste. *J. Dairy Sci*. 104, 1251-1261.

Martini, M., Altomonte, I., Sodi, I., Vasylieva, Y., Salari, F. (2023) Sterol, tocopherol, and bioactive fatty acid differences between conventional, high-quality, and organic cow milk. *J. Dairy Sci.* 106, 8239-8248.

Mathur, H., Beresford, T.P., Cotter, P.D. (2020) Health benefits of lactic acid bacteria (LAB) fermentates. *Nutrients* 12, 1679.

Mayo, B., Rachid, C.T.C.C., Alegría, Á., Leite, A.M.O., Peixoto, S.R., Delgado, S. (2014) Impact of next generation sequencing techniques in food microbiology. *Curr. Genom.* 15, 293-309.

Mazdeh, Z.F., Sasanfar, S., Chalipour, A., Pirhadi, E., Yahyapour, G., Mohammadi, A., Rostami, A., Amini, M., Hajimahmoodi, M. (2017) Simultaneous determination of preservatives in dairy products by HPLC and chemometric analysis. *Int. J. Anal. Chem.* 2017, 1-9.

Mazurek, J., Salehi, E., Propes, D., Holt, J.O., Bannerman, T., Nicholson, L.M., Moolenaar, R.L. (2004) A multistate outbreak of *Salmonella enterica* serotype *Typhimurium* infection linked to raw milk consumption-Ohio, 2003. *J. Food Prot.* 67, 2165-2170.

Mehdizadeh Gohari, I., Navarro, M.A., Li, J., Shrestha, A., Uzal, F., McClane, B.A. (2021) Pathogenicity and virulence of *Clostridium perfringens*. *Virulence* 12, 723-753.

Mehra, R., Singh, R., Nayan, V., Buttar, H.S., Kumar, N., Kumar, S., Bhardwaj, A., Kaushik, R., Kumar, H. (2021) Nutritional attributes of bovine colostrum components in human health and disease: A comprehensive review. *Food Biosci*. 40, 100907.

Menegueti, M.G., Gaspar, G.G., Laus, A.M., Basile-Filho, A., Bellissimo-Rodrigues, F., Auxiliadora-Martins, M. (2018) Bacteremia by *Leuconostoc mesenteroides* in an immunocompetent patient with chronic Chagas disease: a case report. *BMC Infect. Dis.* 18, 1-3.

Mensah, S.E.P., Sessou, P., Nata, C., Adjahoutonon, K., Yélindo, K.B., Lahamy, O., Farougou, S. (2019) Mode d'utilisation des antibiotiques dans les élevages de bovin laitier sédentaires au nord-ouest du Bénin. *J. Anim. Plant Sci.* 42, 7198-7206.

Méthode alternative à la norme ISO 11290-1 (2017) validée conformément à la norme ISO 16140-2:2016.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E.M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., Wilkenning, J., Edwards, R. (2008) The metagenomics RAST server-a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* 9, 1-8.

Michael, W. Mullan, A. (2019) Are we closer to understanding why viable cells of *Mycobacterium avium* subsp. *paratuberculosis* are still being reported in pasteurised milk? *Int. J. Dairy Technol.* 73, 332 -344.

Miloradovic, Z., Smigic, N., Djekic, I., Tomasevic, I., Kljajevic, N., Nedeljkovic, A., Miocinovic, J. (2018) The influence of NaCl concentration of brine and different packaging on goat white brined cheese characteristics. *Int. Dairy J.* 79, 24-32.

Mladenović, K.G., Grujović, M., Kiš, M., Furmeg, S., Tkalec, V.J., Kocić-Tanackov, S.D. (2021) *Enterobacteriaceae* in food safety with an emphasis on raw milk and meat. *Appl. Microbiol. Biotechnol.* 105, 8615-8627.

Mokoena, M.P. (2017) Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini review. *Molecules* 22, 1255.

Monfredini, L.U.C.A., Settanni, L., Poznanski, E., Cavazza, A., Franciosi, E. (2012) The spatial distribution of bacteria in Grana-cheese during ripening. *Syst. Appl. Microbiol.* 35, 54-63.

Montanhini, M.T.M., Montanhini, R.N., Pinto, J.P.N., Bersot, L.S. (2013) Effect of temperature on the lipolytic and proteolytic activity of *Bacillus cereus* isolated from dairy products. *Int. Food Res. J. 20*, 1417-1420.

Montgomery, H., Haughey, S.A., Elliott, C.T. (2020) Recent food safety and fraud issues within the dairy supply chain (2015-2019). *Glob. Food Sec.* 26, 100447.

Moonga, H.B., Schoustra, S.E., Van den Heuvel, J., Linnemann, A.R., Samad, M.S., Shindano, J., Smid, E.J. (2020) Composition and diversity of natural bacterial communities in *Mabisi*, a traditionally fermented milk. *Front. Microbiol*. 11, 1816.

Morales, P., Feliu, I., Fernández-García, E., Nuñez, M. (2004) Volatile compounds produced in cheese by *Enterobacteriaceae* strains of dairy origin. *J. Food Prot.* 67, 567-573.

Morandi, S., Cremonesi, P., Silvetti, T., Brasca, M. (2013) Technological characterisation, antibiotic susceptibility and antimicrobial activity of wild-type *Leuconostoc* strains isolated from north Italian traditional cheeses. *J. Dairy Res.* 80, 457-466.

Morandi, S., Pica, V., Masotti, F., Cattaneo, S., Brasca, M., De Noni, I., Silvetti, T. (2021) Proteolytic traits of psychrotrophic bacteria potentially causative of sterilized milk instability: Genotypic, phenotypic and peptidomic insight. *Foods 10*, 934.

Morris, E.K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T.S., Meiners, T., Müller, C., Obermaier, E., Prati, D. Socher, S.A. (2014) Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. *Ecol. Evol.* 4, 3514-3524.

Mosher, J.J., Bowman, B., Bernberg, E.L., Shevchenko, O., Kan, J., Korlach, J., Kaplan, L.A. (2014) Improved performance of the PacBio SMRT technology for 16S rDNA sequencing. *J. Microbiol. Methods* 104, 59-60.

Motato, K.E., Milani, C., Ventura, M., Valencia, F.E., Ruas-Madiedo, P., Delgado, S. (2017) Bacterial diversity of the Colombian fermented milk "*Suero Costeño*" assessed by culturing and high-throughput sequencing and DGGE analysis of 16S rRNA gene amplicons. *Food Microbiol*. 68, 129-136.

MPD (2022) Plan national de développement 2018-2025. Accessed on 15 August 2022 at file:///C:/Users/home/Downloads/mpd_plan-national-developpement_2018-2025_final_14_janv.pdf.pdf

Mtshali, K., Khumalo, Z.T.H., Kwenda, S., Arshad, I., Thekisoe, O.M.M. (2022) Exploration and comparison of bacterial communities present in bovine faeces, milk and blood using 16S rRNA metagenomic sequencing. *PloS One* 17, e0273799.

Mudgal, S.P., Prajapati, J.B. (2017) *Dahi*-An Indian naturally fermented yogurt. In: Shah, N.P. (eds) Yogurt in health and disease prevention. *Elsevier Inc*. pp. 353-369.

Nahidul-Islam, S.M., Kuda, T., Takahashi, H., Kimura, B. (2018) Bacterial and fungal microbiota in traditional Bangladeshi fermented milk products analysed by culture-dependent and culture-independent methods. *Food Res. Int*. 111, 431-437.

Nájera, A.I., Nieto, S., Barron, L.J.R., Albisu, M. (2021) A review of the preservation of hard and semi-hard cheeses: Quality and safety. *Int. J. Environ. Res. Public Health*. 18, 9789.

Nakamura, S., Morimoto, Y.V., Kudo, S. (2015) A lactose fermentation product produced by *Lactococcus lactis subsp. lactis*, acetate, inhibits the motility of flagellated pathogenic bacteria. *Microbiol*. 161, 701-707.

Nakano, M.M., Dailly, Y.P., Zuber, P., Clark, D.P. (1997) Characterization of anaerobic fermentative growth of *Bacillus subtilis*: identification of fermentation end products and genes required for growth. *J. Bacteriol.* 179, 6749-6755.

Na-Kyoung, L., Kyoung, J.H., Sung-Ho, S., Su, J.E., Si-Kyung, L., Hyun-Dong, P. (2015) Multifunctional effect of probiotic *Lactococcus lactis* KC24 isolated from kimchi. *LWT-Food Sci. Technol.* 64, 1036-1041.

Nami, Y., Vaseghi Bakhshayesh, R., Mohammadzadeh Jalaly, H., Lotfi, H., Eslami, S., Hejazi, M. (2019) Probiotic properties of *Enterococcus* isolated from artisanal dairy products. *Front. Microbiol.* 10, 300.

Natrella, G., Difonzo, G., Calasso, M., Costantino, G., Caponio, F., Faccia, M. (2020) Evolution of VOC and Sensory Characteristics of Stracciatella Cheese as Affected by Different Preservatives. *Foods* 9, 1446.

Negash, A.W., Tsehai, B.A. (2020) Current applications of bacteriocin. *Int. J. Microbiol.* 2020, 4374891.

NF EN ISO 15214, (1998) Microbiologie des aliments - Méthode horizontale pour le dénombrement des bactéries lactiques mésophiles - Technique par comptage des colonies à 30 degrés C.

NF EN ISO 16649-1 (2018) Microbiologie alimentaire - Méthode horizontale pour le dénombrement des *Escherichia coli* bêta-glucuronidase positive - Partie 1: Technique de comptage des colonies à 44 °C au moyen de 5-bromo4-chloro-3-indolyl bêta-D-glucuronate.

NF EN ISO 6579-1 (2017) Microbiologie des aliments - Méthode horizontale pour la recherche des *Salmonella* spp. Indice de classement : V08-013. 99. NF EN ISO 10272-1, 2017.

NF EN ISO 6887-5 (2010) Microbiologie des aliments - Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique - Partie 5: Règles spécifiques pour la préparation du lait et des produits laitiers.

NF EN ISO 6888-3 (2003) Microbiologie des aliments - Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (*Staphylococcus aureus* et autres espèces) - Partie 3: recherche et méthode NPP pour les faibles nombres.

NF ISO 21527-1 (2008) Microbiologie des aliments - Méthode horizontale pour le dénombrement des levures et moisissures - Partie 1: Technique par comptage des colonies dans les produits à activité d'eau supérieure à 0,95.

NF ISO 7932 (2004) Amendement 1 (2020) Microbiologie des denrées alimentaires et des aliments pour animaux. Méthode horizontale pour le dénombrement des *Bacillus cereus* présumés. Technique de comptage des colonies à 30 degrés C

Nyokabi, S., Luning, P.A., de Boer, I.J.M., Korir, L., Muunda, E., Bebe, B.O., Lindahl, J., Bett, B., Oosting, S.J. (2021) Milk quality and hygiene: Knowledge, attitudes and practices of smallholder dairy farmers in central Kenya. *Food Control*. 130, 108303.

Nocera, F.P., Attili, A.R., De Martino, L. (2021) *Acinetobacter baumannii*: Its clinical significance in human and veterinary medicine. *Pathogens* 10, 127.

Oki, K., Dugersuren, J., Demberel, S., Watanabe, K. (2014) Pyrosequencing analysis of the microbial diversity of airag, khoormog and tarag, traditional fermented dairy products of Mongolia. *Biosci. Microbiota Food Health* 33, 53-64.

Okry, F., Kpenavoun Chogou, S., Fanou-Fogny, N., Moumouni, I., Hounhouigan, J. (2017) Local governance of a dairy sector in Benin Republic: actors, roles, power relationships and perception of interdependency. *Asian J. Agric. Ext, Econ. Sociol*. 21, 1-14.

Olaniran, O.B., Iroko, O.T., Famojuro, T.I., Oluwatobi, O.B., Adeleke, O.E. (2022) Microbiological quality assessment of dairy product: detection of extended spectrum beta lactamase genes in bacterial isolates from yoghurt sold in Sagamu Metropolis, Ogun State, Nigeria. *DUJOPAS.* 8, 159-172.

Ortakci, F., Broadbent, J.R., Oberg, C.J., McMahon, D.J. (2015) Late blowing of Cheddar cheese induced by accelerated ripening and ribose and galactose supplementation in presence of a novel obligatory heterofermentative nonstarter *Lactobacillus wasatchensis*. *J. Dairy Sci.* 98, 7460-7472.

Ounit, R., Wanamaker, S., Close, T.J., Lonardi, S. (2015) CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative k-mers. *BMC Genom.* 16, 1-13.

Owusu-Kwarteng, J., Akabanda, F., Agyei, D., Jespersen, L. (2020) Microbial safety of milk production and fermented dairy products in Africa. *Microorganisms* 8, 752.

Oyedeji, J.B., Green, E., Jeff-Agboola, Y.A., Olanbiwoninu, A.A., Areo, E., Martins, I.E., El-Imam, A.M.A., Adebo, O.A. (2023) Chapter 31 - Presence of pathogenic microorganisms in fermented

foods. In: Adebo, O.A., Chinma, C.E., Obadina, A.O., Soares, A.G., Panda, S.K., Gan, R.Y. (eds.) Indigenous fermented foods for the tropics *Academic Press*. pp. 519-537.

Özcelik, S., Kuley, E., Özogul, F. (2016) Formation of lactic, acetic, succinic, propionic, formic and butyric acid by lactic acid bacteria. *LWT* 73, 536-542.

Parente, E., Ricciardi, A., Zotta, T. (2020) The microbiota of dairy milk: A review. *Int. Dairy. J.* 107, 104714.

Parker, M., Zobrist, S., Donahue, C., Edick, C., Mansen, K., Nadjari, M.H.Z., Heerikhuisen, M., Sybesma, W., Molenaar, D., Diallo, A.M., Milani, P., Kort, R. (2018) Naturally fermented milk from northern Senegal: Bacterial community composition and probiotic enrichment with *Lactobacillus rhamnosus*. *Front. Microbiol*. 9, 2218.

Parra-Flores, J., Flores-Soto, F., Flores-Balboa, C., Alarcón-Lavín, M. P., Cabal-Rosel, A., Daza-Prieto, B., Springer, B., Cruz-Córdova, A., Leiva-Caro, J., Forsythe, S., Ruppitsch, W. (2023) Characterization of *Cronobacter sakazakii* and *Cronobacter malonaticus* strains isolated from powdered dairy products intended for consumption by adults and older adults. *Microorganisms* 11, 2841.

Paswan, R., Park, Y.W. (2020) Survivability of *Salmonella* and *Escherichia coli* O157: H7 pathogens and food safety concerns on commercial powder milk products. *Dairy*, 1, 189-201.

Peng, C., Sun, Z., Sun, Y., Ma, T., Li, W., Zhang, H. (2021) Characterisation and association of bacterial communities and

nonvolatile components in spontaneously fermented cow milk at different geographical distances. *J. Dairy Sci.* 104, 2594-2605.

Peng, Z., Li, Y., Yan, L., Yang, S., Yang, D. (2023) Correlation analysis of microbial contamination and alkaline phosphatase activity in raw milk and dairy products. *Int. J. Environ. Res. Public Health* 20, 1825.

Perez, R.H., Zendo, T., Sonomoto, K. (2014) Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. *Microb. Cell Fact.* 13, 1-13.

Petti, C.A., Polage, C.R., Schreckenberger, P. (2005) The role of 16S rRNA gene sequencing in identification of microorganisms misidentified by conventional methods. *J. Clin. Microbiol.* 43, 6123-6125.

Pietrzak-Fiećko, R., Kamelska-Sadowska, A.M. (2020) The comparison of nutritional value of human milk with other mammals' milk. *Nutrients* 12, 1404.

Pollitt, E., Crusz, S., Diggle, S. (2016) *Staphylococcus aureus* forms spreading dendrites that have characteristics of active motility. *Sci. Rep.* 5, 17698.

Porcellato, D., Aspholm, M., Skeie, S.B., Mellegård, H. (2019) Application of a novel amplicon-based sequencing approach reveals the diversity of the *Bacillus cereus* group in stored raw and pasteurized milk. *Food Microbiol.*81, 32-39.

Porcellato, D., Aspholm, M., Skeie, S.B., Monshaugen, M., Brendehaug, J., Mellegård, H. (2018) Microbial diversity of consumption milk during processing and storage. *Int. J. Food Microbiol*. 266, 21-30.

Prabhurajeshwar, C., Chandrakanth, R.K. (2017) Probiotic potential of Lactobacilli with antagonistic activity against pathogenic strains: An in vitro validation for the production of inhibitory substances. *Biomed. J.* 40, 270-283.

Quigley, L., O'Sullivan, O., Beresford, T.P., Ross, R.P., Fitzgerald, G.F., Cotter, P.D. (2012) A comparison of methods used to extract bacterial DNA from raw milk and raw milk cheese. *J. Appl. Microbiol.* 113, 96-105.

Quigley, L., O'Sullivan, O., Beresford, T.P., Ross, R.P., Fitzgerald, G.F., Cotter, P.D. (2012) High-throughput sequencing for detection of subpopulations of bacteria not previously associated with artisanal cheeses. *Appl. Environ. Microbiol.* 78, 5717-5723.

Quijada, N.M., Hernández, M., Rodríguez-Lázaro, D. (2020) High-throughput sequencing and food microbiology. *Adv. Food Nutr. Res.* 91, 275-300.

Quinto, E.J., Jiménez, P., Caro, I., Tejero, J., Mateo, J., Girbés, T. (2014) Probiotic lactic acid bacteria: a review. *Sci. Res*. 5, 49908.

Raevuori, M., Genigeorgis, C. (1975) Effect of pH and sodium chloride on growth of *Bacillus cereus* in laboratory media and certain foods. *Appl. Microbiol*. 29, 68-73.

Rahmeh, R., Akbar, A., Kishk, M., Al-Onaizi, T., Al-Azmi, A., Al-Shatti, A., Shajan, A., Al-Mutairi, S., Akbar, B. (2019) Distribution and antimicrobial activity of lactic acid bacteria from raw camel milk. *New Microbes New Infect*. 30, 100560.

Rahnama, H., Azari, R., Yousefi, M.H., Berizi, E., Mazloomi, S.M., Hosseinzadeh, S., Derakhshan, Z., Ferrante, M., Conti, G.O. (2023)

A systematic review and meta-analysis of the prevalence of *Bacillus cereus* in foods. *Food Control* 143, 109250.

Rai, R., Shangpliang, H.N.J. Tamang, J.P. (2016) Naturally fermented milk products of the Eastern Himalayas. *J. Ethn. Foods* 3, 270-275.

Rai, R., Tamang, J.P. (2022) In vitro and genetic screening of probiotic properties of lactic acid bacteria isolated from naturally fermented cow-milk and yak-milk products of Sikkim, India. *World J Microbiol Biotechnol*. 6, 25.

Rakhmanova, A., Khan, Z.A., Shah, K. (2018) A mini review fermentation and preservation: role of lactic acid bacteria. *MOJ Food Process. Technol.* 6, 414-417.

Ramos, G.L.P.A., Vigoder, H.C., Nascimento, J.S. (2021) Technological applications of *Macrococcus caseolyticus* and its impact on food safety. *Curr. Microbiol*. 78, 11-16.

Reis, J.A., Paula, A.T., Casarotti, S.N., Penna, A.L.B. (2012) Lactic acid bacteria antimicrobial compounds: characteristics and applications. *Food Eng. Rev*. 4, 124-140.

Ribeiro Júnior, J.C., de Oliveira, A.M., Silva, F. de G., Tamanini, R., de Oliveira, A.L.M., Beloti, V. (2018) The main spoilage-related psychrotrophic bacteria in refrigerated raw milk. *J. Dairy Sci.* 101, 75-83.

Ribeiro, S.C., Coelho, M.C., Todorov, S.D., Franco, B.D.G.D.M., Dapkevicius, M.L.E., Silva, C.C.G. (2014) Technological properties of bacteriocin-producing lactic acid bacteria isolated from Pico cheese an artisanal cow's milk cheese. *J. Appl. Microbiol*. 116, 573-585.

Rocha, R., Velho, M.V., Santos, J., Fernandes, P. (2021) *Serra da estrela pdo* cheese microbiome as revealed by next generation sequencing. *Microorganisms* 9, 2007.

Romi, W., Ahmed, G., Jeyaram, K. (2015) Three-phase succession of autochthonous lactic acid bacteria to reach a stable ecosystem within 7 days of natural bamboo shoot fermentation as revealed by different molecular approaches. *Mol. Ecol.* 24. 13237.

Rossi, C., Serio, A., Chaves-López, C., Anniballi, F., Auricchio, B., Goffredo, E., Paparella, A. (2018) Biofilm formation, pigment production and motility in *Pseudomonas* spp. isolated from the dairy industry. *Food Contr.* 86, 241-248.

Rossi, F., Amadoro, C., Colavita, G. (2019) Members of the *Lactobacillus* genus complex (LGC) as opportunistic pathogens: a review. *Microorganisms* 7, 126.

Rukure, G., Bester, B.H. (2001) Survival and growth of *Bacillus cereus* during *Gouda* cheese manufacturing. *Food Control*. 12, 31-36.

Saad, A., Ibrahim, G., Ismail, T., Mohamed, A. (2017) Microbiological studies on some spoilage and food-poisoning microorganism in raw milk. *Suez Canal Vet. Med. J*. 22, 43-54.

Saad, N.M., Amin, W.F., Mostafa, S.M. (2018) Detection of *Acinetobacter* species in milk and some dairy products. *Assiut. Vet. Med. J.* 64, 34-40.

Sacramento, O.R.H., (2008) Contribution à l'amélioration de la conservation du fromage Peulh par l'effet combiné du séchage et de l'emballage sous vide. Thèse d'Ingénieur Agronome, Faculté des Sciences Agronomiques (FSA), Université d'Abomey-Calavi (UAC). 60 p.

Saeed, A.H., Salam, A.I. (2013) Current limitations and challenges with lactic acid bacteria: a review. *Sci. Res.* 4, 40133.

Sakkas, L., Moutafi, A., Moschopoulou, E., Moatsou, G. (2014) Assessment of treatment of various types of milk. *Food Chem.* 15, 293-301.

Sarkar, S. (2015) Microbiological considerations: pasteurized milk. *Int. J. Dairy Sci.* 10, 206-218.

SAS (2013) Base, 2nd Edition, SAS ® 9.4 Procedures Guide: Statistical Procedures. *Cary, NC, USA: SAS Institute Inc.*

Scatamburlo, T.M., Yamazi, A.K., Cavicchioli, V.Q., Pieri, F.A., Nero, L.A. (2015) Spoilage potential of *Pseudomonas* species isolated from goat milk. *J. Dairy Sci*. 98, 759-764.

Schnur, S.E., Amachawadi, R.G., Baca, G., Sexton-Bowser, S., Rhodes, D.H., Smolensky, D., Herald, T.J., Perumal, R., Thomson, D.U., Nagaraja, T.G. (2021) Antimicrobial activity of sorghum phenolic extract on bovine foodborne and mastitis-causing pathogens. *Antibiotics* 10, 1-12.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chenb, W. (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proc. Nat. Acad. Sci. USA* 109, 6241-6246.

Sessou, P., Boko, C., Hounmanou, G., Osseni, S.D., Hounkpe, E., Azokpota, P., Youssao, I., Sohounhloue, D., Farougou, S. (2016) Preservation of traditional cheese *Wagashi* using essential oils: impact on microbiological, physico-chemical and sensorial characteristics. *Br. Microbiol. Res. J.* 15, 1-13.

Sessou, P., Farougou, S., Azokpota, P., Youssao, I., Yèhouenou, B., Ahounou, S., Sohounhloué, D.C.K. (2013) Endogenous methods for preservation of *Wagashi*, a Beninese traditional cheese. *Afr. J. Agric. Res.* 8, 4254-4261.

Sessou, P., Keisam, S., Gagara, M., Komagbe, G., Farougou, S., Mahillon, J., Jeyaram, K. (2023) Comparative analyses of the bacterial communities present in the spontaneously fermented milk products of Northeast India and West Africa. *Front. Microbiol.* 14, 1166518.

Sessou, P., Keisam, S., Tuikhar, N., Gagara, M., Farougou, S., Jeyaram, K. (2019) High-Throughput Illumina MiSeq amplicon sequencing of yeast communities associated with indigenous dairy products from republics of Benin and Niger. *Front. Microbiol.* 10, 594.

Shangpliang, H.N.J., Rai, R., Keisam, S., Jeyaram, K., Tamang, J.P. (2018) Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by high-throughput amplicon sequencing. *Sci. Rep*. 8, 1532.

Shangpliang, H.N.J., Sharma, S., Rai, R. Tamang, J.P. (2017) Some Technological Properties of Lactic Acid Bacteria Isolated from *Dahi* and *Datshi*, naturally fermented milk products of Bhutan. *Front. Microbiol*. 8, 216942.

Shangpliang, H.N.J., Tamang, J.P. (2021) Phenotypic and genotypic characterisation of lactic acid bacteria isolated from exotic naturally fermented milk (cow and yak) products of Arunachal Pradesh, India. *Int. Dairy J.* 118, 105038.

Sharma, H., Ozogul, F., Bartkiene, E., Rocha, J.M. (2023) Impact of lactic acid bacteria and their metabolites on the techno-functional properties and health benefits of fermented dairy products. *Crit. Rev. Food Sci. Nutr.* 63, 4819-4841.

Sharma, S., Kandasamy, S., Kavitake, D., Shetty, P.H. (2018) Probiotic characterization and antioxidant properties of *Weissella confusa* KR780676, isolated from an Indian fermented food. *LWT* 97, 53-60.

Sieuwerts, S., Bron, P.A., Smid, E.J. (2018) Mutually stimulating interactions between lactic acid bacteria and *Saccharomyces cerevisiae* in sourdough fermentation. *LWT Food Sci. Technol.* 90, 201-206.

Singh, A., Ramachandran, A. (2020) Assessment of hygienic milking practices and prevalence of bovine mastitis in Small Dairy farms of peri-urban area of Jaipur. *Indian J. Community Med*. 45, S21-S25.

Singh, P., Singh, R.V., Gupta, B., Tripathi, S.S., Tomar, K.S., Jain, S., Sahni, Y.P. (2018) Prevalence study of *Salmonella* spp. in milk and milk products. *Asian J. Dairy Food Res.* 37, 7-12.

Sobeih, A., AL-Hawary, I., Khalifa, E., Ebied, N. (2020) Prevalence of *Enterobacteriaceae* in raw milk and some dairy products. *Kafrelsheikh Vet. Med. J*. 18, 9-13.

Song, Y., Sun, Z., Guo, C., Wu, Y., Liu, W., Yu, J., Menghe, B., Yang, R., Zhang, H. (2016) Genetic diversity and population structure of *Lactobacillus delbrueckii* subspecies *bulgaricus* isolated from naturally fermented dairy foods. *Sci. Rep*. 6, 22704.

Soule, H.A., Djènontin, J.P.A., Kpera, G.N., Mensah, G.A. (2017) Production laitière bovine au Nord-Bénin. Bulletin de la recherche Agronomique du Bénin 81, 18-25.

Sounon, K.L.S.A., Aho, E., Lesse, P., Ickowicz, A., Messad, S., Lesnoff, M., Houinato, M.R.B. (2019) Evaluation de la valeur économique directe de l'élevage bovin au nord-Bénin. *Int. J. Biol. Chem. Sci.* 13, 8-17.

Sow, E. (2014) Caractéristiques et dominantes pathologiques des élevages bovins laitiers dans les régions de Thiès et de Diourbel. Thèse de doctorat en Médecine Vétérinaire de l'Université Cheick Anta Diop de Dakar, École Inter Etats des Sciences et Médecine Vétérinaires: Sénégal, 112p.

Stenfors, A.L.P., Fagerlund, A., Granum, P.E. (2008) From soil to gut: *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiol. Rev.* 32, 579-606.

Sun, L., D'Amico, D.J. (2021) Composition, succession, and source tracking of microbial communities throughout the traditional production of a farmstead Cheese. *mSystems* 6, 1-18.

Sunagawa, S., Mende, D.R., Zeller, G., Izquierdo-Carrasco, F., Berger, S.A., Kultima, J.R., Coelho, L.P., Arumugam, M., Tap, J., Nielsen, H.B., Rasmussen, S., Brunak, S., Pedersen, O., Guarner, F., de Vos, W.M., Wang, J., Li, J., Doré, J., Ehrlich, S.D., Stamatakis, A., Bork, P. (2013) Metagenomic species profiling using universal phylogenetic marker genes. *Nat. Methods* 10, 1196-1199.

Tabla, R., Gómez, A., Simancas, A., Rebollo, J.E., Molina, F., Roa, I. (2016) *Enterobacteriaceae* species during manufacturing and ripening of semi-hard and soft raw ewe's milk cheese: Gas production capacity. *Small Rumin. Res.* 145, 123-129.

Tabla, R., Gómez, A., Simancas, A., Rebollo, J.E., Molina, F., Roa, I. (2018) Early blowing in raw goats' milk cheese: gas production capacity of *Enterobacteriaceae* species present during manufacturing and ripening. *J. Dairy Res.* 85, 331-338.

Tamang, J.P. (2022) “Ethno-microbiology” of ethnic Indian fermented foods and alcoholic beverages. *J. Appl. Microbiol*. 133, 145-161.

Tamang, J.P., Jeyaram, K., Rai, A.K. Mukherjee, P.K. (2021) Diversity of beneficial microorganisms and their functionalities in community-specific ethnic fermented foods of the Eastern Himalayas. *Food Res. Int*. 148, 110633.

Tenorio-Salgado, S., Castelán-Sánchez, H.G., Dávila-Ramos, S., Huerta-Saquero, A., Rodríguez-Morales, S., Merino-Pérez, E., Roa de la Fuente, L.F., Solis-Pereira, S.E., Pérez-Rueda, E., Lizama-Uc, G. (2021) Metagenomic analysis and antimicrobial activity of two fermented milk *kefir* samples. *MicrobiologyOpen* 10, e1183.

Tirloni, E., Bernardi, C., Stella, S. (2021) *Pseudomonas* spp.: are food grade organic acids efficient against these spoilage microorganisms in fresh cheeses ? *Foods* 10, 891.

Tuikhar, N., Keisam, S., Labala, R.K., Ramakrishnan, P., Arunkumar, M.C., Ahmed, G., Biagi, E. Jeyaram, K. (2019) Comparative analysis of the gut microbiota in centenarians and young adults shows a common signature across genotypically non-related populations. *Mech. Ageing Dev.* 179, 23-35.

Verruck, S., Balthazar, C.F., Rocha, R.S., Silva, R., Esmerino, E.A., Pimentel, T.C., Freitas, M.Q., Silva, M.C., da Cruz, A.G., Prudencio, E.S. (2019) Dairy foods and positive impact on the consumer's health. *Adv. Food Nutr. Res*. 89, 95-164.

Vidic, J., Chaix, C., Manzano, M., Heyndrickx, M. (2020) Food sensing: detection of *Bacillus cereus* spores in dairy products. *Biosensors* 10, 10030015.

Vinderola, G., Ouwehand, A., Salminen, S., Von Wright, A. (2019) Lactic acid bacteria: microbiological and functional aspects. In: Salminen, S., Von Wright, A., Ouwehand, A. (eds) *CRC Press.* pp. 375-396.

Von Gastrow, L., Madec, M.N., Chuat, V., Lubac, S., Morinière, C., Lé, S., Santoni, S., Sicard, D., Valence, F. (2020) Microbial diversity associated with Gwell, a traditional French mesophilic fermented milk inoculated with a natural starter. *Microorganisms* 8, 982.

Wahengbam, R., Thangjam, A.S., Keisam, S., Asem, I.D., Ningthoujam, D.S., Jeyaram, K., (2020) Ethnic fermented foods and alcoholic beverages of Manipur. In: Tamang, J.P. (eds) Ethnic fermented foods and beverages of India. *Sci. History Cult*. 5, 349-519.

Walsh, A.M., Crispie, F., Daari, K., O'Sullivan, O., Martin, J.C., Arthur, C.T., Marcus, J., Claesson, K., Scott, K.P. Cotter, P.D. (2017) Strain-level metagenomic analysis of the fermented dairy beverage nunu highlights potential food safety risks. *Appl. Environ. Microbiol.* 83, e01144-17.

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., Geng, W. (2021) Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Front. Bioeng. Biotechnol.* 9, 612285.

Weber, M., Geißert, J., Kruse, M., Lipski, A. (2014) Comparative analysis of bacterial community composition in bulk tank raw milk by culture-dependent and culture-independent methods using the viability dye propidium monoazide. *J. Dairy Sci*. 97, 6761-6776.

Wemmenhove, E., van Valenberg, H.J.F., Zwietering, M.H., van Hooijdonk, T.C.M., Wells-Bennik, M.H.J. (2016) Minimal inhibitory concentrations of undissociated lactic, acetic, citric and propionic acid for *Listeria monocytogenes* under conditions relevant to cheese. *Food Microbiol*. 58, 63-67.

Widyastuti, Y., Febrisiantosa, A. (2014) The role of lactic acid bacteria in milk fermentation. *Food Nutr. Sci.* 5, 435-442.

Wittwer, G. (2024) The economic impacts of a hypothetical foot and mouth disease outbreak in Australia. *Aust. J. Agric. Resour. Econo.* 68, 23-43.

Wolfe, B.E., Button, J.E., Santarelli, M. Dutton, R.J. (2014) Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity. *Cell* 158, 422-433.

Wu, X., Lu, Y., Xu, H., Lv, M., Hu, D., Lui, Z., Liu, L., Wang, Z., Feng, Y. (2018) Challenges to improve the safety of dairy products in China. *Trends in Food Sci. Technol.* 76, 6-14.

Xu, J. (2006) Microbial ecology in the age of genomics and metagenomics: Concepts, tools, and recent advances. *Mol. Ecol.* 15, 1713-1731.

Yang, X. (2014) *Moraxellaceae*. In: Batt, C.A., Tortorello, M.L. (eds) Encyclopedia of food microbiology (Second Edition), *Academic Press* pp. 826-833.

Yerlikaya, O. (2019) Probiotic potential and biochemical and technological properties of *Lactococcus lactis ssp. lactis* strains isolated from raw milk and kefir grains. *J. Dairy Sci.* 102, 124-134.

Yeung, M. (2012) ADSA Foundation Scholar Award: Trends in culture-independent methods for assessing dairy food quality and safety: Emerging metagenomic tools. *J. Dairy Sci.* 95, 6831-6842.

Yu, Z., Peng, C., Kwok, L.Y., Zhang, H. (2021) The bacterial diversity of spontaneously fermented dairy products collected in northeast asia. *Foods* 10, 2321.

Zannou, O., Agossou, D.J., Koca, I. (2018) Traditional dairy products in the Republic of Benin: *Wagashi* and dèguè. *Global Sci. J.* 6, 630-650.

Zhang, K., Jia, M., Guo, Z., Li, Y., Li, B., Li, X. (2021) Evaluation of bacterial diversity of traditional cheese in Tarbagatay Prefecture, China, and its correlation with cheese quality. *Food Sci. Nutr.* 9, 3155-3164.

Zhao, Z., Ning, C., Chen, L., Zhao, Y., Yang, G., Wang, C., Chen, N., Zhang, Z., Li, S. (2021) Impacts of manufacture processes and geographical regions on the microbial profile of traditional Chinese cheeses. *Food Res. Int.* 148, 110600.

Zhong, Z., Hou, Q., Kwok, L., Yu, Z., Zheng, Y., Sun, Z., Menghe, B., Zhang, H. (2016) Bacterial microbiota compositions of naturally fermented milk are shaped by both geographic origin and sample type. *J. Dairy Sci.* 99, 7832-7841.

Zuniga, M., Pardo, I., Ferrer, S. (1993) An improved medium for distinguishing between homofermentative and heterofermentative lactic acid bacteria. *Int. J. Food Microbiol.* 18, 37-42.

Annexes

Annexe A: Articles supplémentaires

Dossou, W.A., Seko Orou, Baké M.T., Komagbe G., Sessou P., Youssao, A.K.I., Farougou S., Hounhouigan D.J., Mahillon J., Mongbo R., Poncelet M., Madode Y.E., Douny C., Scippo M-L., Clinquart A., Azokpota P. (2022) Processing and preservation methods of *Wagashi Gassirè*, a traditional cheese produced in Benin, *Heliyon* 8, e10605.

Dossou, W.A., Seko Orou, B.M.T., Komagbe, G., Sessou, P., Youssao, A.K.I., Farougou, S., Hounhouigan, J.D., Mahillon, J., Mongbo, R., Poncelet, M., Boutaleb, S., Gobert, S., Madode, Y.E., Azokpota, P., Clinquart, A., Scippo, M.L., Douny, C. (2024) Nutritional Composition and Chemical Safety of *Wagashi Gassirè* Cheese Sold in the Southern Benin Markets. *Dairy* 5, 271-286.

Séko Orou, B.M.T., Komagbé, G. Dossou, W.A., Benon Monra, A., Farougou, S., Clinquart, A., Poncelet, M., Mongbo, R.L. (2023) Fulani cheese *Wagashi Gassirè* market tested by covid-19 in Benin. *A.J.H.S.S.R.* 7, 121-132.

Annexe B: Communications scientifiques

1. Communications orales

Komagbe, G.S., Sessou, P., Mahillon, J., Farougou, S. (2022) Caractéristiques microbiologiques du *Wagashi Gassirè*, un fromage traditionnel produit au Bénin. **Microbiologie et Santé Publique** (Bénin).

Komagbe, G.S., Sessou, P., Mahillon, J., Farougou, S. (2023) Dominant cultivable yeast community in naturally fermented milk produced in Benin. **Premières journées scientifiques de Nutrition et sciences des aliments de la Faculté des Sciences et Techniques de l'Université Abdou Moumouni de Niamey** (Niger).

2. Posters

Komagbe, G.S., Sessou, P., Jeyaram, K., Farougou, S., Mahillon, J. (2021) Bacterial diversity of two types of *Wagashi Gassirè*, a traditional Beninese cheese, using High-Throughput Amplicon Sequencing. **Microbiology Society-Annual Conference Online 2021** (En ligne).