The timeline of progress in fermented food research in the Republic of the Congo: A meta-analytic review

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ABSTRACT

Approximately 61 reports on traditional fermented food (TFF) were published in the Republic of Congo between 1986 and 2024. Research on fermented foods has experienced many delays but has seen significant growth. Several studies related to TFFs have been conducted on bacterial identification, antibacterial and antifungal properties, extraction of flavonoid and phenolic compounds, extraction of biosurfactant and bacteriocins, bacteria-bacteria, bacteria-yeast interactions, enzyme engineering, bioethanol production, soil bioremediation, protein expression and purification, and gene cloning. To date, no review has offered an objective meta-analysis of all previous studies on TFFs. This review aims to establish a research timeline by tracking previously documented scientific publications in terms of TFF year after year while analyzing, commenting, and taking stock of scientific information. The reader is guided through the highlights of pioneers in the history of TFF research.

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The historical background vision

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In 1968, the Republic of Congo became a member of the African and Malagasy Council for Higher Education (CAMES, in French). CAMES is an intergovernmental institution for the integration of higher education systems. The CAMES institution aims to establish permanent scientific and cultural cooperation between member states, to draw up agreements between the member countries on higher education and research, and to contribute to their implementation (https://www.lecames.org/). The policy of this institution has engendered an innovative vision that has encouraged research and development in member countries [1]. The Republic of Congo has not missed this subregional political opportunity to implement the recommendations of CAMES (Figure 1).

Looking back in the history of science in Africa, we recall that in 1987, more precisely from June 25 to 30, the first meeting of scientists in Africa was organized in Brazzaville at the initiative of the political powers. It created the Pan African Union of Science and Technology with its headquarters in Brazzaville. Therefore, it must continue to be said that CAMES initiatives indirectly or directly gave rise to the scientific days that were organized as part of the celebration of the African and National Days of Scientific Renaissance. It is celebrated on

June 30, each year. Subsequently, the Day of Scientific Renaissance was institutionalized in Congo through the signing and publication on 5 Decree No. 97-248 of August 1997.

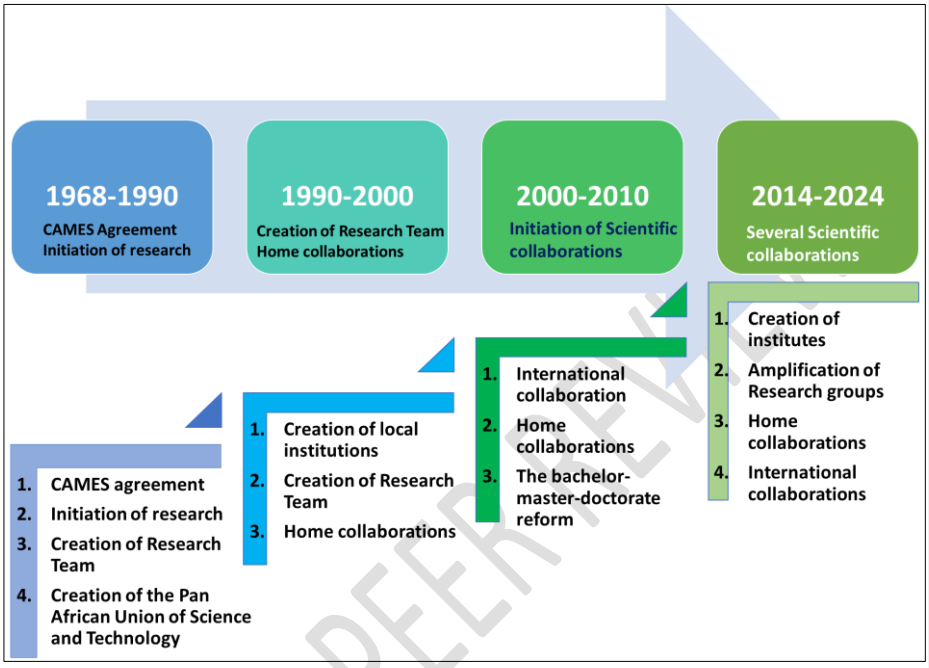

Figure 1: The timeline of the implementation of research activities on traditional fermented foods in the Republic of Congo.

The Beginnings of a Research Activity on TFFs

Simon Kéléké (associate assistant), Simon Charles Kobawila (CAMES Full Professor), and Professor Delphin Louembé (CAMES Full Professor) **(Figure 2),** were pioneers of the scientific development in the Republic of Congo in terms of TFF [2]. At the beginning of their scientific research, they met an eminent scientist by the name of Professor Pascal Lissouba, who declared, I quote, "do anything, do something, otherwise you will be disconnected from science» [Faîtes n'importe quoi mais faîtes quelque chose sinon vous serez déconnectés de la Science]". It is from his advice and other approaches that Professors Delphin Louembé and Simon Charles Kobawila embarked on the study of fermented foods in the Republic of Congo. That is, personal communication with Professor Delphin Louembé. Up-to-date D.TFFs statistics Louembé's scientific contributions refer to 23 research works, with 417 citations and 7,144 reads (https://www.researchgate.net/scientific-contributions/D-Louembe-43764307).

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The contributions of Simon Charles Kobawila's research statistics across all fields reveal 46 research works with 597 citations and 11,946 reads (https://www.researchgate.net/scientific-contributions/Simon-Charles-Kobawila-16113765).

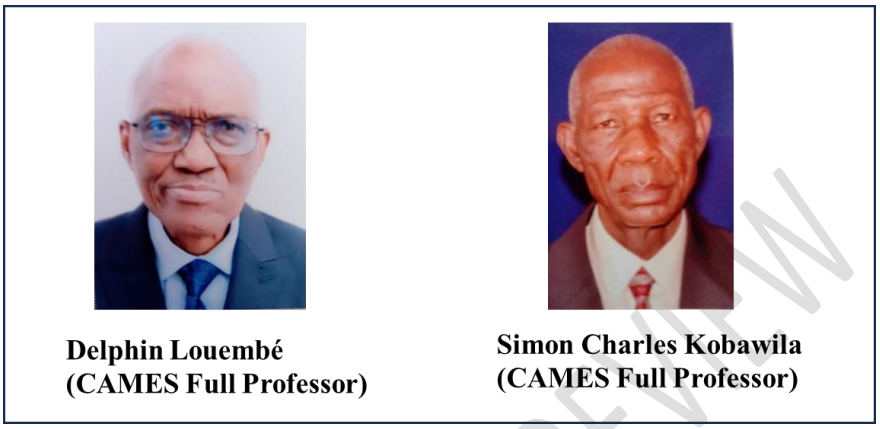

Figure 2: Pioneers of scientific development on Traditional Fermented Foods (TFF) in the Republic of Congo

Since time immemorial, in the 11 departments of the Republic of Congo, artisanal knowledge has made it possible to exploit the potential of fermentation processes to produce fermented foods and beverages that still exist today without scientific knowledge. These endogenous processes have been key to the valorization of agro-resources.

In this context, research on fermented foods began between 1975 and 1986. The research was oriented toward biotechnology, including the evaluation of physicochemical and microbiological parameters, in an attempt to improve the quality of these foods [3].

The advantage of these studies lies in the fact that fermentation technologies are transmitted from generation to generation. This approach facilitates ethnobotanical surveys that allow researchers to obtain TFF manufacturing diagrams of TFFs [4]. Many endogenous technologies are transmitted from parents to daughters and sons by women. The foods used for fermentation include, among others, roots, leaves, seeds, and fruits [4].

Various fermented foods indigenous to the Republic of Congo have been the subjects of preliminary studies [3]. This includes the pulp of *Landolphia jumelei* (fermented pulp of a berry) and ntoba mbodi (fermented leaves of Manihot esculenta (cassava)), fermented cassava

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tubers ('bikedi') of *Manihot esculenta* then processed to provide a staple food among Congolese populations, tutu (starch and gelled mass of cassava flour) and chikwangue, which is a bread cooked from fermented cassava dough [5, 6]. Poto-poto, which does not close the list of fermented products, is a fermented dough made from local corn (*Zea mays* L.) [4].

It was especially after 1990 that studies on indigenous fermented foods really reached a particular peak (**Figure 1 and Table I**) [4]. These improvements have made it possible to improve nutritional quality and optimize production [7-10]. At the same time, traditional fermented foods and beverages that have also been the subject of some studies include 'samba' (palm wine from the oil palm *Elaeis guineensis* Jacq [11], in the mango pulp cultivar [12], poto-poto (a cornbread dough), 'Mokiki' (fermented tubers and retted cassava) [13, 14], in yonga (a traditional fermented beverage from palm wine) [15] and Ntoba mbodi [7, 8].

Scientific Collaboration Based on Fermented Foods

In the 2000s (**Figure 1 and Table I**), studies focused not only on organoleptic quality and mineral content, but also on biodiversity, poto-poto, and Ntoba mbodi [15-20]. A previous study showed that retting performed with 'pieds de cuve' were able to reduce the odors of the retting tubers and the duration of retting from 24 to 36 h [21].

However, it should be noted that the identification methods were based on the cultural characteristics of microorganisms, including bacteria, yeasts, and fungi [17, 18]. The microbial composition of traditional fermented foods. Poto poto (a maize dough) was studied using a culture-independent approach using temporal temperature gel electrophoresis (TTGE). The sequencing of DNA bands from TTGE gels corresponding to a maize dough sample revealed the presence of *Lactobacillus gasseri*, *Enterococcus* sp., *E. coli*, *L. plantarum / paramundarum*, *L. acidophilus*, *L. delbrueckii*, *Bacillus* sp., *L. reuteri*, and *L. casei*. [22, 23]. Another study combined an integrated experimental approach to denaturing gradient gel electrophoresis (DGGE) analysis of total DNA from cassava dough [19]. The enzymatic hydrolysis of cyanogenic glycosides (linamarin and lotaustralin) leads to the production of hydrogen cyanide. Linamarin accounts for more than 80% of cassava cyanogenic glucosides [24]. Fermentation, boiling and ensiling are efficient techniques for the removal of cyanide from cassava peels [25].The reduction in the cyanide content during cassava root and leaf fermentation was taken into account to produce 'bikedi' (fermented tubers) and 'ntoba mbodi' [26].

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Fermented foods and beverages had begun to attract the curiosity of new generations of scientists. From 2008, more refined publications appeared in highly reviewed journals [4, 22,

27, 28]. To combat child malnutrition taking into account available local resources, modifications were made to the traditional process of producing fermented corn dough and preparing porridge by adding malt and carbonate before or after fermentation. This combination allowed for an increase in added nutritional value and resulted in a high energy density porridge fluidity [29].

Using species-specific PCR and 16S rRNA gene sequencing and cluster analysis of RAPD-PCR fingerprints, *Lactobacillus plantarum* and *L. fermentum* were identified. In the same study, bacteriocins secreted by *L. plantarum* and *L. fermentum* were found to have a wide spectrum of bacterial inhibition, including *Escherichia coli*, *Salmonella enterica*, *Enterobacter aerogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Enterococcus faecalis* [22]. A great study allowed to evaluate and improve the nutritional quality of fermented corn pasta and porridge [30]. To contribute to industrial development, initiatives have been launched. A study was conducted for the production of a traditional maize beer from maize [31].

The Rise of Scientific Research in Traditional Fermented Foods

From 2014 **(Figure 1 and Table I)** another new dynamic appeared, including new techniques in molecular biology identification, proteomics, and metagenomics that explain the meteoric rise of research [32-35].

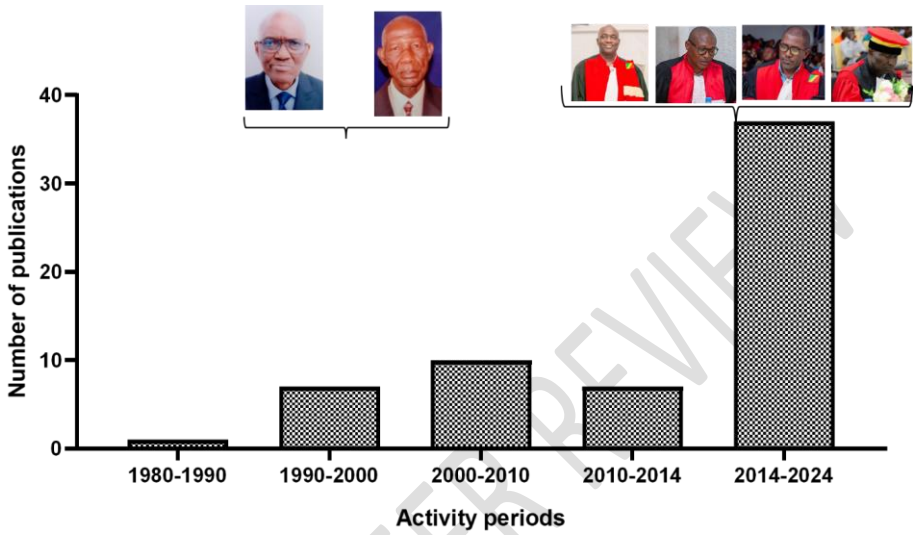
The period of 1980-1990 had been provided 1 publication, 7 for 1990-2000, 10 for 2000-2010, 7 for 2010-2014 and 37 for 2014-2024. The number of publications from the period 1990-2000 to the period 2014-2024 as a percentage represents an increase of 428.571% from the period 1990-2000 (Figure 3A). The explanation would be found in the amplification of the workforce and the universities reforms through the Bachelor-Master-Doctorate (BMD) system, which was one of the CAMES subregional recommendations.

The pioneers of this period in research on TFFs are Christian Aimé Kayath (CAMES Lecturer) with 70 research, 550.3 research interest score, 327 citations, and 48,618 reads (https://www.researchgate.net/profile/Aime-Christian-Kayath/stats/reads). Saturnin Nicaise Mokemiabeka (CAMES Lecturer) with 32 research, 164.3, research interest score, 128 citations and 9,061 reads (https://www.researchgate.net/profile/Mokemiabeka-Nicaise/stats).

Alain Brice VOUIDIBIO MBOZO (CAMES Lecturer) and Etienne Nguimbi (CAMES Full Professor) with 51 research, 180.2 research interest score, 139 citations and 9,835 reads (https://www.researchgate.net/profile/Etienne-Nguimbi-2/stats). All of the authors are professors and researchers at Marien Ngouabi University in Brazzaville. These pioneers

introduced new aspects and new techniques of molecular biology and proteomics in the study of TFFs **(Figure 3B).**

(A)



(B)

Figure 3: Pioneers of scientific development on traditional fermented foods (TFF) in the Republic of Congo from 2014 to 2024.

In the wake of the new dynamic, new species have been identified from Ntoba mbodi. This is the case of *Lysinibacillus loembei* [32, 36].

New initiatives focused on the microbiological and biochemical evaluation of volatile compounds produced in Nsamba (palm wine) and bikedi (retted cassava dough) [37] and crushed red pepper from *Capsicum frutescens* [35, 38], chikwangue, Mbala pinda (chikwangue mixed with peanut paste) [35, 39], Ntoba mbodi [40-42], Safou pulp [43], 'bikedi', sugar cane wine (Loungwila/Lungwila) [44, 45], ginger juice [46], pineapple wine, grapefruit wine (vulgarly called 'Let me sleep'), ginger juice [46], banana wine (Mbavu) [47, 48] sugarcane wine lees (*Saccharum officinarum*) [49], and corn malt [50, 51]. The focus on traditional fermented foods has not only been on prebiotic and probiotic bacteria [40]. Pathogenic bacteria such as *Bacillus* cereus and *Staphylococcus aureus* have been extensively studied [52, 53]. Bacteria of the genus *Bacillus* obtained from local fermented foods (fermented cassava, palm wine, wine ginger, Ntoba mbodi) were characterised [40, 54].

The biochemical evaluation of volatile compounds showed that 86% esters (ethyl caprylate, ethyl decenoate, N-ethyl decanoic, ethyl laurate) and decanoic acid. In terms of «bikedi», 43% terpenes and 37% alcohols were found: estragol, limonene, linalol, myrcene, and menthol [37]. Investigation of *Bacillus* biodiversity and safety involved in alkaline fermentation of cassava leaves (*Manihot esculenta* Crantz) for the production of Ntoba Mbodi has been carried out [42]. Citrus fruits are an integral part of agro-resources that undergo fermentation processes. The pulp of Safou has been characterised with respect to the bacteria that participate in its softening [43].

The development of a highly efficient consortium of bacteria of the genus *Bacillus* allowed reducing the retting time of cassava. These bacteria showed the ability to ferment cassava tubers in a 2 days alone and then in consortium in less time [55, 56]. Organic ferment is in the process of obtaining a patent and being marketed.

More refined studies using DNA technologies (PCR and derivatives, RT-PCR, RLFP, and sequencing) on many genes have been associated with the understanding of interactions in fermented foods. Fibrinolytic enzymes with a biopharmaceutical scope have been better characterised [34, 57-59]. The antimicrobial resistance profile of *B. cereus* and *Staphylococcus* isolated from traditional fermented had been done with a promising future [52, 60]. Antibiotic resistance has not spared bacteria of the genus Bacillus isolated from fermented foods [40].

Table I: Scientific work carried out and documented in the Republic of Congo for the period 1986 to 2024.

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Years	Number of Publications	Topics	References
1986	1	Preliminary study of the microbiology of palm wine *Elaeis guineensis*	[3]
1992	1	Weaning Porridges in the Congo: Composition, nutritional value and methods of use	[7]
1993	1	Lactic acid bacteria during retting: Characterization and evolution	[13]
1995	1	Palm wine: Microbiological and biochemical study in republic of Congo	[11]
1996	3	Microbiological and biochemical characterisation of cassava retting, a traditional lactic acid fermentation for the production of foo-foo (cassava flour) production	[8]
		Microbiological and biochemical studies of the the poto-poto of maize porridge poto-poto ,	[10]
		Microbiological and biochemical characteristics of Cassava retting	[14]
1997	1	Evolution of the content of cyanide compounds in cassava tubers during the opening-Linamarasic activity of lactic acid bact	[9]
2002	1	Retting of cassava tubers from 'pied de cuve" made from retted cassava	[21]
2003	3	Lactic acid bacteria from fermented corn dough in Congo.	[18]
		Microbiological study of fermented cassava leaves: "Ntoba Mbodi"	[17]
		Identification, isolation and quantification of representative bacteria from fermented cassava dough using an integrated approach of culture-dependent and culture-independent methods	[19]
2004	2	Variability and improvement of traditional technology for the production of fermented corn dough in Co	[16]
		Lactic acid bacteria tolerant to acidity and biliary salts of yonga, a traditional fermented beverage in Congo	[15]
2005	1	Reduction of cyanide content during the fermentation to produce bikedi and ntoba mbodi, two food products of the Congo.	[26]
2006	1	Culture-independent analysis of the microbial composition of traditional African fermented foods poto poto and degue using three different DNA extraction methods	[23]
2008	1	Lactobacillus strains isolated from poto poto, a Congolese fermented maize product, and genetic fingerprints of their plantaricin operons	[22]
2009	1	Evaluation and improvement of the nutritional quality of fermented corn pasta and porridge in Congo	[30]

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2010	1	Genotypic diversity of lactic acid bacteria isolated from traditional African alkaline-fermented foods	[33]
2011	2	Softening and Mineral Content of Cassava Leaves (Manihot esculenta Crantz) During the Fermentation to Produce Ntoba mbodi	[20]
		Traditional retting of cassava roots in the ponds or the rivers	[28]
2012	2	Response mechanisms of lactic acid bacteria to alkaline environments: a review	[27]
		Effect of malt incorporation on the fluidity and density of the porridge of corn-araichidedate to infants and young children	[29]
2013	2	Physico-chemical characterisation of Brew during the Brewing Corn Malt in the Production of Maize Beer in Congo	[31]
		Effect of the degree of maturation on the kinetics of ethyl fermentation of mango pulp cultivar BOKO	[12]
2014	5	Toward the Understanding of Fermented Food Biotechnology in Congo Brazzaville	[4]
		Behaviour of Fermentable Sugars in the Traditional Production Process of Cassava Bioethanol	[61]
		Volatile compounds produced in two traditional fermented foods of the Congo: Nsamba (palm wine) and bikedi (retted cassava dough)	[37]
		Optimisation of Growth, Fibrinolytic Enzyme Production, and PCRAmplification of Encoding Fibrinolytic EnzymeGene in Bacillus amyloliquefaciens Isolated from Ntoba mbodi at Brazzaville	[34]
		Evaluation of traditional technology for the production of lungwila, a sugarcane wine of sugarcane of Congo	[45]
2015	1	Lysinibacillus louembei sp. nov., a spore-forming bacterium isolated from Ntoba Mbodi, alkaline fermented leaves of cassava from the Republic of the Congo	[32]
2016	4	Microbiological and biochemical evaluation of crushed red pepper from Capsicum frutescens preserved in jars and manufactured in local markets in Republic of Congo	[38]
		Correlation between the initial content of starch in the Mash of Cassava (*Manihot esculenta)* in fermentation and Temperature of Distillation of alcohol Fermented Wort in the Course of Bioethanol Production in the Production of Bioethanol in Congo	[62]

		Physiological Characterisation of Staphylococci and Micrococci Isolated from Fermented Cassava Leave (Manihot esculenta Crantz), Ntoba mbodi	
		Toward the Understanding of Fermented Food Biotechnology in Congo Brazzaville	[4]
2017	3	Potential Spore-Forming Probiotics Isolated from Ntoba mbodi, Alkaline Fermented Leaves of Cassava from the Republic of the Congo	[40]
		Investigation of the diversity and safety of the predominant Bacillus pumilus sensu lato and other Bacillus species involved in the alkaline fermentation of cassava leaves for the production of Ntoba Mbodi	[42]
		Production, partial purification, and SDS-PAGE profiles of Caseinolytic Enzyme in two Bacillus strains isolated from fermented cassava leaves "ntoba mbodi" in congo Brazzaville.	[54]
2018	2	Assessment of dominant bacterial strains isolated from Ntoba mbodi, an indigenous African alkaline fermented food, and their potential enzyme activities	[41]
		Production, Variability and N- Terminal Sequences of Fibrinolytic Enzymes Produced by Bacillus Strains Isolated from Fermented Cassava Leaves 'Ntoba Mbodi' at Brazzaville, Republic of Congo	[57]
2019	3	The Genus Lysinibacillus: Versatile Phenotype and Promising Future	[63]
		Environmental heterogeneity of Staphylococcus species from alkaline fermented foods and associated genetic elements of toxins and antimicrobial resistance genetic elements	[52]
		Giving More Benefits to Biosurfactants Secreted by Lactic Acid Bacteria Isolated from Plantain Wine Using Multiplex PCR Identification	[47]
2020	3	First Development of a Biotechnological Ferment Based on a Consorsium of the Bacillus Genus for the Optimisation of the Fermentation Process of Cassava Tubers	[56]
		A Quick Biochemical Comparison Between the Traditional Fermentation of Plantain Wine and Banana Wine Produced in Republic of Congo	[48]
		Invasion of epithelial cells is related to the Secretion of Biosurfactant through the Type 3 Secretion System (T3SS) of Shigella flexneri	[64]
2021	4	Involvement of Bacillus species in understanding the softening process of Safou Pulp (Dacryodes Edulis H.J.Lam)	[43]

		The Diversity of the Bacterial Community of Fermented Pepper in Brazzaville Revealed by Illumina Miseq of 16S rRNA Gene	[35]
		New Phylogenetic Molecular Markers in Bacteria of the Genus Bacillus: Fibrinolytic Proteases	[65]
		Profiling of indigenous biosurfactant-producing Bacillus isolates in the Bioremediation of soils contaminated by Petroleum Products and Olive Oil	[66]
2022	3	Diversity of the Bacterial Community of a Congolese Traditional Fermented Food in Congo, 'Pandé', Revealed by Illumina MiseqTM Sequencing of 16S rRNA Gene	[67]
		Molecular Identification and Phylogenetic Classification of Antibacterial Substance Isolated Bacteria Isolated from Mbala Pinda, a Traditional Congolese Traditional Food	[39]
		Antibiotic resistance profile of Bacillus cereus strains isolated from soil and Pepper in Brazzaville	[60]
2023	4	Molecular identification of Bacillus species and yeast isolated from food sources and their interaction with the Lysinibacillus louembei strain	[58]
		Molecular Profiling of Sugarcane Wine, a Traditional Fermented Beverage (Loungouila) from Madingou	[44]
		The behaviour of grains in the course of the Smothering Phase of the traditional corn malting process (Zea mays sp.) in the production of Lotoko, a Brandy of the Basin of Congo	[51]
		Selection and characterisation of yeast isolates with the amylolytic ability of corn malt (Zea mays sp.)	[50]
2024	5	Antiseptic efficacy of a Soap Made from Biosurfactants Isolated from Bacillus and Lactobacillus against Pathogenic Bacteria	[68]
		Traditional Process and Identification of Lubo or Sugar Cane Wine Lees Yeast Flora (Saccharum officinarum) in 15 Workshops in the Niari Valley in Congo	[49]
		The Bacillus Species Consortium as a New Starter in the Optimisation of Cassava Tuber Retting	[55]
		Involvement of the Bacillus SecYEG Pathway in Biosurfactant Production and Biofilm Formation	[69]

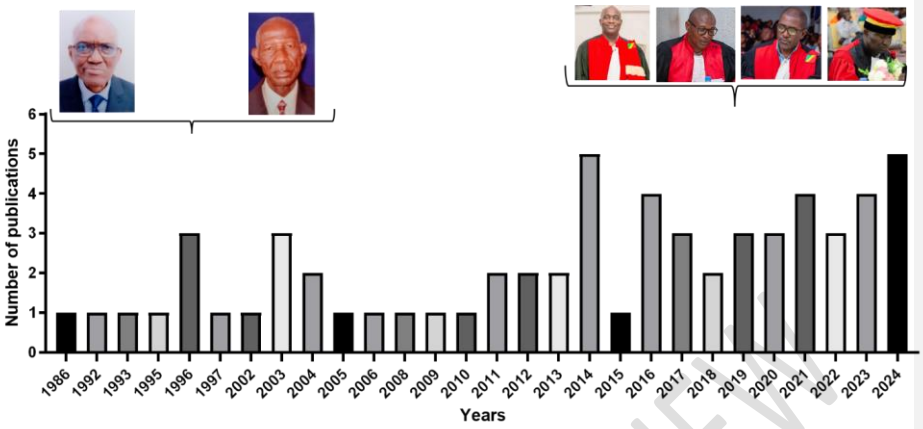

Figure 4: Timeline based on the number of scientific publications.

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Advances in modern research and bioactive compounds.

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Initially, studies on TFFs were only orientated toward biochemical and microbiological characterisation [70]. Modern concepts of microbiology have not been left out of studies on fermented foods. This refers to the study of antibacterial activities [47], multidrug resistance to antibiotics [32, 60], biofilm formation [58, 69], bacterial physiology related to quorum sensing and quorum quenching [69] , and in the production of bioethanol (Figure 3) [61, 62].

The molecular isolation of TFF of has allowed the identification of several bacterial species, including lactic acid bacteria, *Bacillus*, and yeasts [33, 44, 65, 67, 71]. The diversity of bacteria is higher in the fermented pulps of Pandé than in the unfermented pulps. Pandé is a traditional fermented food from the Republic of the Congo made from pulps of *Raffia* spp. [67]. Using new techniques in biochemistry and molecular biology, it has been shown that traditional fermented foods and beverages of the Republic of Congo contain biomolecules that are released by *Bacillus* into the extra bacterial environment. Analysis by one-dimensional SDS-PAGE and then by Maldi-Tof using Lougwila samples (fermented sugarcane beverage), has shown the presence of proteases, amylases, cellulases, and pectinases [44]. Biosurfactant and bacteriocins have also been isolated in the supernatant [44, 47, 54, 71]. Bacteria of the genus *Bacillus* isolated from traditional fermented foods have been the subject of numerous studies. Hydrolase secretion, biosurfactant secretion and extraction, bacterial physiology, bacteria-bacteria, and bacteria-yeast interactions were characterised [46-48, 56, 58, 59, 67, 69, 71] (Figure 3).

The biosurfactant extracted from *Bacillus* isolated from traditional fermented food has antibacterial [46, 71], antifungal [47], antibiofilm properties [58, 69] and antiquorum detection

[72]. In the presence of lipopeptide biosurfactants such as surfactin, iturin, lichenisin, soponin, fengycin, pathogenic bacteria such as *P. aeruginosa*, *Shigella flexneri* M90T 5a [64], *Salmonella enterica*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus mirabilis*, *Streptococcus* agalactiae, and *Enterobacter cloacae* cannot grow [47, 68, 73-77]. The antibacterial properties of the biosurfactant isolated from home-fermented food (HFF) could also involve pathogenic gastrointestinal *Escherichia coli*. A study of pathogenic gastrointestinal *E. coli* serotypes is to be carried out. Strains of *E. coli* are classified according to the properties of their virulence: enteropathogenic *E. coli* (EPEC), enterotoxinogenic *E. coli* (ETEC), enterohaemorrhagic E. coli (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), verotoxinogenic *E. coli* (VTEC), diffusely adherent *E. coli* (DAEC) and necrotoxinogenic *E. coli* (NTEC) [78, 79].

Indirectly, biosurfactants, extracted from bacterial species of the genus *Bacillus* and *Lysinibacillus* isolated from fermented foods, have shown a promising future in terms of their ability to decontaminate soils contaminated with heavy and light hydrocarbons [63, 66, 71].

A study has evaluated the potential application of microbial biosurfactants in the pharmaceutical, cosmetic, and personal care industries. The article has focused on biosurfactant production, their characterisation, and application in biotechnological fields. Studies highlight the use of biosurfactants as antiseptics in the formulation of medicinal soaps [68].

Traditional fermentation of foods improves their digestibility and nutritional values. Microorganisms produce interesting compounds, such as vitamins and polyphenols, that increase their antioxidant power and contribute to cardiovascular health [80-82]. Flavonoids and polyphenolic compounds are powerful natural antioxidants in food [83] . The study of fermented ginger juice has highlighted these compounds [46].

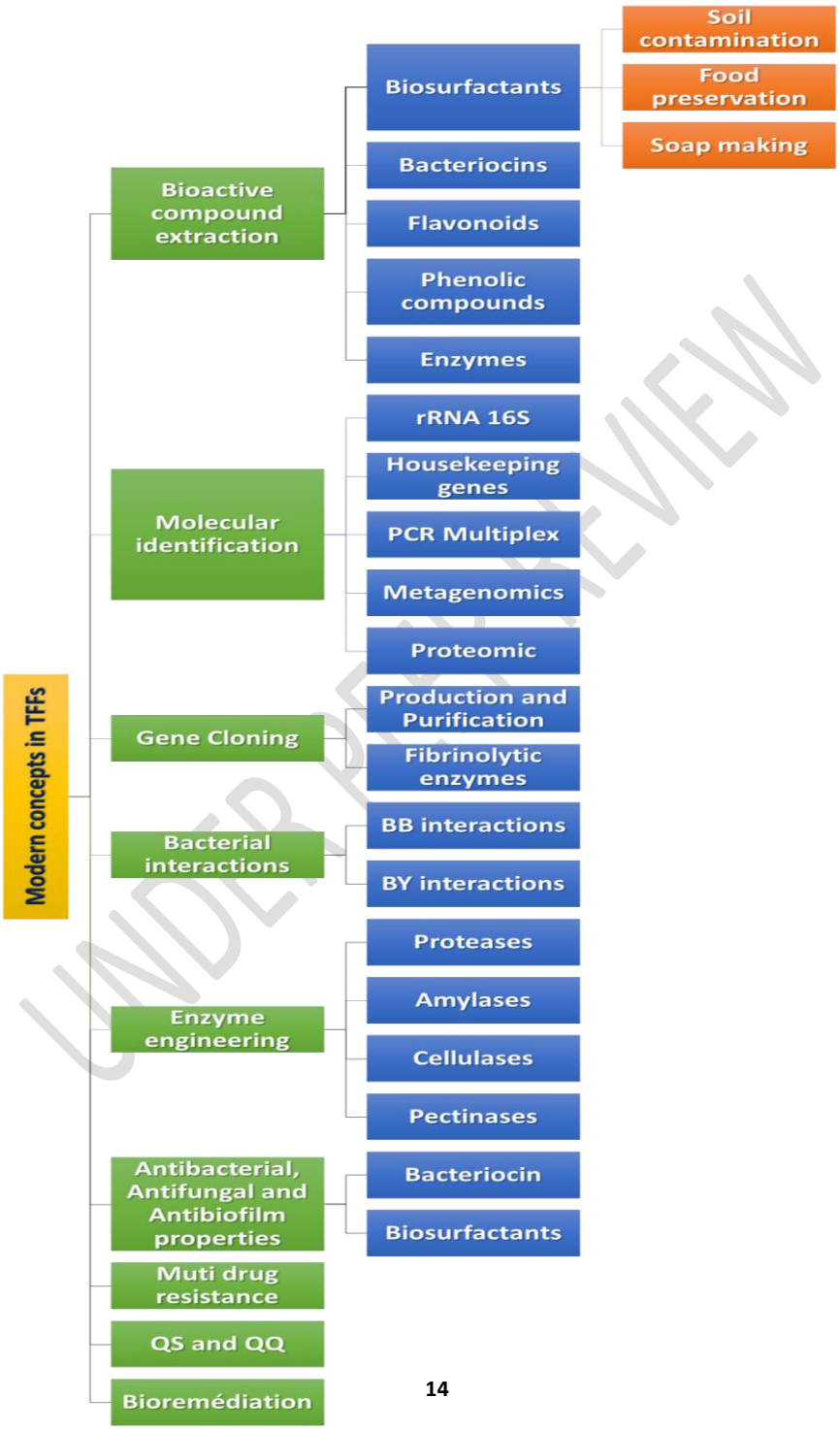

Figure 3: Advances in modern research and bioactive compounds. QQ: quorum sensing, QQ: quorum quenching, BY: Bacteria yeast, BB: Bacteria-bacteria. TFF: Traditional Fermented Foods

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Database Scientific Contributions

Scientific contributions are based not only on knowledge of traditional fermented foods and nucleotide and protein sequence databases published in NCBI-National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), DDBJ-DNA Data Bank of Japan (https://www.ddbj.nig.ac.jp/index-e.html), and in ENA-European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home). Several new sequences related to bacteria isolated from TFFs have been identified and published. Genes have been better characterized, and sequences have been incorporated into databases. Approximately 200 accession numbers related to TFFs were published from 2000 to 2024 (https://www.ncbi.nlm.nih.gov/nuccore/?term=Kayath).

Conclusion

We do not claim to have confirmed that all documented studies on TFFs have been cited. Some doctoral theses and master's or bachelor's dissertations have not been cited. Nevertheless, this meta-review allowed us to explain in simple terms the strategy, although laborious but smart, that has been implemented to conduct research in a world where it is sometimes difficult to be accepted as a scientist, especially when the subjects do not directly interest human health. The review also allowed us to show the limits of collaboration that exist between scientists. There has certainly been a lot of compartmentalization, but the coaching of newcomers has allowed for a great comeback. We expect that the next generation of TFF will contribute to research.

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