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Microbial ecology and fermentation of *Coffea canephora*

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Recent investigations into the microbiota and fermentation of *Coffea canephora* fruits and beans have yielded limited advancements globally, highlighting a developing field of study. Consequently, this review seeks to consolidate existing literature through an analysis focusing on the microbiota and fermentation processes inherent in the postharvest processing of Canephora coffee. To this end, a comprehensive examination of the principal microorganisms inherent to this species, the application of starter cultures in fermentation, and the repercussions of fermentation on the chemical and sensory attributes of the beverage will be expounded. These investigations underscore the influence exerted by the fermentation process and the introduction of microorganism inoculation on Canephora coffees' chemical composition and sensory characteristics. Fermentation emerges as a mechanism facilitating the modification of coffee flavor and aroma, thereby presenting avenues for innovative enhancements in producing distinct Canephora coffee beverages. Research to assess the microbiota of Canephora coffees from various origins has the potential to advance our understanding of the microbial ecology specific to this species. Such studies will play a crucial role in identifying pertinent starter cultures that could be used to produce high-quality coffees.

KEYWORDS

coffee fermentation, coffea canephora, microbiota, fermentation, starter culture

1 Introduction

Brazil is at the forefront of coffee production, with an average output of 50.68 million 60-kg bags of processed coffee in the last 10 years. The world's largest producer and second-largest consumer of coffee are some of the titles Brazil has obtained over the years. The country invests in the cultivation of the two most important species, *Coffea arabica* and *Coffea canephora*, the latter being the second most traded species in the world, with the most cultivated botanical varieties being Robusta and Conilon coffee (Conab Companhia Nacional de Abastecimento, 2023).

Compared to other species, *C. canephora* coffees are fuller-bodied but more bitter, less aromatic, and less acidic than *C. arabica*, which attributes it to a lower quality beverage (Brando, 2004). However, this issue has been overcome due to technological advances in and out of the field. Brazilian Canephora coffees (Conilon and Robusta) of exceptional quality are ascending to the status of specialty coffees, marking a shift in the industry's perception (Baqueta et al., 2023).

Pre- and postharvest processing steps influence beverage quality, significantly improving the final quality (Velásquez and Banchón, 2022). In postharvest processing,

TABLE 1 Country of origin, processing, identification method, bacterial and fungal genera identified in different varieties of Canephora coffees.

Country	Variety	Processing	Identification method	Identified bacterial genera	Identified fungal genera	Reference
India	Robusta	Wet	Morphological and biochemical tests, and enzyme production	*Streptococcus*, *Pseudomonas*, *Flavobacterium* and *Proteus*	*Saccharomyces* and *Schizosaccharomyces* sp	Agate and Bhat, (1966)
Congo	Robusta	Wet	Morphological and biochemical tests, and enzyme production	-	*Candida*, *Saccharomyces*, *Rhodotorula* and *Torulopsis*	Van Pee and Castelein, (1971)
India	Robusta	Semi-dry	Morphological and biochemical tests	*Bacillus* sp., *Lactobacillus* sp., *Leuconostoc* sp., *Pseudomonas* sp., and *Flavobacterium* sp	*Saccharomyces* sp., *Schizosaccharomyces* sp. *Aspergillus* sp., *Cladosporium* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., and *Rhizopus* sp	Velmourougane (2013)
Cameroon	Robusta	Dry	DGGE +16S and 26S rDNA sequencing	*Microbacterium* sp., *Enterobacter*, *Pantoea* sp. and Enterobacteriacea	*Aspergillus*, *Hanseniaspora* and *Wallemia*	Hamdouche et al. (2016)
Brazil	Conilon Emcaper 8151–Robusta Tropical	Dry	Maldi-Tof +16S rDNA and ITS sequencing	*Acinetobacter*, *Bacillus*, *Cellulosimicrobium*, *Citrobacter*, *Dermacoccus*, *Enterobacter*, Enterobacteriacea *Enterococcus*, *Escherichia*, *Kosakonia*, *Lactobacillus*, *Leuconostoc*, *Micrococcus*, *Pantoea*, *Pectobacterium*, *Pseudomonas*, *Raoultella*, *Salmonella*, *Staphylococcus*, *Stenotrophomonas* and *Streptomyces*	*Candida*, *Hanseniaspora*, *Meyerozyma* and *Pichia*	Pereira et al. (2021)
India	Robusta	Semi-dry	16S rDNA and ITS sequencing	*Acetobacter*, *Flavobacterium*, *Halomonas*, *Hydrogenophaga*, *Lactobacillus*, *Lactococcus*, *Nitrospora*, *Paracoccus*, *Pediococcus* and *Pseudomonas*	*Basidiobolus*, *Candida*, *Geotrichum*, *Kazachstania*, *Kurtzmaniella*, *Meyerozyma*, *Mortierella*, *Pichia*, *Wallemia* and *Wickerhamomyces*	Aswathi et al. (2022)

DGGE, denaturing gradient gel electrophoresis. MALDI-TOF, matrix-assisted laser ionization time-of-flight mass spectrometry.

the fermentation method has contributed to the improvement of sensory profiles, becoming an alternative approach for increasing the quality of Canephora coffees (Agnoletti et al., 2022; Bravim et al., 2023; Cassimiro et al., 2023).

Coffee fermentation can occur spontaneously or using starter cultures (Schwan et al., 2023). During this process, intense microbial activity occurs, and many biochemical reactions occur inside and outside the bean. The coffee epiphytic microorganisms metabolize the sugars in the mucilage and produce compounds of interest, such as organic acids, esters, and alcohols. These volatile and non-volatile compounds produced during fermentation directly affect the beverage´s quality (Silva, 2015; Martinez et al., 2019; Bravim et al., 2023).

Thus, it is essential to deepen the knowledge of the coffee microbiota, which will facilitate the selection of microorganisms with specific characteristics that can be used as starter cultures during fermentation (Schwan et al., 2023). Using selected bacteria and yeasts is an economically viable alternative to obtain coffees with different flavors and aromas. In addition, it contributes to greater standardization of the process, reduction of fermentation time, inhibition of the growth of undesirable microorganisms, and production of promising compounds for improving the quality of the beverage (da Silva et al., 2021; Prakash et al., 2022; Bravim et al., 2023; Cassimiro et al., 2023).

Studies on the fermentation of Canephora coffees began recently in Brazil, with a limited number of advances in this field. Given the above, this review aims to condense the literature through a critical analysis addressing the microbiota and fermentation processes associated with the postharvest processing of Canephora coffee. Thus, an overview of the main microorganisms found in this species, starter cultures used in fermentation, and the impact of fermentation on the chemical and sensory characteristics of the beverage will be presented.

2 Natural microbiota of canephora coffee

Coffee naturally contains a microbiota composed of several microbial groups, including lactic acid bacteria (LAB), acetic acid bacteria (AAB), bacteria of the *Enterobacteriaceae* family, yeasts, and filamentous fungi. These microbial communities are involved in the fermentation of nutrients present in coffee fruits, such as carbohydrates, proteins, amino acids, and organic acids, and influence the chemical composition of the beans and the beverage. Due to their favorable water activity and nutritional composition, freshly picked coffee fruits offer ideal conditions for the multiplication of microorganisms, whose metabolic activity can occur up to water activity values of 0.6.

The multiplication of microorganisms in coffee depends on several factors, such as humidity, temperature, water activity, nutrient availability, pH, and enzyme production (Silva et al., 2008). Some studies have been conducted to quantify and identify the presence of microorganisms in Canephora coffees, including both Robusta coffee and, more recently, Conilon coffee (Table 1).

The predominance of different microbial groups is influenced by factors such as the origin and production environment of the coffee, the variety, fruit maturity, and the processing method. Recent studies conducted on Brazilian Canephora (Conilon) showed that, for both dry and wet processed coffees, the bacterial population is predominant over the fungal population (Pereira et al., 2021; Bravim et al., 2023). In Conilon coffee, the most common bacterial genera were *Bacillus* and *Staphylococcus*, while the predominant yeasts were *Candida*, *Meyerozyma,* and *Pichia* (Pereira et al., 2021). Some of these isolates were evaluated for use in the fermentation of Conilon coffee and showed excellent results in improving the quality of the beverage and controlling the population of filamentous fungi. The microbial profile is influenced by the use of starter cultures, which can either restrict or encourage the growth of particular species. Microbial populations are regulated by intrinsic and extrinsic factors, substrate competition and metabolite production. The presence of filamentous fungi is undesirable as it imparts negative sensory traits to the beverage and some species produce mycotoxins. The suppression of filamentous fungi may be attributed to competition for space and the secretion of hydrolytic enzymes by bacteria and yeasts. *Meyerozyma caribbica* inoculated in natural coffee improved the cup score in 1.52 points and reduced 90% of fungal filamentous fungi population when compared to spontaneous fermentation. In wet-processed Conilon coffee *Meyerozyma guilliermondii* and *Bacillus licheniformis* used as starter cultures stimulated the multiplication of lactic acid bacteria. Besides that, *Bacillus licheniformis* was correlated with volatile compounds such as 5-methylfurfural, two-oxobutyl acetate, 1-furfurylpyrrole, and maltol, which contributed to desirable sensory notes of almonds, caramel, and chocolate in the cupping test. *M. guilliermondii* combined with the SIAF method increased the coffee score by over three points, improved the production of desirable volatile compounds (2-methylpyrazine, two-ethylpyrazine, furfuryl alcohol, and 5-methylfurfural), enhanced the perception of sweetness and the sensory complexity of Conilon coffee. *Leuconostoc mesenteroides* resulted in a beverage with notes of caramel, fruits, and spices in wet-processed Conilon coffee subject to SIAF method (Bravim et al., 2023; Cassimiro et al., 2023; da Silva et al., 2021; do Rosário et al., 2023).

The need for more studies to better elucidate the natural microbiota of Brazilian Canephora coffees is evident, especially for the botanical variety Robusta, which is cultivated and has stood out in the Amazon region of Brazil. Knowledge of the natural microbiota of Brazilian canephora coffees may contribute to identifying and selecting starter cultures with the potential to control coffee fermentation better, resulting in beverages with distinct sensory characteristics.

3 Fermentation process: dry, wet, semi-dry, and self-induced anaerobiosis fermentation (SIAF)

In obtaining coffee beans, the fruits can be subjected to different types of postharvest processing, which influences the quality of the product. Four processes will be described below (Figure 1): dry or natural processing, wet processing, semi-dry or pulped natural processing, and self-induced anaerobiosis fermentation (SIAF).

3.1 Dry or natural processing

The dry method, natural processing, is popular in Brazil, especially for *C. canephora* coffees. In this process, the whole fruits are placed on concrete, asphalt, or suspended terraces, under the sun, or in mechanical dryers to ferment and dry.

When drying in the sun, the product's final quality will depend on the region's climatic conditions concerning rainfall, temperature, relative air humidity, and insolation. In mechanical drying, parameters such as temperature and relative humidity are better controlled, promoting faster and more uniform drying.

Spontaneous microbial fermentation may occur during sun drying because yeasts and bacteria are part of the natural microbiota of the fruit (Duong et al., 2020). Mechanical drying prevents the occurrence of spontaneous fermentation since the reduction in moisture occurs more quickly. Fermentation occurs because the fruit contains mucilage, and the pulp comprises water, proteins, fibers, sugars, cellulose, pectic substances, and ash (Pereira et al., 2021).

Some studies have shown that fermentation can occur through different mechanisms, and there is still no conclusion on what causes the phenomenon in coffee. One theory is that fermentation may occur due to the action of enzymes endogenous to the fruits or by the action of microorganisms that produce enzymes such as pectin lyase and polygalacturonase (Schwan et al., 2012). These enzymes aid in the degradation of mucilage, whose components are converted into ethanol, lactic acid, butyric acid, acetic acid, and other higher carboxylic acids (Silva et al., 2000).

The fermentation occurs during the first days of drying, while drying occurs until the beans reach between 11% and 12% moisture. After drying, the beans are removed from the husks and parchment. They are stored under controlled temperature and relative humidity until the beans are distributed in the market.

3.2 Wet processing

In this process, the husks are removed, and the beans containing mucilage are placed in containers with water, where the fermentation process begins and lasts between 24 and 48 h (Silva, 2015). Next, the beans wrapped in parchment are dried in open air on wooden frames with screens, which allow spontaneous fermentation, or in mechanical dryers.

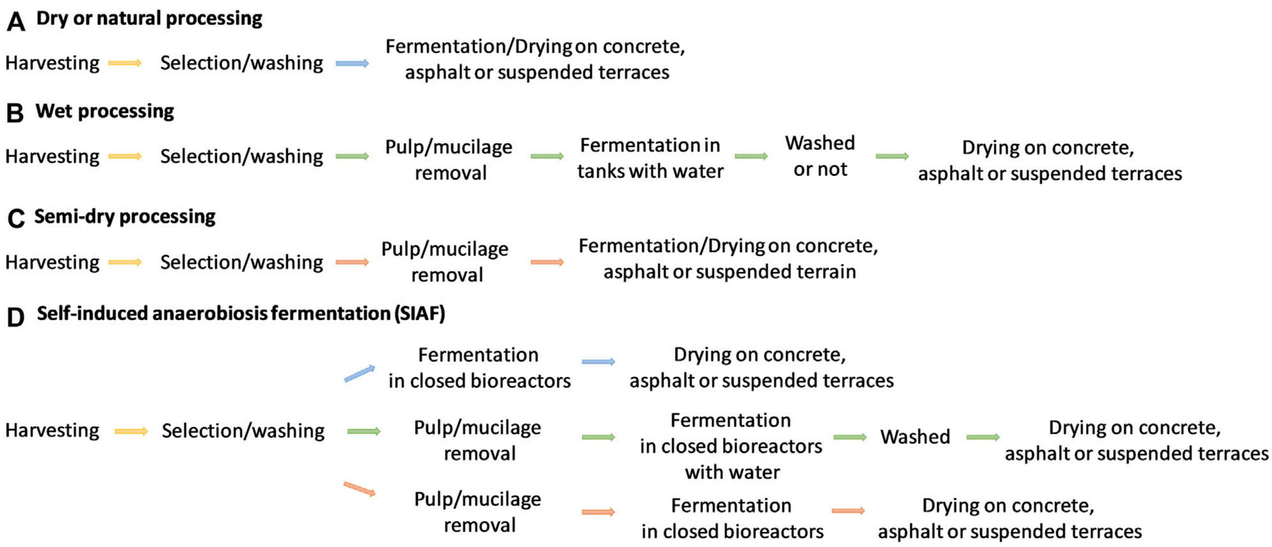

FIGURE 1
Schematic representation of postharvest processing (dry, wet and semi-dry) and self-induced anaerobiosis fermentation (SIAF).

3.3 Semi-dry or pulped natural processing

The semi-dry processing method is a variation of the wet process and an intermediate between the dry and wet methods. It is also known as "cherry peeled" (CP), "natural pulping," or "pulped coffee," where the beans are removed from the husk but without the removal of mucilage. These beans, containing mucilage and parchment, are placed to dry in the sun, allowing spontaneous fermentation, or in mechanical dryers, where fermentation does not occur. In both processes, drying occurs until the humidity reaches 11%–12%.

3.4 Self-induced anaerobiosis fermentation (SIAF)

In this method, the whole coffee or pulped or peeled fruits, wrapped in parchment and mucilage, are placed in a closed container to maintain anaerobiosis (Pereira et al., 2022). This container does not allow air entry, and the oxygen present is consumed by the metabolism of the plant material or microorganisms, producing CO_2, in addition to metabolites that influence the quality of the product.

4 Use of starter cultures in fermentation

Starter cultures have long produced fermented foods and beverages from various products, including grains, cereals, milk, meat, soy, fruits, and vegetables (Steinkraus, 2004). The use of starter cultures contributes to a faster and more consistent fermentation process, which may improve the sensory quality of the product (Silva, 2015).

In coffee processing, the inoculation of microorganisms during fermentation is a relatively recent approach. In Brazil, studies that evaluated the use of yeasts isolated from the coffee itself to ferment Arabica coffee began in 2014 (Evangelista et al., 2014), while research on Canephora coffee (Conilon) was published in 2021 (da Silva et al., 2021). The starter cultures can be applied in all fermentation process, dry, semi-dry, wet and SIAF method.

In selecting suitable starter cultures, it is essential to consider the ability to grow and survive in the coffee environment, produce enzymes to decompose mucilage and pulp, and inhibit the growth of unwanted microorganisms such as toxigenic fungi. Furthermore, the starter culture must produce metabolites that improve the beverage's sensory quality and do not produce toxic compounds (Silva, 2015). Besides that, the coffee's species, variety, and processing must be considered.

Bacteria, especially LAB, yeasts, filamentous fungi, and enzymes produced by these microorganisms, can be used in coffee fermentation (Table 2). Research has shown that using yeasts and bacteria in Canephora coffees may be advantageous for inhibiting the fungal population, producing volatile compounds, improving the cup test score, and obtaining a beverage with desirable sensory notes. All these advantages add value to the product, allowing producers to receive a better sales price for coffee, as they can offer consumer market coffees with different sensory profiles. Studies with the Robusta botanical variety from Brazil still need to be conducted to evaluate both the microbiota of these coffees and the use of starter cultures in fermentation.

5 Impact of fermentation on chemical and sensory characteristics

The postharvest processing method influences the product quality, as it affects the chemical and sensory characteristics of the coffee beans. Coffee aroma and flavor are the result of the interactions between compounds such as short-chain organic acids, aldehydes, ketones, sugars, proteins, amino acids, fatty acids, and phenolic compounds (trigonelline and chlorogenic acids) (Alcantara et al., 2021).

TABLE 2 Country of origin, fermentation process, microorganisms or enzymes used in the fermentation of Canephora coffee and main results obtained.

Country	Variety	Fermentation or type of coffee used (whole fruits or beans)	Microorganism or enzyme used in fermentation	Main results	Reference
India	Robusta	Pulped beans	Pectinase produced by *Aspergillus niger*	Reduction of fermentation time	Murthy and Naidu, (2011)
Indonesia	Robusta	Beans	1% Kefir +2% lactose	Increase in the number of volatile compounds	Harada, (2019)
Brazil	Conilon Emcaper 8151–Tropical Robusta	Whole fruits fermentation and dry processing	*Meyerozyma caribbica* and *Pichia kluyveri* isolated from Conilon coffee	Reduction in the fungal population during fermentation, improvement in the overall score in the cup test, and perception of difference by consumers in coffees inoculated with *M. caribbica*	da Silva et al. (2021)
India	*Coffea canephora* var. S274 and var. Peridenia	Solid-state fermentation of beans	*Aspergillus* and *Mucor*	Higher levels of pyrazines in coffees inoculated with *Aspergillus oryzae* and furans for those fermented with *Mucor plumbeus*	Tang et al. (2021)
India	Robusta variety *Coffea congensis* × *Coffea canephora* (CXR)	Solid-state fermentation of pulped beans	*Saccharomyces cerevisiae* isolated from cocoa	Reduction in fermentation time, increase in the number of volatile compounds, improvement in sensory quality	Prakash et al. (2022)
Brazil	*Coffea canephora* var. Conilon	Peeled and pulped beans (CD) and natural coffee (NC), with or without the addition of water	*Saccharomyces cerevisiae*, *Klebsiella* and lactic acid bacteria (species not reported)	Higher score of natural coffee inoculated with *S. cerevisiae* after 120 h of fermentation without addition of water	Zani Agnoletti et al. (2022)
Brazil	Conilon Vitória	Pulped beans wet processed for 48 h	*Bacillus licheniformis* and *Meyerozyma guilliermondii* isolated from Conilon coffee, separately and in coculture	Stimulation of the multiplication of the LAB population during fermentation and production of volatile compounds that resulted in positive sensory attributes in the beverage	Bravim et al. (2023)
Brazil	Conilon LB1	Whole fruits wet processed and SIAF for 72 h	*Leuconostoc mesenteroides, Lactiplantibacillus plantarum, Torulaspora delbrueckii, Saccharomyces cerevisiae* separately and in coculture	Inoculation with *L. mesenteroides* obtained the best sensory score and the beverage was characterized by notes of caramel, fruits, and spices	Cassimiro et al. (2023)
Brazil	Conilon K61	Whole fruits in the SIAF method for 120 h	*Meyerozyma guilliermondii* isolated from Conilon coffee	*M. guilliermondii* increased the score by over 3 points compared to spontaneous fermentation	do Rosário et al. (2023)

Some of these compounds are naturally present in fruits and, consequently, in beans, while others are produced by the metabolism of yeasts and bacteria during fermentation (Velásquez and Banchón, 2022). During fermentation, some of these microorganisms can generate volatile and non-volatile precursors, improving the sensorial quality of the beverage (Schwan et al., 2022).

Bacillus licheniformis inoculation had a positive impact on wet-processed Conilon coffee. The fermented coffee was correlated with the volatile compounds 5-methylfurfural, two-oxobutyl acetate, 1-furfurylpyrrole, and maltol that contributed sensorial notes of almonds, caramel, and chocolate (Bravim et al., 2023).

In another recent study with Conilon coffee, SIAF favored the development of *Leuconostoc mesenteroides* CCMA1105 and *Lactobacillus plantarum* CCMA 1067 (Cassimiro et al., 2023). Consequently, the production of lactic and acetic acids intensified, contributing to the beverage's acidity. Furthermore, some esters were detected only in the inoculated treatments, such as benzene acetic acid, ethyl ester; benzoic acid, 2-hydroxy, ethyl ester-; two-ethyl hexyl salicylate, and isopropyl myristate, contributing with sweet and fruity notes.

Regarding yeasts, *Saccharomyces cerevisiae* was inoculated in Conilon coffee (Agnoletti et al., 2022) and Robusta coffee (Prakash et al., 2022). In Conilon coffee, the different types of fermentation studied were discriminated by differences in the concentrations of chlorogenic acids, caffeine, γ-butyrolactone, lipids, sugars, and acetic acid. In Robusta coffee, the balance of volatile and non-volatile compounds in coffee inoculated with *S. cerevisiae* improved sensory attributes, contributing chocolate, sweet, fruity, caramel, and floral notes.

Meyerozyma caribbica CCMA 1738 and Pichia kluyveri CCMA 1743 were inoculated in dry-processed Conilon coffee (da Silva et al., 2021). Among the volatile compounds detected, the pyrazine class was predominant. Coffee inoculated with *M. caribbica* showed an increase of more than one point in the score given by trained and certified tasters. Furthermore, untrained tasters could also perceive the differences in the sensorial profile of this coffee, with fruity, almondy, chocolate, and nutty notes.

In another study, the comparison was made between the inoculation of *Meyerozyma guilliermondii* and spontaneous fermentation over 5 days in a closed bioreactor using the SIAF method for Conilon coffee (do Rosário et al., 2023). The findings revealed an elevation in the cup test score (>3 points) and a notable enhancement in the production of desirable volatile compounds (2-methyl pyrazine, furfuryl alcohol, 5-methyl furfural), leading to an improved perception of sweetness and sensory complexity in Canephora coffee.

These studies highlight the significant impact of the fermentation process and the inoculation of microorganisms on Canephora coffees' chemical composition and sensory profile. Thus, fermentation makes it possible to enhance and modify the flavor and aroma of the coffee, opening paths for innovation in the production process of unique Canephora coffees.

6 Conclusion

Understanding the microbiology and fermentation process is essential to produce high-quality coffees. The microbiota of Canephora coffee fruits plays a key role during fermentation, and different factors, such as origin, variety, and processing methods, influence it. The microorganisms naturally present in the fruit are responsible for producing volatile and non-volatile compounds that affect the flavor and aroma of the beverage, allowing the development of coffees with unique profiles.

In addition, the use of starter cultures and even coculture of yeasts and bacteria has shown promise in the fermentation of Canephora coffees. These factors contributed to producing volatile compounds, inhibiting undesirable microorganisms, and reducing fermentation time, resulting in beverages with different sensory attributes.

However, further studies on the microbiology and fermentation of Coffea canephora must be conducted to improve knowledge about the species. Thus, studies on the microbiota and selection of starter cultures are essential to understanding the dynamics of the fermentation process in Robusta and Conilon coffees, which will contribute to increasing the quality of the beverage.

Author contributions

PB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing. JC: Writing–original draft. PM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft. RS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing.

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