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Heat-stable Camel Milk-base Culture Medium Efficiency to Improve Microbiological Analysis of Raw Camel Milk

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ABSTRACT

The development of an optimal culture medium for microbiological analysis is a significant advancement in the field. The conventional PCA for RAMF enumeration may not accurately assess raw camel milk's microflora, potentially compromising food safety evaluations. Our study addresses this crucial issue. We developed CaM-PCA medium using sterilized whole camel milk and compared it with standard PCA using twenty-three raw milk samples. Enumeration results were analyzed using Mann-Whitney test and exclusive isolates were identified through 16S rDNA sequencing. CaM-PCA yielded significantly higher RAMF counts (8.50±0.45 log10 CFU ml-1) than PCA (8.20±0.60 log10 CFU ml-1), P=0.048. Three isolates exclusive to CaM-PCA were identified as *Metabacillus* sp., *Hafnia alvei* and *Enterobacter hormaechi* subsp. *hoffmannii*, demonstrating CaM-PCA's enhanced ability to detect spoilage and opportunistic pathogens in raw camel milk.

Key words: Bacterial isolation, Camel milk, Culture medium, *Enterobacter*, Food safety, *Hafnia alvei*, *Metabacillus*, Microbial enumeration, RAMF.

Raw camel milk has gained significant popularity as a functional and nutraceutical food beyond traditional arid regions due to its unique bioactive compounds and health benefits (Tidjani *et al*., 2025). This trend reflects increasing health consciousness among consumers and has expanded into new applications, including infant formulas for those with cow milk allergies (Ho *et al*., 2022).

Microbiological safety concerns arise from raw camel milk's diverse microbial flora, which contains both beneficial microorganisms and potential pathogens. These concerns are heightened by improper handling and poor hygiene, especially in developing countries where foodborne illnesses significantly impact public health (Tomar and Tiwari, 2024).

As global consumption increases, processing methods like pasteurization, microwave treatment and controlled fermentation are essential for ensuring safety and extending shelf life (Lankri *et al*., 2024). Standard microbiological assessment typically employs methods for enumerating re*via*ble mesophilic aerobic microflora (RAMF), such as total *via*ble count (TVC) or aerobic plate count (APC), with plate count agar (PCA) serving as the primary culture medium (Drici *et al*., 2023).

However, PCA may not effectively recover the complete range of cultivable microbes in raw camel milk, potentially underestimating microbial populations. Addressing this limitation, researchers have developed camel milk-plate count agar (CaM-PCA), an innovative medium designed to enhance accuracy, sensitivity and efficiency in RAMF enumeration and detection of specific bacterial species in raw camel milk.

This specialized culture medium offers an effective solution for improving microbiological assessment and enhancing safety control of raw camel milk as it continues to gain popularity as a nutraceutical dairy product with exceptional health properties.

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Raw camel milk sampling and preparation

Twenty-three raw camel milk samples were collected from food stores (n=15) and farms (n=8) in Southern Algeria in February-March 2022. This small sample size reflects challenges obtaining raw camel milk in the region, where production is seasonal, limited and mostly consumed

locally. Samples underwent immediate on-site California mastitis test and pH measurement before transport in cool boxes (6°C) within 20 minutes, following standard protocols to maintain sample integrity (Kroger 1985). Upon arrival at the Sciences and Environment research laboratory (SCIENV-C1810200, University of Tamanghasset), samples were subjected to pH remeasurement and microbiological analysis for RAMF enumeration to assess microbial load.

Sterilized milk-based culture medium CaM-PCA composition and preparation

Raw camel milk samples were tested for stability under microwave or autoclave sterilization at original pH or when adjusted to pH 7.1-7.2 with 0.1 M NaOH (Davies and White, 1966) The novel CaM-PCA medium was developed based on milk plate count agar (APHA and ISO 4833 standards), replacing skimmed milk with 1% (v/v) heat-stable camel milk (SP1). Composition included 5.00 g peptone water, 1.00 g glucose, 2.50 g yeast extract, 10 mL whole camel milk and 10 g agar per liter of distilled water. After boiling and complete dissolution, the medium was bottled, autoclaved (115°C or 121°C for 15 minutes), cooled to 50°C and poured into plates (14 mL/dish). Plates were dried inverted for 72 hours at 24°C before use.

RAMF spread plating and SP SDS culture methods

Standard surface spreading (Johns and Mcnabb, 1930) and SP-SDS (Thomas *et al*., 2015) methods were used to ensure that the RAMF enumeration was performed in duplicate through predried PCA and CaM-PCA Petri dishes. Tenfold serial dilution were performed from 10-1 to 10-6 for all raw camel milk samples and then 100 µL or 10 µL of each sample or serial dilution was plated or spotted on both PCA and CaM-PCA culture media. The readings were taken after 48 hours of incubation at 30°C and the results are expressed in colony-forming units/mL (CFU/mL). CFU/mL counts were obtained from plates and spots as duplicates of appropriate dilutions of each raw camel milk sample.

Data analysis

Statistical analysis of the RAMF count was performed with trial version 2024.2.0 of XLSTAT Premium. The RAMF data were converted to base-10 logarithms of CFU/mL of the raw camel milk samples (log10 CFU/mL). Then, accounting for the small sample size (twenty three samples), the data were tested for the normality parameter using the Shapiro Wilk test. Finally, because the data did not follow the normal distribution rules, a nonparametric Mann Whitney test at a significance level of 5% was applied to compare the two studied groups, with PCA and CaM-PCA culture media used as independent variables and the microbiological count used as the dependent variable.

Stability of raw camel milk after sterilization

The twenty-three raw camel milk samples collected, all four samples from SP1 maintained their fluid structure and homogeneous appearance after autoclaving (115°C or 121°C for 15 minutes) or microwave treatment, regardless of pH adjustment (Table 1). In contrast, the remaining nineteen samples (83%) lost structure in sterilization step, showing coagulation and whey separation. One sample from SP4 exhibited browning after heat treatment, indicating Maillard reaction occurrence. The poor heat stability observed in most samples aligns with previous research on camel milk proteins (Farah and Atkins, 1992; Alhaj *et al*., 2011; Ho *et al*., 2022; Zhang *et al*., 2023) . This instability stems from the absence of β-lactoglobulin and reduced κ-casein levels (only 5% of total casein versus 13.6% in bovine milk), leading to protein coagulation within 2-3 minutes at 120°C (Farah and Atkins, 1992). The browning observed in SP4 samples likely resulted from Maillard reactions between lactose, casein lysine and whey proteins (Mohamed *et al*., 2022; Zhao *et al*., 2023). The remarkable stability of SP1

Table 1: CMT and average pH on-site measurement and sterilization effects on the structure of the 23 fresh raw whole camel milk samples.

Sample origin (n)	Milking type	CMT	Average pH at the sampling point, Mean±SD	Camel milk structure after heat treatment			
				Program for microwave treatment at two successive power levels*		Autoclave at 115°C or 121°C for 15 minutes	
				At original pH	After adjustment to pH 7	At original pH	After adjustment to pH 7
SP1 (4)	Individual camel milk	Negative	6.60±0.12	NPP	NPP	NPP	NPP
SP2 (11)	Camel milk mixture	Negative	6.56±0.10	PP	NPP	PP	NPP
SP3 (4)	Camel milk mixture	Negative	6.52±0.09	PP	NPP	PP	NPP
SP4 (4)	Camel milk mixture	Negative	6.58±0.11	PP	NPP	NPPB	PPB

Abbreviations: CMT: California mastitis test.

Individual camel milk: Hand milked from a single female camel; Camel milk mixture: A mixture of camel milk obtained by hand milking from several female camels; NPP: No protein precipitation; NPPB: No protein precipitation and camel milk browning; PP: Protein precipitation; PPB: Protein precipitation with camel milk browning.

Notes:

* Cycle 1: 600 W/5 minutes, Cycle 2: 480 W/4 minutes.

samples (17%) represents a novel finding in camel milk heat treatment. This stability may be attributed to optimal κ-casein concentration, as adding 1-2 mg/mL κ-casein increases heat stability at pH 6.7-6.9 (Kappeler *et al*., 1998; Alhaj *et al*., 2011). Other factors may include protein compositions similar to cow milk, low lactose content, or the presence of heat stabilizers like phosphates or citrates (Mohamed *et al*., 2022; Zhao *et al*., 2023).

RAMF enumeration on PCA versus CaM-PCA culture media

All twenty-three raw camel milk samples showed higher colony counts on CaM-PCA than on PCA medium at the same dilution (Table 2). Nonparametric Mann-Whitney test revealed significantly higher RAMF counts ($p=0.048$, $\alpha=0.05$) on CaM-PCA (8.50 log10 CFU/mL ± 0.45) compared to PCA (8.20 log10 CFU/mL ± 0.60). Box plot analysis confirmed higher values with lower variation on CaM-PCA (SD=0.446) versus PCA (SD=0.595) (Fig 1). The CaM-PCA medium showed superior performance in enumerating microorganisms from raw camel milk, likely due to providing essential nutrients and growth factors specific to the camel milk ecosystem that are absent in conventional PCA. This parallels the development of milk plate count agar with 0.1% skimmed milk for dairy product analysis (Wehr and Frank, 2004).

Molecular identification of CaM-PCA specific isolates

Three bacterial isolates (CaM-T9, CaM-T13 and CaM-T20) were uniquely detected on CaM-PCA medium. Based on 16S rRNA sequence similarity using the EzBioCloud database, CaM-T13 (PQ260741) was assigned to *Hafnia alvei* (99.54% similarity), CaM-T20 (PQ260742) to *Enterobacter hormaechi* subsp. *hoffmannii* (99.65% similarity) and CaM-T9 (PQ260744) to the *Metabacillus* genus (98.84% similarity with *M. halosaccharovorans* and *M. schmidteae*) (Fig 2). The unique detection of *Metabacillus* in raw camel milk using CaM-PCA highlights its enhanced cultivation capabilities. Species of *Metabacillus* genus are typically found in extreme environments, likely entered the milk through environmental contamination. Their spore-forming nature raises food safety concerns as these spores can resist pasteurization (Patel and Gupta 2020). Similarly, the opportunistic pathogens *Hafnia alvei* and *Enterobacter hormaechi* subsp. *hoffmannii* indicates potential contamination through environmental sources, animal

Table 2: pH measurement and average $\log_{10}$CFU/mL RAMF enumeration on PCA and CaM-PCA culture media for the 23 fresh raw camel milk samples.

Sample number	Sample code	pH at the sampling point for each individual sample	Value in conventional PCA culture medium, Mean±SD	Value in the new CaM-PCA culture medium, Mean±SD
1	S1	6.62	6.99±0.09	8.15±0.01
2	S2	6.43	7.37±0.04	8.29±0.02
3	S3	6.70	7.51±0.05	8.22±0.01
4	S4	6.65	7.51±0.01	8.19±0.02
5	S5	6.63	8.07±0.27	8.35±0.07
6	S6	6.70	8.34±0.01	8.22±0.22
7	S7	6.45	8.13±0.21	8.21±0.13
8	S8	6.61	7.93±0.28	8.07±0.16
9	S9	6.52	7.82±0.88	8.02±0.51
10	S10	6.64	8.64±0.19	8.34±0.27
11	S11	6.61	8.45±0.03	8.49±0.02
12	S12	6.70	7.75±0.64	9.06±0.03
13	S13	6.47	8.34±0.48	8.65±0.25
14	S14	6.42	8.68±0.03	8.74±0.25
15	S15	6.46	7.25±0.07	7.50±0.26
16	S16	6.55	8.73±0.01	8.97±0.10
17	S17	6.43	8.50±0.04	8.72±0.35
18	S18	6.64	8.57±0.12	8.35±0.49
19	S19	6.47	8.43±0.07	8.61±0.13
20	S20	6.71	8.91±0.04	9.09±0.29
21	S21	6.62	8.66±0.11	9.01±0.44
22	S22	6.50	8.85±0.17	9.11±0.01
23	S23	6.43	8.86±0.06	8.92±0.20

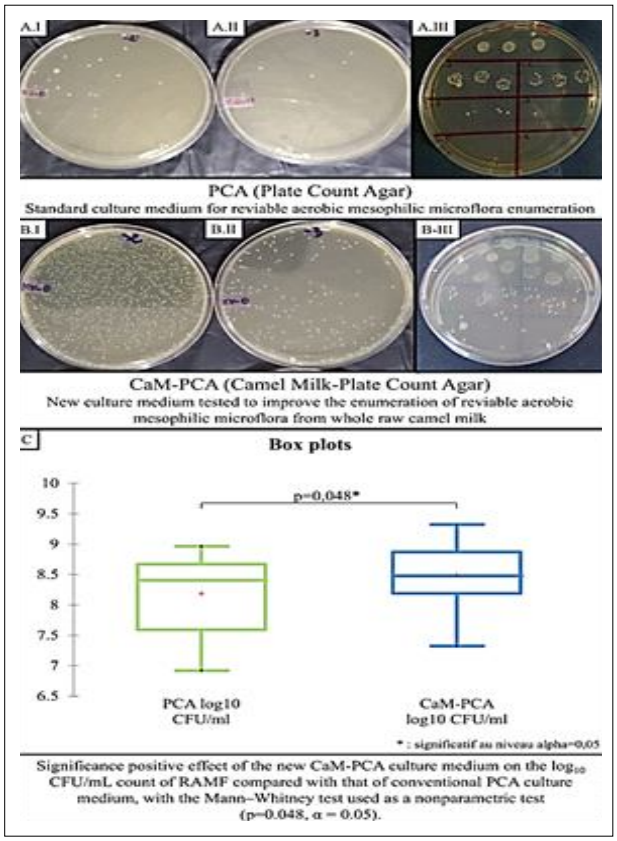

Fig 1: Enumeration of re*via*ble aerobic mesophilic microflora (RAMF) on PCA (A.I, A.II, A.III) and CaM-PCA (B.I, B.II, B.III) culture media *via* the spread method (A.I, A.II, B.I, B.II) or the SP SDS method (A.III, B.III) and statistical analysis *via* the Mann Whitney test as a nonparametric test with α = 0.05 as the significance level.

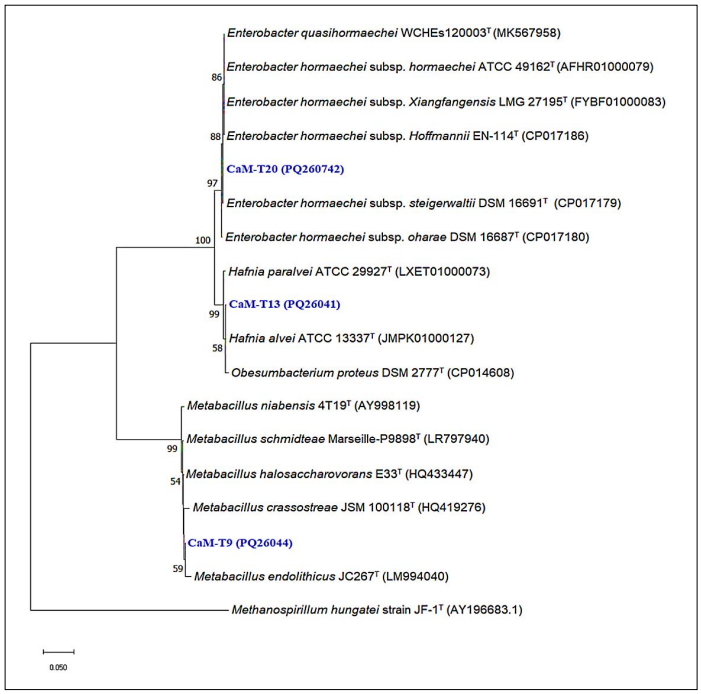

Fig 2: Evolutionary relationships of the isolates CaM-T9 (PQ260744), CaM-T13 (PQ260741) and CaM-T20 (PQ260742) with other species, determined *via* the neighbour-joining method in MEGA11.

contact, human handlers, or dairy equipment biofilms. The psychrotrophic nature of *Hafnia alvei* poses particular concerns for milk quality in cold storage (Tabla *et al*., 2016).

CONCLUSION

This study introduces CaM-PCA, an innovative culture medium that significantly outperforms conventional PCA for raw camel milk microbial analysis, successfully detecting previously unidentified bacterial species while improving RAMF count sensitivity. Future research should incorporate broader sampling strategies across diverse geographical regions over extended periods to strengthen statistical robustness and facilitate more thorough characterization of unique bacterial communities in camel milk. These advancements are crucial for preserving this nutritionally valuable food source for nomadic communities while establishing rigorous hygiene practices throughout the production chain as camel milk's popularity continues to grow globally.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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