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Chapter

Quality and Safety of Bovine Raw Milk: Present Challenges and Technological Solutions

Patricia Munsch-Alatossava and Tapani Alatossava

Abstract

The dairy, as an essential agricultural activity, plays a vital role in global food production systems. Irrespective of the regions or countries, milk producers are confronted with several and sometimes contradictory challenges: achieving food security, reaching economic profitability while responding to sustainable development goals. The quality and safety of raw milk are essential for the manufacture of dairy products. For the preservation of raw milk, two options are proposed by the FAO: cold storage or the activated lactoperoxidase system. Worldwide, mostly farmers attempt to "produce" raw milk for which the bacterial content does not exceed 100,000 (10^5) cfu/ml: due to the particularities of milk production and handling and numerous contamination risks, it is difficult to always reach this goal. Our group has evaluated an N_2 gas flushing-based technology, as a supplementary or alternative hurdle to prevent bacterial development, such as to preserve the quality and safety of raw milk during its storage and transportation from farms to processing sites. We discuss here its potential compared to other options.

Keywords: food security and quality, raw milk, spoilage, bacteria, psychrotrophs, antibacterial, N_2 gas flushing

1. Introduction

Like other agricultural sectors presently challenged by environmental constraints, the dairy sector is also pushed to move towards environmental sustainability and is urged to change practices.

Despite alarming predictions, the OECD-FAO outlook for the period 2018–2027 projects an increase by 22% of the world milk production; India and Pakistan are expected to jointly account for 32% of the global milk production; for Europe, the estimations of global exports of dairy commodities are in favour of 27–29% increase for the same time period [1]. In Africa, the consumption of milk and dairy products is also expected to increase due to the population and urbanisation increase and due to economic development [2].

If processed products, like cheese or butter, still dominate the consumption of dairy products in the developed world, fresh dairy products are mostly preferred in developing regions [1].

Dairy farms around the world still show a highly contrasted picture: milk is either produced in small holder farms, where it is mainly served for family

consumption, or in large modern dairy farms equipped with rotary milking parlour or milking robots designed for up to thousands of cows.

However, irrespective of the type of farming or the local environmental constraints, raw milk as a particularly rich media is highly perishable. The quality of raw milk largely determines the quality of products manufactured at the dairy; but, milk also constitutes a health issue if consumed raw, especially.

2. Composition, physico-chemical and antimicrobial properties of bovine raw milk

Milk is synthetised in the secretory cells of the alveolar epithelium (also called alveolar cells) and further secreted into the lumen, the core of the alveolus. Alveoli, the functional units of milk synthesis and secretion, are spherical bodies found only in the mammary gland, the unique organ of the mammals. The four mammary glands of the cow (female bovine) form the single anatomic unit called udder.

Milk is the first and essential food for the newborn of the mammal, and accordingly, milk needs to fulfil all its *nutritional* needs. These needs are varying among mammalian species and consequently, the composition of milk varies considerably in carbohydrate (mainly milk sugar called lactose, as energy source), protein (source of amino acids for protein synthesis), lipid (energy source and membrane components) and mineral contents (**Table 1**). For example, bovine (cow) milk has lower lactose and higher protein contents than human (mother's) milk. In addition, the milk composition varies during the lactation period and especially, during the first days that follow calving.

The nutritional properties are not only the important characteristics of milk, but additional *protective* and *regulatory* functions are of importance, too. The protective functions are related to the survival of the newborn in the presence of various environmental microbes. Bovine milk contains antimicrobial elements like leucocytes (somatic cells), immunoglobulins, lactoperoxidase, antiadhesive glycoconjugates (oligosaccharides linked to lipids and proteins) of milk fat globule membrane (MFGM) and sialic acid residues of oligosaccharides (**Table 1**). The protective functions also may include particular prebiotics like amino sugars of the oligosaccharides that contribute to establish the optimal microbiome in the gastro-intestinal track of the newborn. The milk components having regulatory functions include hormones (e.g. insulin, somatotropin, and growth hormone), regulatory proteins (e.g. cytokines), particular bioactive lipids, and membrane-enclosed extracellular vesicles (EV) like exosomes containing bioactive miRNAs and proteins [3, 4].

All these intrinsic components of milk are of crucial importance for the growth and development of the newborn. The balance and the spectrum of the milk components are unique for each mammalian species. Because the cow (*Bos taurus*) is globally and economically the most important dairy husbandry animal, the knowledge on the bovine raw milk has the most significant impact on the dairy industry.

2.1 Sources of microbial contaminations in fresh raw milk

Raw milk is widely considered as sterile in the lumen of the alveolus in the case of a healthy cow; bacteria may be however transmitted to milk via the cow's blood in case of systemic infection. The intrinsic features of raw milk and its handling favour the presence and growth of many microbes; consequently, various viruses, moulds, yeasts, and especially bacteria take advantage of raw milk production conditions to either persist or proliferate. Bacteria by exhibiting contrasted roles in raw milk can be truly categorised as "good, bad or ugly": for ages, some are key

Principal components	Relative amounts
Water	87% (79% free, 8% bound in casein micelles)
Carbohydrates	**4.6%**
Lactose	4.6
Oligosaccharides	Traces
Lipids	**4.1%**
Neutral lipids	4.0
Polar lipids	0.05
Proteins	**3.3%**
Caseins	2.6
Whey proteins	0.6
MFGM-associated proteins	0.07
Minerals	**0.7%**
K	0.15
Ca	0.13
Cl	0.11
Phosphates	0.09
Na	0.04
Other organic compounds	**0.32%**
Citrate/citric acid	160 mg per 100 g
NPN (non-protein nitrogen)	60 mg per 100 g
Gases	
CO_2/bicarbonate	10 mg per 100 g
N_2	1.6 mg per 100 g
O_2 (fresh-cold stored)	0.15–0.6 mg per 100 g
Major redox systems	
Dehydro/ascorbic acid (C vitamin)	2.0 mg per 100 g (E_0 + 0.07 V)
Riboflavin (B2 vitamin)	0.2 mg per 100 g (E_0–0.20 V)
Milk redox potential:	
Fresh raw milk (air free)	E_h + 0.05 V
Cold stored raw milk (air dissolved)	E_h + 0.20 – +0.30 V
Major antimicrobial elements	
Leucocytes (somatic cells)	Average 150,000 cells per ml
Immunoglobulins	0.08% (77% IgG, 17% IgA, 6% IgM)
Lactoperoxidase (with SCN^- and H_2O_2)	
MFGM-associated glycoconjugates[b, c]	
Sialic acid residues of oligosaccharides[c]	

[a]*Most presented data is collected from [5].*
[b]*Ref. [6].*
[c]*Ref. [7].*

Table 1.
Some characteristics of bovine raw milk[a].

agents in the manufacture of numerous milk-based products reflecting traditions and cultures around the world; others are involved in the spoilage of raw milk and dairy products; finally, some are authentic pathogens causing severe illnesses, which have largely contributed to build the reputation that raw milk is a vehicle for spreading diseases.

Depending on the ambient conditions, the farming practices or the health of the animals, various contamination sources raise the bacterial load in raw milk. The sources of bacterial contaminations can be categorised as such:

i. The cow's udder: the teat surface can present a quite highly diverse bacterial population. Milk ducts of the udder carry epithelium-adhering commensals (like streptococci, staphylococci and micrococci), and possibly pathogens from with and within the udder. Three categories of bacterial pathogens can be distinguished: human pathogens, such as Mycobacteria or *Brucella*, which originate from within or outside the udder; others like *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Escherichia coli*, causing bovine mastitis, can also enter raw milk; finally, direct or indirect faecal contaminations lead to the addition of *Salmonella* or *Campylobacter* to raw milk.

ii. The air and soil: these factors impact differently on whether the animals are fed in- or out-doors.

iii. The farmer or the milk handler: the absence of good hygiene practices favours the distribution of contaminants and their entrance in milk. The level of contaminations promoted by a farmer or a milk handler depends on how close is the contact with raw milk: this aspect is seriously considered that as some carriers of *Salmonella* are prevented to handle milk in dairies.

iv. Water and beddings: when employed for milk production, water should be of high microbiological quality as either pathogenic or saprophytic bacteria present in water can contaminate raw milk. Several reports highlighted the crucial role of water as a major source of pseudomonal raw milk contamination [8–10]. Beddings materials may, especially at winter, carry high bacterial loads (10^8–10^{10} cfu/g), which may contaminate teats [11].

v. The dairy equipment: in the case of insufficient cleaning and disinfection, milk cans, tanks, milking machines, and pipelines constitute major contamination sources [11]. The design of certain components presenting "dead ends" constitutes ideal shelters for the settlement of biofilms: milk residues aggregate with bacteria in difficult to clean areas, detach time after time from the surface and hence raise the bacterial load in raw milk.

Due to numerous contamination sources, a rather diverse microbiota is present in raw milk; many Gram (+) or Gram (−) bacterial representatives can be present in significant numbers; their relative importance is variable and greatly depends on the elapsed time since milking. Gram (+) dominates in fresh raw milk, whereas Gram (−) takes over after cold storage [11]. If it is possible to relate high levels of coliforms to faecal contaminations, it may be more difficult to trace the source of the ubiquitous pseudomonads.

Many reports often highlight multiple, sometimes similar, contamination sources.

For example, in Brazil, the difficulties for farmers to fulfil the goals set by the Ministry of Agriculture were attributed to several causes: at first, most dairy farms

had their water contaminated with coliforms [12]. A water of poor quality combined to poor hygienic conditions (for example, in the case of insufficient cleaning of tanks) raise the contamination of raw milk; the authors also mentioned insufficient training of the farmers, which resulted in a not regular cleaning of the udders before and after milking; moreover, a majority of farmers did not systematically control mastitis in their animals [12].

In Tanzania, which belongs to the East African Community Countries (EACC) organisation, two bacteriological criteria were defined for total bacterial counts (TBCs) and for total coliform counts (TCCs) [13]: grade I, II and III raw milk is characterised by total bacterial counts below 2.10^5, between 2.10^5 and 10^6, and between 10^6 and 2.10^6 cfu/ml, respectively; raw milk of very good and good quality is characterised by coliform counts below 10^3, and between 10^3 and 5.10^4 cfu/ml, respectively. Three major factors, that impacted milk quality and caused milk-borne diseases, were identified: the doubtful health status of animals, the lack of good milking and handling practices, and the distribution, which occurs out of relevant regulations [2].

In Western African countries (Burkina Fasso, Mali, and Senegal), a campaign entitled "My milk is local" aims to replace the large consumption of imported milk powders and urges local farmers to respond to increasing demands. Producers, mainly organised as small holders, suffer to meet the challenge of producing raw milk of sufficient microbiological quality. A study reported that over 75% of raw milk samples exhibited excessive bacterial counts, with an average of 4.5×10^7 cfu/g of raw milk; the poor microbiological quality of raw milk at the farm level was due to contaminations resulting from a lack of adequate equipment and facilities, of good hygiene practices along the collection and processing steps [14].

Raw milk is frequently identified as a source of food-borne disease outbreaks. However, the consumption of raw milk continues in low-income countries because of traditions and lack of processing facilities; in high-income countries, the consumption of raw milk is encouraged by certain lobbies and life style groups for health benefit claims that vary from superior nutritional properties, lower allergenicity, reduced lactose intolerance or more efficient antimicrobial systems. In practice, the use of raw milk remains marginal, and fortunately, the consumption of milk mostly relies on processed dairy products.

3. Bacteriological quality criteria for industrial processes

The importance of bacteria in raw milk, as in other food products, is reflected by the fact that the microbiological quality criterion is a "bacteriological criteria".

Preserving and controlling the quality of raw milk is a worldwide concern and is reflected by a "common" criteria of total bacterial counts (TBCs) around 100,000 (10^5) cfu/ml (**Figure 1**).

In the European Union, the directive No 853/2004 defines "Raw milk" as the secretion of the mammary gland of farmed animals that has not been subjected to temperature above 40°C, or undergone "any treatment that has an equivalent effect" [15]. The directive also indicates that the milk, must be "cooled immediately" to not more than 8°C in case of daily collection, or not more than 6°C, if the collection does not occur daily. For cow milk, the bacteriological standard should be lower or equal to 100,000 cfu/ml (determined from the rolling geometric average over a 2-month period with at least two samples per month); for food business operators, the bacteriological level should be below or at 300,000 cfu/ml (**Figure 1**).

In Finland, for example, regarding the criteria for raw milk quality, three classes are defined depending on the bacterial load at farm level: E (excellent), I (first

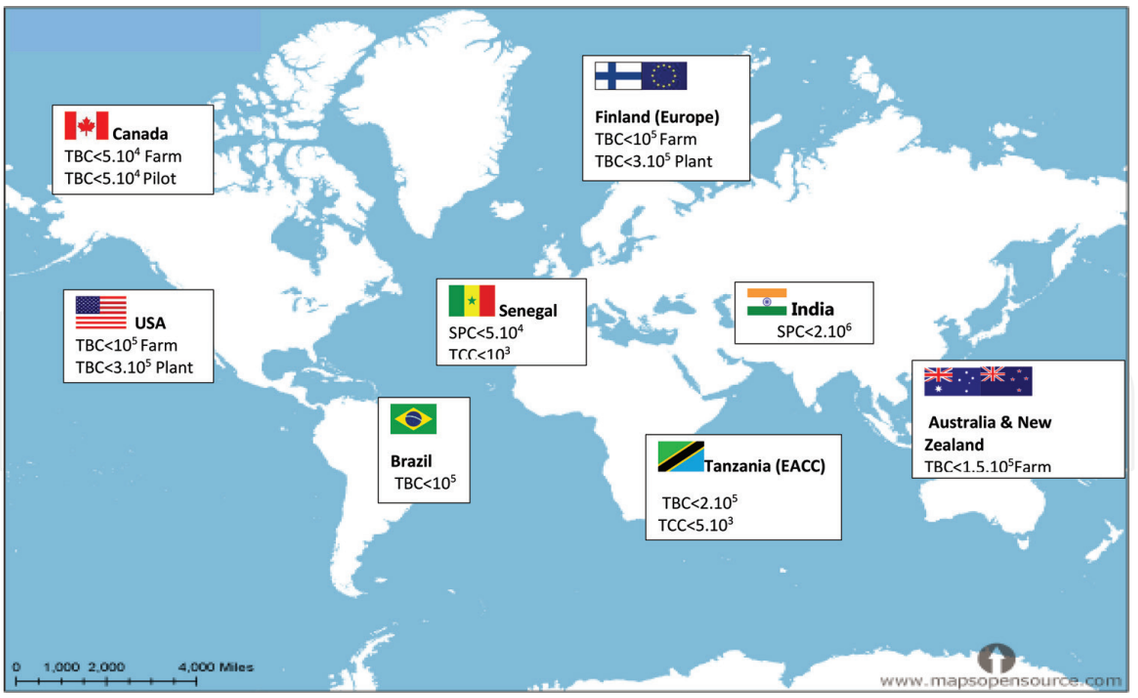

Figure 1.
The about 10^5 cfu/ml "Total bacterial counts in raw milk" world challenge [12–15, 26, 27]. Note: TBC, total bacteria counts; TCC, total coliform counts; SPC, Standard Plate Count.

class) and II (non-acceptable for dairy processes), for bacterial counts <50,000, ranging between 50 and 100,000, and exceeding 100,000 cfu/ml, respectively. In 2017, the bacterial content was on average of 5200 cfu/ml (equivalent to 3.71 $\log_{10}$ units) at farm level [16].

The microbiological criteria rely on a culture-based reference method, SPC, which accounts for Standard Plate Count agar method. Total colony forming units of bacteria, eventual yeast and moulds are determined per ml or g of milk, after 32°C for 48 h or 30°C for 72 h incubation: the so-called pour-method is recommended for the analyses [17]. Concerning bacteria, particularly viable mesophiles, aerobes and facultative anaerobes can be enumerated.

As for any method of analyses, drawbacks exist concerning the SPC method:

- The method is time-consuming as the bacterial load is only revealed after 2–3 days incubation (to overcome the time obstacle, the dairy industry uses a particle counting-based method).

- The results are method-dependent: as observed with raw milk samples, the spread-method (another common method employed for microbiological analyses) yields a slightly higher amount of colonies despite the fact that the pour-method supports growth on and within the agar; based on some analyses of raw milk samples, the spread-method yielded an average of 0.17 log unit more bacterial colonies than the pour-method ([18] and unpublished data): for example, if the pour-method would yield 1000 bacteria/ml, the spread-method would enable to enumerate 1500 bacteria/ml, for the same raw milk sample.

- The analyses may lead to underestimated bacterial contents as a large fraction of bacteria are not cultivable [19], and common bacteria can enter a viable but not cultivable (VBNC) stage [20].

- The plating result does not inform about the presence of pathogens.

- The SPC method also ignores important bacterial groups, when considering further treatments to which raw milk is subjected: the level of Gram (+) bacterial types, such as *Bacillus*, *Paenibacillus*, and *Clostridium* present as spores in raw milk, can only be revealed after a preliminary heat treatment of the raw milk samples. Hence, key spoilage bacteria that limit the shelf life of HTST milk [21, 22] are not considered by SPC.

Recently, high-throughput DNA sequencing or molecular barcoding approaches, as non-culture-based methods, were also applied to raw milk, and allowed a more accurate estimation of microbial/bacterial diversity in samples [23, 24].

However, the difficulties and costs for sampling and testing food materials also apply to raw milk testing: in USA, it was already estimated that one analysis of a cost of 5 $ (US dollars) would result in an annual cost of 150 million $, if performed daily at the farm level; similarly, if every milk tanker would be tested at the processing plant for one microbial agent, this would lead to a cost of 21 millions $ [25]. With such costs, it seems impossible to identify all risks for raw milk.

4. Effects of the storage time and temperature on bacterial growth in raw milk

4.1 Importance of low temperature

Irrespective the ecosystem, the temperature is a key determining factor of bacterial growth. Low temperature was implemented to preserve the quality of raw milk until the processing stage. When the number of dairy plants was reduced, the raw milk had to be transported over longer distances from farms to dairies. Longer distances result in increased time that elapses between milking and processing stage.

Milk, which leaves the udder, is at a temperature of about 35°C: a study that evaluated the impact of the storage temperature on bacterial growth in raw milk showed that high temperatures promoted rapid and intense bacterial growth. Importantly, the study also illustrated the "time limited effect" of cold storage at 5°C, as the bacterial growth was only inhibited for 36 h, after which a moderate increase was noticed [28]. Some reports mention "the critical age" (the time after which bacterial growth is observed) to be slightly above 48 h [29]: these variations may reflect differences in initial bacterial levels, in bacterial diversity, or in variable levels of the natural antimicrobial systems present in raw milk.

The temperature value, even at low temperature range, is of crucial importance: an illustration can be seen with the 12 raw milk samples considered in experiments I and II, listed in Table 1 [30]; for I and II, the initial average counts in log-units were 3.9 and 4.03, respectively; it can be observed that 4 days cold storage at 4 and 6°C, respectively, yielded bacterial counts of 6.4 and 7.8 log-units, respectively; the 2° shift showed an about 1.4 log-units (equivalent to a factor of 25) higher bacterial level at 6 compared to 4°C. A 2 log-units (a factor of 100) difference in psychrotrophic *Pseudomonas* levels was also reported by another study that compared optimal (4°C) and suboptimal (6°C) storage temperatures [31].

4.2 Consequences of cold storage

At bacterial population level, cold storage results in the replacement of Gram (+) by Gram (−) bacteria [11]. DGGE-based studies first highlighted that bacterial diversity in raw milk decreased during cold storage [32–34]. That cold storage-impacted bacterial diversity in raw milk was also evidenced by the determination of the amount of

Operational Taxonomic Units (OTUs) in initial and cold-stored raw milk samples: for example, after 3–4 days at 6°C, only 33% of the initial OTUs were recovered in our studies and some bacterial types did not survive the low-temperature storage condition [24].

5. Spoilage features of psychrotrophs

The reputation of psychrotrophs as key spoiling bacteria in cold-stored raw milk is extensively documented, due to their production of various enzymes, which can degrade the major milk constituents. The heat treatments, such as pasteurisation or UHT, which target bacterial populations do not affect much the hydrolytic enzymes, characterised by remarkable heat stability. Recently, the heat stability of proteases, lipases and phospholipases from selected raw milk isolates was determined in one study: after 142°C for 4 s, *Acinetobacter* frequently showed remaining lipase and phospholipase activities, whereas *Pseudomonas* exhibited highest protease activities [35].

Consequently, the spoilage is not limited to raw milk but occurs also at the level of milk-derived products: various defects or technological failures were linked to enzymatic activities [36]. Psychrotrophs and their enzymes significantly impact the quality of dairy products, which implies economic consequences.

If proteases and lipases received major attention, less studies describe other enzymatic types; however, the production of phospholipase C (PLC) was evidenced for key spoiling genera such as *Pseudomonas* and *Bacillus*. PLC causes the disruption of the milk fat globule membrane (MFGM) and is also described as a heat stable enzyme [35–37]. In our recent investigations concerning phospholipids, by a lipidomics-based approach, we also observed that PLC production was a common feature of psychrotrophic bacteria; but, consequent to bacterial growth in raw milk during its cold storage, we also evidenced the presence of various types of bacterial phospholipases that promoted hydrolysis of phosphatidylcholine, sphingomyelin, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine species, together with an increase of phosphatidic acid; the changes imply the implication of phospholipases C, A, D and sphingomyelinase C activities, and show that phospholipolysis in raw milk involves many enzymatic types [38]. The analyses also revealed the presence of various lysophospholipids (LPLs) (resulting from PLA activity) in cold-stored raw milk: the fact that numerous reports described multiple physiological or pathophysiological roles of LPLs [39, 40] calls for further investigations on the significance of LPLs in cold-stored raw milk.

Like for the producing bacteria, only considered as problematic when associated to technological failures, the enzymes synthesised by these bacteria are also mainly considered under a technological point of view and to a lesser extent on their eventual impact on human health.

6. Antibiotic resistance of psychrotrophs

Earlier observations showed that bacterial isolates, retrieved from raw milk samples that apparently spent a longer time in cold storage, also exhibited higher antibiotic resistance (AR) or multi AR features [41]. We also observed that psychrotrophic bacterial populations are more risky in terms of AR compared to their corresponding mesophiles [30].

Recently, a study that evaluated the efficiency of the activated lactoperoxidase system (LPS) and N_2 gas flushing to hinder bacterial growth in raw milk showed that N_2 seemed to favour a more diverse bacterial community at 6°C, less heavily loaded with antibiotic multi-resistance features, compared to LPS [42].

Numerous reports pointed *Pseudomonas* and *Acinetobacter* as key genera associated with raw milk and spoilage of dairy products [11, 24, 35, 43–45]. The WHO, which considers that AR is nowadays one of the highest threats to global health, to food security and development, has ranked the bacterial species *Pseudomonas aeruginosa* and *Acinetobacter baumannii* as critical priorities regarding AR [46].

7. Methods to inhibit or inactivate raw milk-associated bacteria

Chemical (addition of CO_2, considered as safe), biochemical (the activated lactoperoxidase system, LPS) or physical (HHP, UHPH and LTP)-based treatments are presently in use or still under evaluation (**Table 2**).

LPS is recommended by the FAO, where economic or technical constraints prevent the use of cooling facilities: following the addition of SCN^- and H_2O_2, the shelf life of raw milk can be extended for 7–8 h under tropical conditions [47, 48].

Method/some features and applications		Advantages	Disadvantages	References
Activated LPS system	Addition of SCN^- and H_2O_2	• Raw milk is kept safe where no cold chain facilities exist	• Requests the addition of chemicals • Time-limited effect	[47, 48]
CO_2	Treatment of raw milk	• Inhibition of bacterial growth for 7 d at 4°C; no pathogen enriched		[49]
	Treatment of raw milk: impact on UHT milk	• Physicochemical and microbiological quality preserved • Proteolysis in UHT milk is delayed	• pH decrease (0.55 pH-unit difference between control and treatment)	[50]
High hydrostatic pressure (HHP) technology	• 100–1200 MPa	• Effective to inactivate microorganisms and food-borne pathogens	• Does not inactivate enzymes and spore	[51]
	• 300 MPa	• Coagulation properties of milk enhanced	• Cheese making properties altered	[52]
Ultra-high pressure homogenisation (UHPH)	• 100–300 MPa	• Equivalent to high pasteurisation 90°C/15 s • Shelf life of 14/18 d	• Physico-chemical changes	[53]
	• 400–600 MPa /800 MPa	• Inactivation of microorganisms and pathogens • Inactivation of enzymes	• Bacteria can recover • Increased level of FFA • Decrease of casein micelle size • Denaturation of whey proteins	[54]
Low temperature plasma (LTP) system	• Whole, semi and skimmed milk spiked with *E. coli*	• Reduction of bacteria by over 4 log-units (cfu/ml) within 20 min		[55]
	• 15 ml raw milk/ treatment for 20 min	• No change for the lipid composition	• Some changes with volatile compounds and increase of total aldehydes	[56]

Table 2.
Methods to tackle bacteria in raw milk.

8. A novel approach for raw milk storage: N_2 gas flushing technology

Two major observations were at the basis of the search and testing of a novel approach to better preserve the quality and safety of raw milk. In low-income countries, considerable amounts of milk are adulterated by the use of various chemicals including antibiotics to inhibit bacterial growth. On the other hand, in high-income countries, it is well known that psychrotrophs mainly considered as benign in their majority are causing significant spoilage of raw milk; our group also observed that these bacteria are heavily loaded with AR determinants.

N_2 gas, a non-finite resource and considered as chemically inert, was therefore tested at laboratory and pilot plant scales [57–59]. The so far established benefits, recorded from raw and pasteurised milk samples and from the treatment of some pure bacterial strains, are summarised in **Figure 2**.

For low- or high-income countries, the N_2 gas flushing technology presents indisputably multiple advantages considering bacteriological, biochemical, technological and nutritional aspects for the preservation of the quality and safety of raw milk.

The N_2 gas flushing technology has been recently granted a patent by the European Patent Office [63]. Future studies should consider the optimisation of the treatments and the completion of the further steps to render N_2 gas flushing technology as fully sustainable at large scale.

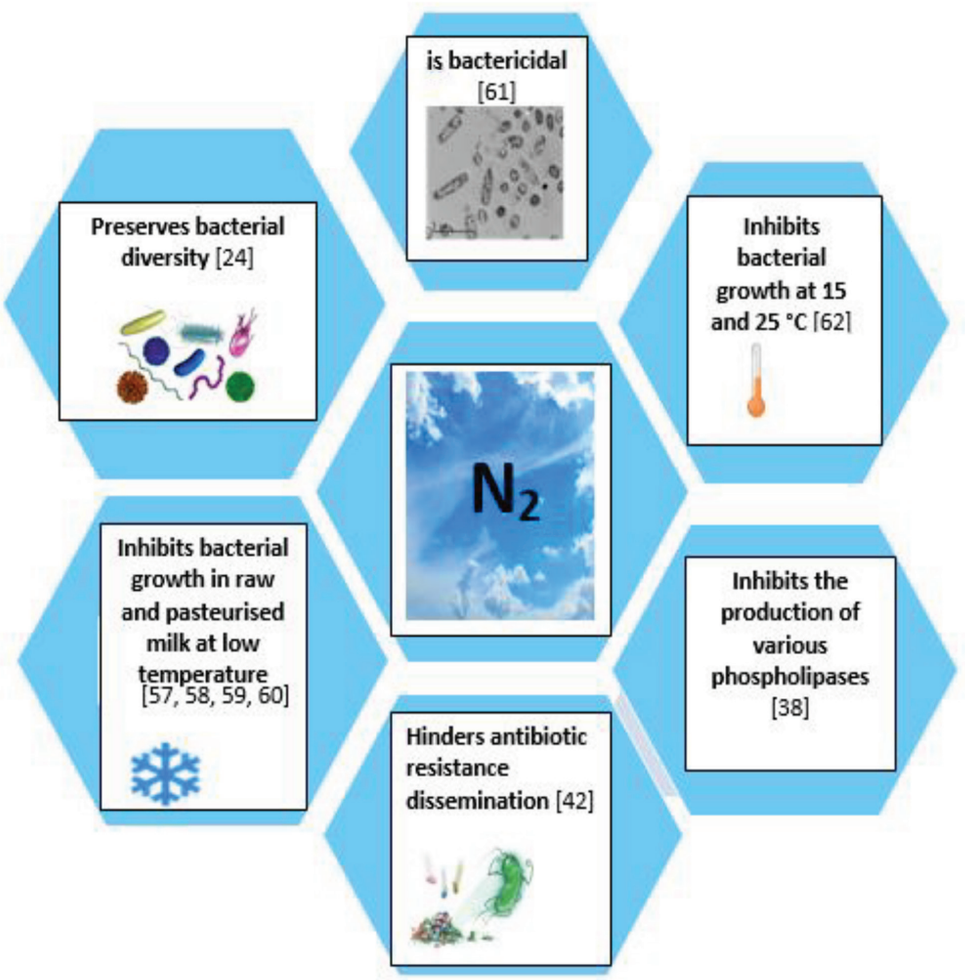

Figure 2.
Impact of N_2 flushing on raw and pasteurised milk-associated bacterial populations, and on some pure strains [24, 38, 42, 57–62].

9. Conclusions

More ancient and also recently conceived strategies that aim to reduce/eliminate bacterial populations in food materials including raw milk, are based on treatments, which are applied when the bacterial level reaches a certain threshold value: around 100,000 (10^5) cfu/ml for raw milk, which was first applied by countries having cold chain facilities; but nowadays, this threshold value is also targeted by many other countries. Depending on the production site, this goal may be difficult to reach.

The N_2 gas flushing of raw milk was initially conceived to be applied at earliest possible in farms until the processing site as an additional hurdle to cold storage, such as to preserve the initial microbiological, biochemical and nutritional features of raw milk along the cold chain of raw milk storage and transportation, before its transformation. The recent observation that N_2 gas flushing was about equivalently inhibitory of bacterial growth in raw milk at milder temperatures (15 and 25°C) compared to LPS [62], offers further perspectives for the method and especially as a replacement of numerous adulterating substances, including antibiotics, added to raw milk.

Strategies that aim to limit or control the bacterial load in raw milk should be designed to simultaneously dispel technological risks and consider human health risks: in a world that struggles with superbugs, it is reasonable to constantly evaluate practices on whether they respond at best to global challenges and interests.

N_2 gas that constitutes 78% of our atmosphere is an unlimited resource. The N_2 gas flushing technology, designed for an "open system" and successfully tested at pilot plant scale, when finalised, would simply "borrow" the gas from the atmosphere. By considering the *Sustainable Development Goals* set up by the United Nations (Agenda 2030) [64], the N_2-based treatment contributes to the achievement of several objectives: by tackling food spoilage, there are perspectives to reduce poverty, improve food security and nutrition, ensure better health, while promoting sustainable economic growth.

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