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The Potential Benefits of N_2 Gas Flushing Technology for Various Dairy Products: A Sustainable Approach That Proved to Be Multiadvantageous for Preserving the Quality and Safety of Raw Milk During Its Storage

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Worldwide, food production systems are confronted with multifaceted challenges. In the context of global climate change, the necessity to feed an expanding population while addressing food insecurity and reducing the tremendous losses and wastage of food places all production steps under considerable pressure. In this context, dairies also face extensive pressure to reduce greenhouse gas emissions, wastewater, and sludge; here, as elsewhere, innovative technological solutions must meet sustainable criteria. To preserve the quality and safety of raw milk during its storage, N_2 gas flushing technology was devised and implemented at laboratory and pilot plant scales: the treatment proved to be multiadvantageous considering microbiological, biochemical, and technological aspects. The proposed study aims to reconsider the benefits of the patented N_2 flushing technology, applied at the "raw milk stage" and evaluate the potential advantages that the treatment would confer, in terms of quality and safety aspects, to various dairy products such as liquid milk products, butters, creams, ice creams, and cheeses, including local and traditional dairy products.

Keywords: sustainability, quality and safety, raw milk, dairy products, N_2 gas flushing technology

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DAIRY SECTOR AND SUSTAINABILITY ISSUES

Milk and dairy products, often associated with specific regions, are the outcome of centuries of traditions and also the result of formidable technological advancement; dairy products constitute a rich source of essential nutrients ensuring a healthy and nutritious diet while contributing to global food security.

Over the projection period of 2020–2029, an annual growth of 1.6% is predicted in global milk production, which would exceed most other main agricultural commodities, with India and Pakistan accounting for more than 30% of the production in 2029; if, for the European Union, the production is expected to grow more slowly than the world average, the predictions indicate that the EU, as the second largest milk producer, will continue to be the main world cheese exporter (OECD/FAO, 2020). According to some authors, the demand for dairy products and new technologies is expected to increase as, compared to poultry and some plant products, less land is needed to produce an equivalent amount of edible milk protein; also, in developing countries, migration from rural to urban regions leads to increased consumption of dairy products (Britt et al., 2018). On the other hand, dairies are challenged by recent changes in consumer preferences for more "green products"; if the growth rates of plant-based dairy substitutes are strong, conflicting views exist regarding their environmental impact and their relative health benefits; consequently, there is uncertainty with respect to their long-term effect on dairy demand (OECD/FAO, 2020).

Similar to other food-producing systems, dairies are also challenged by climate change which already leads to more frequent drought or flood events resulting in milk yield variability and price volatility (Britt et al., 2018; OECD/FAO, 2020). In addition to environmental considerations, moderate or severe food insecurity affects ∼2 billion people in the world; on the other hand, approximately one-third of all the food produced for human consumption is lost or wasted every year; in Europe alone, ∼20% of dairy products equivalent to 29 million tons are concerned by the loss or wastage (FAO, 2015; FAO et al., 2020). Indisputably, higher environmental constraints lead to a situation where every production stage must implement sustainable criteria.

Between 2005 and 2015, greenhouse gas (GHG) emissions from dairy farms increased by 18% when milk production increased by 30%; however, over the same period, GHG/kg of milk declined by ∼11% due to better management and improved animal productivity (FAO and GDP, 2019). In 2015, the UN-Paris Climate Change Conference requested that all sectors undertake urgent actions on climate change. Some authors underlined that great care should be given to decisions, as in the case of dairy products, the carbon footprint (CF) was shown to be dependent on product types and production systems. For example, milk produced in a pasture-based system had an 18% lower CF compared to a semiconfinement system; similarly, cheese produced from milk, which originated from a pasture-based system, presented an 11% lower CF compared to a semiconfined system (Vergé et al., 2013; Laca et al., 2020). Since the 1960s, the global temperature has continuously increased, and most forecasts expect continuity in the increase; if climate change-triggered disorders are already impacting food production systems, even more severe weather incidents such as excessive rainfall or longer drought periods are expected to occur (Britt et al., 2018). The food sector is also well known for being one of the highest water consumptions per unit of production; this ascertainment also characterizes the dairy sector, where cleaning-in-place (CIP) and pasteurization units are particularly demanding; stringent hygiene conditions, prevailing in dairies, also imply the production of considerable amounts of wastewater (Rad and Lewis, 2014; Boguniewicz-Zablocka et al., 2019).

In the process of reducing GHG emissions, and water and energy consumption while contributing to guaranteeing food security, the dairy sector clearly faces many simultaneous challenges. Given the complexity of the issues with conflicting constraints, new technological solutions with limited environmental impact are needed.

MILK, ORIGINALLY A RAW FOOD MATERIAL

Storage Conditions

It is trivial to affirm that raw milk of good microbiological quality guarantees obtaining dairy products of good quality, and "poor quality" milk at the farm level cannot be improved in the dairy: this point is, for example, illustrated by the study of Sorhaug and Stepaniak (1997), which showed that raw milk having supported the growth of 5.5 log cfu/ml psychrotrophic bacteria led to gelation of UHT milk. For preserving raw milk, refrigeration and the activation of the lactoperoxidase system are the two means approved by the FAO-WHO Codex Alimentarius with the precision that "cooling to 4°C maintains the original quality of the milk and is the method of choice for ensuring good-quality of milk for processing and consumption" (FAO, 2021). A previous report indicated that "cooling of fresh raw milk to below 4°C as soon as possible after milking, and within 3–4 h at most, is recognized as the best way of preserving quality and avoiding spoilage" (FAO, 2016). These recommendations are interpreted as shown in **Table 1**.

Investigations on bacterial multiplication in raw milk during storage highlighted the crucial importance of the temperature factor and the combination of temperature/time factors, as even low temperatures can promote significant qualitative and quantitative changes in bacterial populations (Chambers, 2002; Lafarge et al., 2004). In the absence of cold chain conditions, raw milk storage is even more complicated: for example, in many African countries, high ambient temperatures or an erratic power supply render the storage of raw milk problematic, with implications on milk safety even after pasteurization and pathogens such as *Mycobacterium bovis*, *Brucella abortus*, and *Coxiella burnetti* are still of concern in the African dairy chain (Owusu-Kwarteng et al., 2020).

Raw Milk Consumption Is Not Discouraged by the Eventual Presence of Pathogens

Between 2007 and 2015, the global burden of foodborne diseases reached 600 million cases with 420,000 turning fatal (WHO, 2015). In the WHO European region, it was evaluated that in 2010 more than 23 million people fell ill after eating contaminated food, resulting in an estimated 4,654 deaths (WHO, 2017a). To draw attention to the problem, the WHO (2021) initiated a "world food safety day" on the 7th of June. For 2019, EFSA (2021) reported 5175 food-borne outbreaks leading to 49,463 cases of illness, 3,859 hospitalizations, and 60 deaths (an increase of 50% compared to 2018 for the latter); milk and cheese were incriminated in 9 and 4 outbreaks, respectively, whereas other dairy products caused 4 outbreaks. Notably, the data for strong-evidenced outbreaks were provided at 75% by only four countries (France, Italy, Poland, and Spain).

The bovine raw milk microbiota is very diverse, and bacteria are categorized as beneficial, pathogenic or associated with

TABLE 1 | Cold storage conditions of raw milk: some examples.

Countries/Region	Temperature/time combinations	References
USA	Grade A raw milk: "cooled to 10°C or less within 4 h or less of the commencement of the first milking; cooled to 7°C or less within 2 h after the completion of milking, provided that the blend temperature after the first milking and subsequent milking does not exceed 10°C"; "all raw milk shall be maintained at 7°C or less until processed…"	FDA (2017)
Australia	"milk should be cooled to 5°C within 3.5 h from the start of the milking"; "other time/temperature combinations are allowed provided the necessary risk assessments are completed by the manufacturer"	Australian Government (2019)
India	Transportation of milk from the collection center to milk processing unit: "raw milk shall be transported from VLC to MCC/BMC/processing unit as applicable within 4 h of milking and it shall be cooled as soon as practicable to a temperature of 5°C or below"	FSSAI (2018)
Europe		
*European Parliament regulation	"milk must be cooled immediately to not more than 8°C in the case of daily collection, or not more than 6°C if collection is not daily" "during transport, the cold chain must be maintained and, on arrival at the establishment of destination, the temperature of the milk… must not be more than 10°C."	EC (2004)
*European Dairy Association (EDA)	"milk cooled to no more than 6°C and kept at that temperature until processing"; "higher temperatures are allowed under specific conditions: right after milking or for technological reasons"	EDA (2020)

**symbol served for the listing of different points.*

spoilage; the presence of authentic pathogens such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, and Shiga toxin-producing *E. coli* (STEC) was reported in milk bulk tanks (Oliver et al., 2005; Quigley et al., 2013; Jaakkonen et al., 2017). The increasing trend to consume more "natural" or minimally processed food also concerns milk and dairy products. Whitehead and Lake (2018) emphasized lower infections and allergic disorders as health benefits when consuming fresh unprocessed milk. Conversely, Costard et al. (2017) underlined that outbreaks due to dairy products in the USA increased over the 15 past years, when in parallel greater access to unpasteurized products was offered, whereas CDC (2019) recommended that public health officials and regulatory authorities should support pasteurization and restrict or prohibit the sale of raw milk and unpasteurized dairy products. If the consumption of raw milk and raw milk-based products is clearly associated with risks, faulty or inadequate pasteurization or postpasteurization contaminations are also associated with outbreaks (Boor and Murphy, 2002; Heuvelink et al., 2009; Robinson et al., 2014; Pärn et al., 2015).

Food Production Systems and Antimicrobial Resistance: Milk Is Also a Concern

It is estimated that antimicrobial resistance (AMR) causes 700,000 deaths per year, and predictions indicate that up to one million lives will be threatened by 2050, in addition to tremendous losses amounting to billions of euros (Dadgostar, 2019; USP, 2020). WHO (2020) pointed out AMR as one of the biggest threats to global health, given the growing list of infections, including foodborne diseases, that are difficult if not impossible to treat because many antibiotics stop being effective. Nevertheless, high amounts of antibiotics are used in food-producing animals, especially in settings of intense animal production, where up to four times more antibiotics are consumed compared to human usage as either prevention or growth-promoting means with the risk that antibiotic-resistant bacterial contamination of humans can occur via direct contact with animals, or through the food chain (WHO, 2017b; Dadgostar, 2019). In the list of antibiotic-resistant "priority pathogens" published by the WHO (2017c), multidrug-resistant *Acinetobacter baumanii* and *Pseudomonas aeruginosa* were ranked as "critical." Notably, species belonging to the *Pseudomonas* and *Acinetobacter* genera were also identified as key spoilage genera of raw milk (Dogan and Boor, 2003; Munsch-Alatossava and Alatossava, 2006; Hantsis-Zacharov and Halpern, 2007; Machado et al., 2015; Gschwendtner et al., 2016). Some investigations showed that some bacteria, typically favored by the cold storage conditions of raw milk and generally considered benign while pointed out as key spoilage agents by dairies, present multiresistance to antibiotics; many psychrotrophic bacteria that dominate the microflora at the time of spoilage even displayed innate multidrug-resistant features; one striking observation, as schematized in **Figure 1**, was that antibiotic resistance was highest in raw milk samples that approximately reached the bacteriological acceptance threshold level (10^5 cfu/ml) at a time when raw milk normally enters the industrial processing stage (EC, 2004; Munsch-Alatossava and Alatossava, 2006, 2007; Munsch-Alatossava et al., 2012). Two recent studies concluded

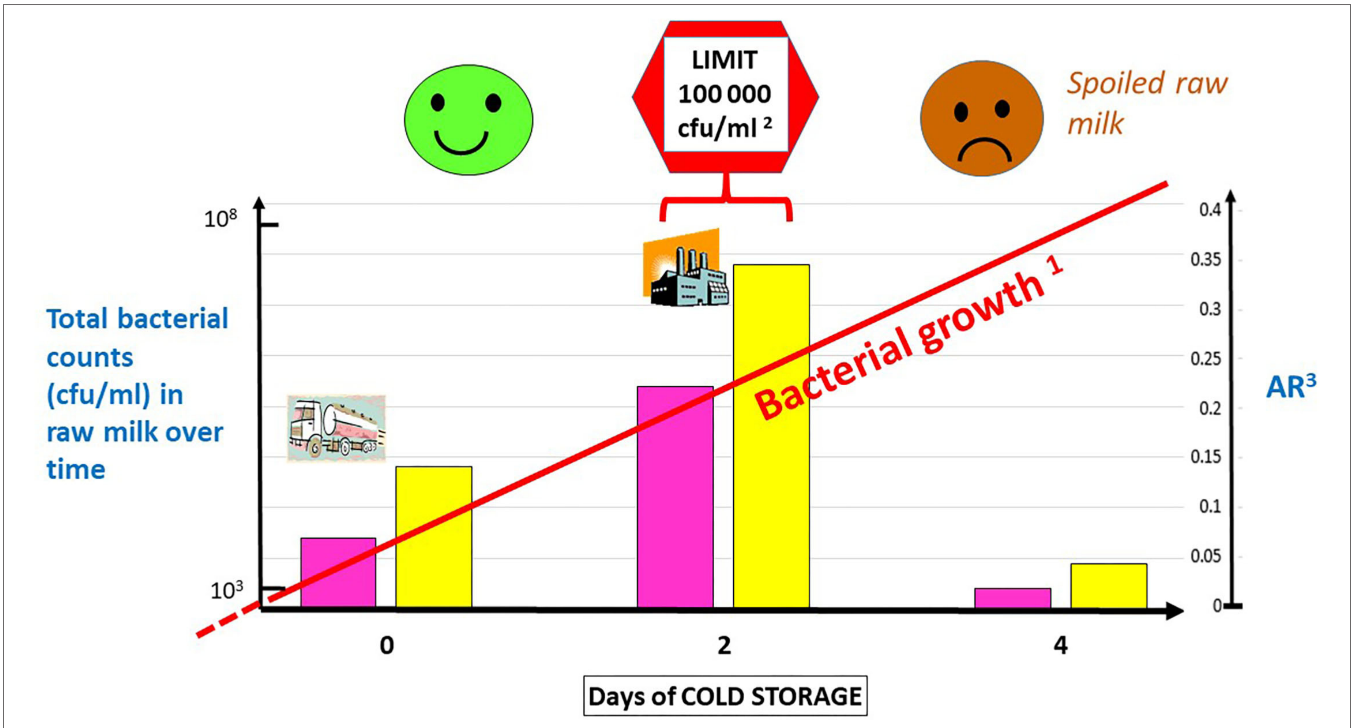

FIGURE 1 | The irony behind the antibiotic resistance (AR) trend evaluated for mesophilic (pink) and psychrotrophic (yellow) bacterial populations considering "total" bacterial growth, during raw milk cold storage. [1]"Total" bacterial growth during cold storage (modified from Chambers, 2002; Munsch-Alatossava et al., 2012). [2]EC (2004). [3]AR as deduced from Rapd, defined in Munsch-Alatossava et al. (2012).

that AMR of raw milk-associated *Pseudomonas* spp. should be taken seriously and that retail raw milk serves as a reservoir of AMR genes (Liu et al., 2020; Meng et al., 2020).

Following its entrance into dairies, raw milk is mostly subjected to various heat treatments such as low pasteurization or UHT (at least 72°C for 15–20 s and at least 135°C for a few s, respectively), that reduce the microbiological risks as most bacterial cells will be degraded.

The literature is rather scarce with respect to heat treatments of foods and AMR, especially on whether heat treatments, that are applied to reduce or eliminate bacterial contaminants, are sufficient to damage AMR genes and prevent their uptake by other bacteria. Two recent studies highlighted potential hazards in pasteurized milk: high prevalence of different plasmid-mediated AMR genes together with the presence of bacterial cells in a viable but nonculturable (VBNC) state; moreover, some AMR genes kept *in vitro* transferability and expression capacity (Taher et al., 2020a,b). The same studies concluded that UHT treatment or sterilization was more effective in decreasing the numbers of staphylococci and their AMR genes, while less favoring the switch to the VBNC state.

On the other side, the fate of residual genomic DNA from dead cells following heat treatments remains questionable, taking into account that three studies showed that autoclave sterilization (121°C/15 min), which is chemically more stringent than UHT sterilization, did not totally degrade DNA (Masters et al., 1998; Yap et al., 2013; Calderón-Franco et al., 2020).

By considering a 10^5 cfu/ml raw milk sample, a rough estimation lead to around 24,500 AMR genes per ml of raw milk (Munsch-Alatossava et al., 2017). Indisputably, further studies are requested to estimate the risks related to the presence and persistence of antibiotic-resistant bacteria and their genes in raw, pasteurized, and UHT milks.

FROM RAW MILK TO DAIRY PRODUCTS: FOOD SAFETY HAZARDS AND SPOILAGE ISSUES

Dairy or milk products are commonly made from the milk of goats, sheep, camels, water buffaloes, and cows (by far the most common throughout the world); the quality of all products is greatly influenced by the initial quality of raw milk.

Estimations regarding milk losses from withdrawal until the processing stages are still scarce and difficult to compare. In Israel, milk industries estimated that psychrotrophs can cause ∼10% loss in milk fats and proteins (Hantsis-Zacharov and Halpern, 2007). A recent study evaluated milk loss on dairy farms in Scotland and concluded that milk loss in primary production was not insignificant (March et al., 2019).

Dairy products comprise liquid milk, milk powder, infant formula, yogurt and other fermented milk, cream, butter, ice cream, and cheese, including traditional products too. Some microbiological, biochemical, and technological complications, reported for major dairy products, are shown below and in **Table 2**.

Heat-treated milk, including UHT milk, faces a range of limiting factors regarding shelf life. If the shelf life of pasteurized milk may be reduced by the presence of spore formers, heat-treated milk products may also be the target of postpasteurization contaminations, whereas heat-resistant spoilage enzymes may cause off-flavors, coagulation or gelation in finished products (Sorhaug and Stepaniak, 1997; Boor and Murphy, 2002; Chambers, 2002; Huck et al., 2007; Ivy et al., 2012). Present limitations concerning shelf life and the reduction of milk waste are crucial issues. To propose actions to reduce milk waste, the Waste and Resources Action Programme (WRAP, 2018) estimated that the most significant amount was discarded in the homes of consumers (87.3%), whereas processing and filling operations led to a loss of 3.9%. If the shelf life of UHT milk is typically 3 months, new demands arise for a shelf-life of up to 12 months for a UHT milk product that should withstand long transport distances and fluctuating temperatures during transit.

According to Wilbey (2002), the spoilage of butter is mainly caused by mold species, or by lipolytic psychrotrophs originating from wash water; poor quality may also be linked to the presence of coliforms. Although butter is not considered a high-risk dairy product, a listeriosis outbreak in Finland in 1999 due to contaminated butter, made from pasteurized cream, affected 25 patients, and was fatal to 6 (Lyytikäinen et al., 2000). Regarding the quality of butter, after some time of cold storage, butter may deteriorate due to fat auto-oxidation, which results in flavor defects (Walstra et al., 2006).

The hygienic state of raw milk is of particular importance concerning creams: strict hygienic conditions together with high-quality ingredients combined with efforts to avoid postpasteurization contaminants are key factors ensuring the quality of creams and ice creams; if postpasteurization contaminants are dominated by Gram-negative bacteria causing taints in ice creams, listeriosis outbreaks linked to ice-cream consumption have been also reported (Papademas and Bintsis, 2002; Pouillot et al., 2016; Rietberg et al., 2016). The quality of creams relies essentially on preventing lipolysis and fat auto-oxidation (Walstra et al., 2006).

Fermented milk products are the result of fermentations usually achieved by lactic acid bacteria starters alone or in combination with yeasts. The characteristic acidity level limits the growth of many undesired microbes; however, outbreaks were described earlier by Robinson et al. (2002), and recently by the Washington State Department (2021), who reported an outbreak of *E. coli* 0157:H7, present in pasteurized yogurt, which led to 17 illnesses.

Altogether, long ripened semi/hard cheeses are considered safe food products. Compared to pasteurized milk cheeses, raw milk-based cheeses are believed to have a more intense flavor due to increased proteolysis and lipolysis achieved by the raw milk

TABLE 2 | Benefits that N_2 gas flushing technology could confer to various dairy products, based on research-evidenced benefits highlighted for N_2-treated raw milk, considering microbiological, biochemical, and technological aspects.

Observations	Question	*Research-evidenced benefits of N_2 flushing treatment*
Raw milk		
Cold storage promotes bacterial growth, alters bacterial diversity and favors AR dissemination (1- 9, 13)	Can the cold chain perform well in the context of global warming?	*Inhibition of bacterial growth associated with spoilage and AR risks (6–9) *Longer preservation of bacterial diversity (7) *Initial bio/chemical features are better preserved (8–12)
Dairy products	**Some reported difficulties**	**Putative benefits if raw milk would have been treated by N_2 gas flushing?**
Liquid milks (Pasteurized milk; ESL and UHT milk)	*Limited shelf life *Flavor defects *Gelation (1–2; 13–14)	*Lower bacterial load implies less spoilage enzymes (proteases and phospho/lipases), which would result in less flavor and biochemical defects and would enable a longer shelf life *Less "risky" bacterial metabolites and genes
Fermented milks (cultured buttermilk, yogurt, kefir, viili…)	*Flavor defects caused by lipolysis or proteolysis *Inhibitory effect of lipolysis on growth of starters (13)	Lower risks for defects
Creams and butters (Non- fermented and fermented types: creams, whipping cream, ice-cream…)	*Flavor defects caused by auto- oxidation of fat (unsaturated FA residues) *Lipolysis *Fat globule aggregation (13)	*Lower bacterial load implies less spoilage enzymes (reduced lipolysis and proteolysis) *The absence of O_2 in raw milk implies lower fat auto-oxidation
Cheeses *Soft cheeses (fresh or mold types) *Semi/hard types	*Pathogens in raw-milk based cheeses *Discoloration defects *Flavor and texture defects (13,15)	*Lower bacterial load implies less spoilage enzymes, less flavor and discoloration defects *Better preservation of bacterial diversity in raw milk (7) could positively affect quality and safety of products

*The relevant references are as follows: (1) Sorhaug and Stepaniak (1997); (2) Chambers (2002); (3) Lafarge et al. (2004); (4) Quigley et al. (2013); (5) Munsch-Alatossava et al. (2012); (6) Munsch-Alatossava et al. (2017); (7) Gschwendtner et al. (2016); (8) Murray et al. (1983); (9) Dechemi et al. (2005); (10) Gursoy et al. (2017); (11) Munsch-Alatossava et al. (2018); (12) Munsch-Alatossava et al. (2019); (13) Walstra et al. (2006); (14) Ivy et al. (2012); (15) Del Olmo et al. (2018). *symbol served for the listing of different points.*

microflora, conferring evident marketing advantages. Outbreaks are often linked to raw (or unpasteurized) milk-based fresh and mold cheeses, with the presence of *E. coli*, *Salmonella enterica*, and *Staphylococcus aureus* and to cheeses made from pasteurized milk, where outbreaks due to *Listeria monocytogenes* were reported by several studies (De Valk et al., 2000; Koch et al., 2010; De Castro et al., 2012; Gaulin et al., 2012; Johler et al., 2015).

Due to their high moisture content, soft cheeses are sensitive to spoilage; in addition, the quality and pH of the washwater are critical factors. Coliforms and psychrotrophic bacteria are especially of concern for cottage cheeses (Farkye and Vedamuthu, 2002).

N_2 GAS FLUSHING BENEFITS FOR DAIRY PRODUCTS DEDUCED FROM RESEARCH-EVIDENCED BENEFITS FOR RAW MILK

Improved storage technology based on modified atmosphere or controlled atmosphere (MA and CA, respectively) is widely employed in the food sector. Concerning raw milk, many studies reported a shelf life extension after the addition of CO_2; however, some disadvantages were also reported, like acidification of raw milk, the modification of sensory properties, or technological complications, for example, at heat-treatment stages (King and Mabbitt, 1982; Calvo and De Rafael, 1995; Ruas-Madiedo et al., 1996; Boor and Murphy, 2002).

Different reports have described the multiple advantages of the application of N_2 gas to raw milk in which microbiological, biochemical, or technological aspects were considered; (Murray et al., 1983; Dechemi et al., 2005; Munsch-Alatossava et al., 2010a,b,c, 2013, 2016, 2017, 2018, 2019; Gschwendtner et al., 2016; Munsch-Alatossava and Alatossava, 2020); in the conditions described by Munsch-Alatossava et al. (2010a, 2017, 2018) and Gschwendtner et al. (2016) bacterial levels were typically lower by an over four log-units factor compared to the nonflushed conditions.

As N_2 gas flushing technology enabled the control of both mesophilic and psychrotrophic bacterial populations present in raw milk, it would certainly contribute to preserving the quality and safety of raw milk from withdrawal until the processing stages, if applied continuously during cold storage (**Figure 2**). The putative benefits that the treatment could confer to different dairy products are listed in **Table 2**.

CONCLUSION

Multiple ways of addressing sustainability exist in dairies, namely, the improvement of production efficiency, the promotion of farm best practice interventions, the protection of carbon sinks (forests and grasslands), the development of a circular bioeconomy, and the modification of feeding practices, including remarkable cattle training (FAO and GDP, 2019; Laca et al., 2020; Dirksen et al., 2021). The sustainability of dairy farms should not be dissociated from their profitability, as a tight interlink exists between economic, social, and environmental

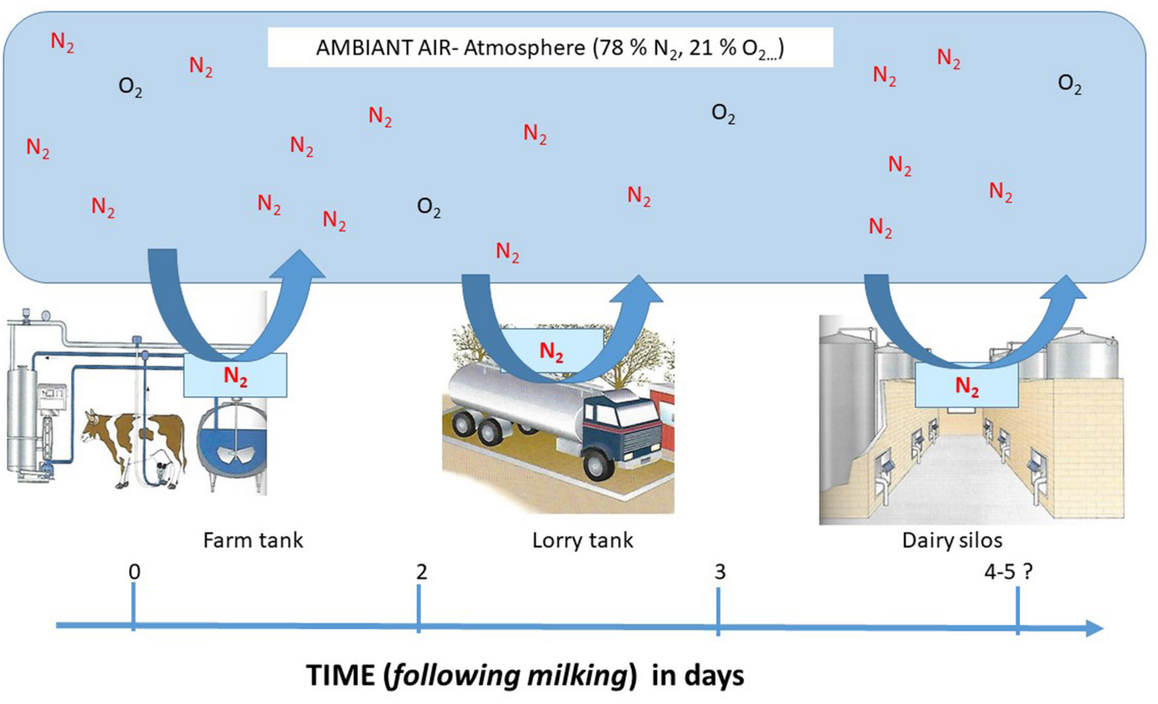

FIGURE 2 | N_2 gas flushing technology is applicable throughout the chain of raw milk storage and transportation, irrespective of its duration. Pictures included in figure were downloaded from the PowerPoint online database by Milk tanks and silo pictures are modified from Tetra Pak (2003).

aspects of sustainability; it was shown, at the farm level, that economic sustainability is crucial to achieving social and environmental survival in the medium and longer term (Britt et al., 2018; Bánkuti et al., 2020; Feil et al., 2020).

Difficulties of transporting raw milk from farms to processing units are obvious considering that milk collection and transport represent a significant share, often above 30% of the milk processing costs; some studies also discussed vulnerabilities linked to high temperatures inside cisterns or the consequences of transport over long distances (Teh et al., 2012; Vithanage et al., 2016; FAO, 2021).

Among the opportunities to reduce waste along the journey of milk, WRAP (2018) recommended a diminution of consumers' fridge temperature or the freezing of milk; as households were found responsible for the highest waste, these actions to address food spoilage sound judicious. However, the consumer is quite deprived at the opening of spoiled milk packages (Munsch-Alatossava and Alatossava, 2020).

Irrespective of the product and the cause of spoilage, addressing spoilage of dairy products should also consider upstream early actions aiming to preserve at maximum the initial features of freshly withdrawn raw milk, from farms until the processing stages; surely, early actions would allow the increase of shelf life and most likely also significantly contribute to the reduction of waste related to liquid milks and dairy products.

Tests performed at laboratory and pilot plant scales with N_2 flushing technology indicated that the treatment could constitute a complementary hurdle to cold storage, and most likely as an alternative for a short time when low temperatures cannot be guaranteed; the treatment seemed even advantageous for out of cold chain conditions (Munsch-Alatossava et al., 2010a,b,c, 2018, 2019; Alatossava and Munsch-Alatossava, 2018).

N_2 gas flushing of raw milk presents all advantages of non-thermal technologies, while relying on N_2, an infinite resource. Indisputably, the treatment would contribute to ensuring the quality and safety of many dairy products.

AUTHOR CONTRIBUTIONS

PM-A wrote the first draft of the manuscript. TA contributed to data compilation, critically read, and commented on the manuscript. Both authors approved the submitted version of the manuscript.

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