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Review

Raw and Heat-Treated Milk: From Public Health Risks to Nutritional Quality

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Abstract: Consumers have recently shown a preference for natural food products and ingredients and within that framework, their interest in consuming raw drinking milk has been highlighted, claiming nutritional, organoleptic and health benefits. However, a public debate has simultaneously emerged about the actual risks and benefits of direct human consumption of raw milk. This paper compares the microbiological, nutritional and sensory profile of raw and heat-treated milk, to evaluate the real risks and benefits of its consumption. In detail, it provides an updated overview of the main microbiological risks of raw milk consumption, especially related to the presence of pathogens and the main outputs of risk assessment models are reported. After introducing the key aspects of most commonly used milk heat-treatments, the paper also discusses the effects such technologies have on the microbiological, nutritional and sensory profile of milk. An insight into the scientific evidence behind the claimed protective effects of raw milk consumption in lactose-intolerant subjects and against the onset of asthma and allergy disorders in children is provided. The emergence of novel milk processing technologies, such as ohmic heating, microwave heating, high pressure processing, pulsed electric fields, ultrasound and microfiltration is also presented as an alternative to common thermal treatments.

Keywords: raw drinking milk; heat-treated milk; microbiological hazards; risk assessment; lactose intolerance; allergies; milk nutritional quality

1. Introduction

In recent decades, a strong desire for things that are natural has appeared and consumers have shown a preference for natural food products and ingredients. Food naturalness is an abstract construct, hard to define and measure but consumers have interpreted it as synonymous with going shopping at farmers' markets, purchasing organic food, consuming seasonal and minimally processed food products [1]. The demand for fresh-like, nutritious products with high organoleptic quality and an extended shelf-life has hence increased more and more.

A few surveys on the importance of food naturalness for consumers—namely the Kampffmeyer Food Innovation Study in 2012 [2] and the Nielsen Global Health and Wellness Survey in 2015 [3]—have recently been undertaken to understand why freshness, naturalness and minimal processing are the most desirable attributes for a food and what is the drive for that trend. It emerged that natural foods are considered healthier than commercial or processed foods [1].

In this context, the consumption of raw milk and products made from it has appeared. A prevalent belief that milk possesses particularly healthy properties and attributes when it is consumed in its raw form has arisen and, as a result of some perceived health benefits, it has become especially consumed by individuals that may have lowered immunity, such as very young, very old or immune-compromised people, as well as persons with specific dietary habits [4].

Over recent decades, a public debate has, nevertheless, emerged about the actual risks and benefits that direct human consumption of raw milk, as a drinking milk, may have. From a science perspective, food naturalness does not straightforwardly imply food healthiness, tastiness and safety. In fact, 27 milk-borne disease outbreaks occurred from 2007 to 2012 in the EU and an association with the consumption of raw drinking milk was claimed [4].

Recently, the European Food Safety Authority (EFSA) has been called upon to provide scientific opinion on the public health risks related to the consumption of raw drinking milk [4]. The hazards related to raw drinking milk are also well evidenced on the websites of authoritative institutions like the Food and Drug Administration (FDA) [5] and the Centers for Disease Control and Prevention [6].

The aim of this work is to compare the microbiological, nutritional and sensory profile of cow's raw and heat-treated milk in order to evaluate the risks and benefits of its consumption and to provide a basis for driving further research on technologies enabling the production of high quality milk. In detail, an updated overview of the main microbiological risks of raw milk consumption is presented, with emphasis on models for quantitative microbial risk assessment. The most common heat-treatments and their effects on the microbiological, nutritional and sensory profile of milk are reported. The effect of cow's raw milk consumption against lactose intolerance and allergy disease risk is discussed. An overview of novel technologies for milk processing, as an alternative to common heating treatments, is finally presented.

2. Method

2.1. Literature Search

The study layout was first designed and an extensive literature search for papers on planned topics was conducted May to August 2017 by two authors (FM and VM). A limited updated literature search was performed also in October 2017.

Major literature databases were used (i.e., SCOPUS, PubMed, ScienceDirect) in order to identify the literature relevant to the topic. The websites of authoritative Institutions were also consulted: the European Food Safety Authority (https://www.efsa.europa.eu), the U.S. Food and Drug Administration (https://www.fda.gov/), the Center for Disease Control and Prevention (https://www.cdc.gov/), Codex Alimentarius (http://codexalimentarius.org) and the Rapid Alert System for Food and Feed—RASFF (https://ec.europa.eu/food/safety/rasff_en). The legal framework for raw milk sale was searched on the Official Journal of the European Union (EUR-Lex, http://eur-lex.europa.eu/homepage.html).

During the search of the major literature databases, time limits were first set—the year of publication was to be later than 2007 in order to collect the most up-to-date published works. Several combinations of terms were used, depending on the following raw milk-related aspects/topics: consumer behavior/perception; raw milk microbial ecology; raw milk pathogenic microorganisms; risk assessment on raw milk consumption; heat treatment technologies; influence of heat treatments on milk microbiological, nutritional and organoleptic profile; raw milk and lactose intolerance; farm milk and asthma/allergy; and novel milk processing technologies.

2.2. Including and Excluding Criteria

Exclusion was applied to duplicate papers, articles not accessible for authors, or research studies dealing with raw milk other than cow's and with raw milk by-products. Reference lists of articles were also scanned to further identify relevant papers that were not found in electronic databases. The screening of the titles and abstracts was performed by two authors (FM and VM). A screening of the full text resulted in a further exclusion of papers. The key information from the selected papers was reviewed, extracted and grouped in order to meet the scientific requirements of each topic section.

3. Results and Discussion

3.1. Raw Drinking Milk Definition and Legal Framework

According to EU legislation, "raw milk" is milk produced by the secretion of the mammary gland of farmed animals, which has not been heated to more than 40 °C or has not undergone any treatment with an equivalent effect [7]. Raw milk intended for human consumption must be free of pathogens in accordance with the food safety requirements of the General Food Law, i.e., Regulation (EC) No. 178/2002 [8].

Moreover, specific microbial criteria are also laid down by Reg. (EC) 853/2004: for raw cows' milk, ≤100,000 CFU/mL for plate count at 30 °C and ≤400,000 CFU/mL for somatic cells are established, whereas for raw milk from species other than cows, a plate count at 30 °C of ≤1,500,000 CFU/mL is allowed. Health requirements for production animals and hygienic requirements on milk production holdings (e.g., premises and equipment, hygiene conditions during milking, milk collection and transport, staff hygiene) are also regulated, in order to guarantee all the above microbiological characteristics.

Handling and sale of raw milk are also regulated by EU legislation and accurate provisions are given by Regulations (EC) No. 853/2004 and 854/2004 [7,9] within the EU Hygiene package. Only authorized producers registered for supplying raw milk through vending machines are allowed to place raw milk dispensers near the farm or at any other location. Milk has to be cooled to 6 °C at the farm immediately after milking and transferred into dedicated vending machines. Temperature must be held at 0–4 °C between the tank and the nozzle of the vending machine. It is not allowed to stock any milk batch in the vending machine more than 24 h, as certain pathogens are capable of multiplying at low temperatures and prolonged storage may boost their growth. Any residual milk must be removed carefully and the machine must be cleaned prior to refilling. Internal and external cleaning procedures for milk vending machines should be part of good hygienic practices (GHPs).

A key issue often associated with the quality of milk dispensed by vending machines is the formation of bacterial biofilms, which may lead to an increased opportunity for microbial contamination [10–12]. *Listeria monocytogenes* has, for instance, the potential to form biofilms on materials such as stainless steel, rubber, or plastic, which are frequently used in milk handling equipment or tanks [10]. Psychrotrophic strains of *Pseudomonas* spp., *Escherichia coli*, as well as *Bacillus* spores are also involved in formation of biofilms on milk handling equipment. The safety issue is that biofilms may survive the cleaning process. To that aim, special cleaning treatments, e.g., pre-rinsing with water, circulation of sanitizing and/or alkali/acidic solutions and final cleaning with water, are required [13].

Finally, consumers have to take some precautions because raw milk transportation from selling points to home, handling and storage practices can also be a critical point and potentially have an impact on the microbiological quality of raw drinking milk. Insulated bags have to be used to transport raw milk, the duration of transportation to consumption spots must be very short, raw milk is to be boiled before consumption and stored between 0 °C and 4 °C, as indicated on vending machines.

3.2. Microbial Ecology in Raw Milk

Milk is estimated to be sterile in healthy udder cells and not to contain bacteria in the mammary gland at the site of its production, unless there is an intra-mammary infection and/or the animal has a systemic disease. The indigenous flora is mainly represented by the genera *Streptococcus*, *Staphylococcus* and *Micrococcus* (>50% of the overall raw milk flora) [14].

However, as soon as milk is excreted it is immediately colonized by a complex microbiota, which is comprised of a significant population of microorganisms that naturally dwell in the teat skin and the epithelial lining of the teat canal. Specifically, the bovine teat surface is colonized by bacteria belonging to the phylum *Firmicutes* (76%), *Actinobacteria* (4.9%), *Proteobacteria* (17.8%) and *Bacteroides* (1.3%) but also *Planctomycetes*, *Verrucomicrobia*, *Cyanobacteria* and *Chloroflexi* at a lower level [15]. Milking equipment [16], location of the animals [17,18], feeding site [19,20], bedding material [21] and lactation stage [22] have an influence on the raw milk microbiota as well.

The biodiversity of raw milk microbiota is represented by a great variety of species belonging to the domains of bacteria and fungi, and is influenced by the profile of initial microflora but also by the raw milk biochemical composition, the near neutral pH (6.4–6.8) and the high water-activity (a_w), which may contribute to favoring their growth.

Raw milk microbiota can be mainly classified into two main groups: spoilage microorganisms (Table 1) and pathogens (Table 2), which are both undesirable in raw milk. Spoilage microorganisms can, in fact, grow rapidly in milk and alter traits like nutritional and sensory quality. Pathogens present in raw milk are a threat for milk safety and key causative agents of human infections; hence, their presence is very unwanted.

3.2.1. Spoilage Microorganisms

The category of spoilage microorganisms comprises different groups, the main being lactic acid bacteria (LAB), psychrotrophic bacterial populations both Gram-negative (−) and Gram-positive (+), which can grow during milk storage at ≤6 °C, coliforms and fungal populations (both yeasts and molds) [14,16].

Lactic Acid Bacteria

Lactic Acid Bacteria are an integral part of raw milk microbiota [16]. Their biodiversity in milk depends on the kind of milk and other external parameters during milking [14]. In raw ewes' milk, LAB flora is dominated by enterococci (~40%), lactococci (14–20%), leuconostocs (8–18%) and lactobacilli (10–30%); in raw goat's milk it is dominated by lactobacilli [14]. However, lactococci and lactobacilli are usually the most frequently identified LAB, thereof *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus fermentum* are the most frequently found species [23]. *Lactobacillus* spp. also have proteolytic activity and can produce aroma compounds and exopolysaccharides.

LAB role as spoilage microorganisms results from their being acid-producing fermentative agents when storage temperatures are sufficiently high for them to outgrow psychrotrophs or Gram-negative aerobic organisms are inhibited [24].

Psychrotrophic Microorganisms

Milk freshly drawn from the udder often does not contain detectable populations of psychrotrophic bacteria [25]. However, after milk collection psychrotrophic bacteria grow also when the cold chain is applied. Despite these microorganisms have optimal and maximal growth temperatures above 15 and 20 °C, respectively [25], they have, in fact, the ability to grow at low temperatures, such as 2–7 °C. This means that over time psychrotrophic populations can develop in cold stored raw milk and their presence in the raw milk microbiota can become a matter of concern. The drawback of psychrotroph presence in milk is their ability to produce extracellular enzymes, mainly proteases and lipases, which are responsible for spoiling milk but also dairy products, as the extracellular enzymes can resist pasteurization and even ultra-high temperature processing [26].

The prompt application of a cooling treatment after milking and of cold temperatures for storage, which are a routine practice to control the microbiological quality and safety of raw milk, are thus not effective to have a fall-off in the growth rate of psychrotrophic bacteria.

The number of psychrotrophic bacteria, which develop after milk collection, depends on the storage temperature, time and hygienic conditions. For instance, under unsanitary conditions more than 75% of the total microflora is represented by psychrotrophs, whereas in case of sanitary conditions the number of psychrotrophic microorganisms is lower than 10% [26].

Psychrotrophic bacteria from numerous genera have been isolated from raw milk. They are represented primarily by the Gram-negative genera *Pseudomonas*, *Aeromonas*, *Serratia*, *Acinetobacter*, *Alcaligenes*, *Achromobacter*, *Enterobacter*, *Chryseobacterium* and *Flavobacterium* (Table 1) [16,25–27].

Pseudomonas spp. and *Enterobacter* spp. are the most abundant in cold stored raw milk. It is estimated that Gram-negative microflora accounts for more than 90% of the total psychrotrophic raw milk microflora [14].

The Gram-positive genera *Bacillus*, *Clostridium*, *Corynebacterium*, *Microbacterium*, *Micrococcus*, *Streptococcus*, *Staphylococcus* and *Lactobacillus* are also commonly found in raw milk but they only account for a small proportion of the psychrotrophic microflora [27]. *Bacillus* spp. are also the main predominant spore-forming bacteria, therefore *B. licheniformis*, *B. cereus*, *B. subtilis* and *B. megaterium* are the most isolated. *B. cereus* is the most common contaminant [24], but *B. subtilis* and *B. licheniformis* are more heat-resistant than *B. cereus* and they spoil sterilized and UHT milk [14]. In 2006, Heyndrickx's group [28] isolated a very heat-resistant mesophilic species of *Bacillus*—i.e., *Bacillus sporothermodurans*—from UHT milk.

The Gram-positive *Arthrobacter* is claimed to enter from the dairy facility, whereas *Corynebacterium* spp. are reportedly found on the teat surface and in the farm environment [16].

The psychrotrophic microflora of raw milk also comprises pathogens like the Gram-negative *Aeromonas hydrophila* and *Yersinia enterocolitica*—the Gram-positive *L. monocytogenes* and toxin-producing strains of *Bacillus cereus*—whose spores can even survive heat treatments in the range of 65–75 °C.

Coliforms

Coliforms are normally found in raw milk with varying levels [14,29]. Their presence is due to different sources, such as water, plant materials, equipment, dirt and feces. High levels of coliforms (e.g., >1000 CFU/mL) generally indicate unsanitary practices on the farm or inadequate refrigeration but also non-adequate management practices, such as milking machine wash failures and fall-offs in the rate of milking units [29].

Attempts have been made to find a correlation between levels of coliform bacteria and the possibility of public health hazards from raw milk consumption. However, so far, no correlation has been identified. A recent survey carried out in the United States [30] demonstrated that coliform counts are not an index of the presence of *B. cereus*, *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* spp. and that subsequently coliform testing of raw milk intended for human consumption cannot be used as a reliable tool for public health risk screening [30,31]. Further investigations are thus required.

Fungi

Yeasts and molds can also be an important population in raw milk. They usually originate from contaminated environment of the dairy farm and/or processing plant but can also derive from the physiological state of the animal, feeding and climatic conditions [16]. The most commonly detected yeasts in raw milk belong to the genera *Candida*, *Cryptococcus*, *Debaryomyces*, *Geotrichum*, *Kluyveromyces*, *Pichia*, *Rhodotorula* and *Trichosporon*. *Debaryomyces hansenii*, *Kluyveromyces marxianus* var. *marxianus* and *Kluyveromyces marxianus* var. *lactis* are of particular interest.

The levels to which molds are present in raw milk are lower than yeasts. The most detected mold genera are *Penicillium*, *Geotrichum*, *Aspergillus*, *Mucor*, *Rhizomucor*, *Rhizopus* and *Fusarium* [14,15]. Interestingly, over the last ten years notifications and alerts from the Rapid Alert System for Food and Feed (RASFF) have been issued for raw milk contaminated by mycotoxins from Italy, Hungary and Slovenia [32].

Table 1. Spoilage microorganisms in raw milk [1].

Lactic Acid Bacteria						**Psychrotrophs**		**Fungi**	
***Lactococcus* spp.**	***Streptococcus* spp.**	***Lactobacillus* spp.**	***Leuconostoc* spp.**	***Propionibacterium* spp.**	***Enterococcus* spp.**	**Gram Positive**	**Gram Negative**	**Yeasts**	**Molds**
L. lactis spp. *cremoris*	*S. agalactiae*	*L. acidophilus*	*L. mesenteroides*	*P. acidipropionici*	*E. durans*	*Arthrobacter* spp.	*Achromobacter* spp.	*Candida* spp. *C. sake* *C. parapsilosis* *C. inconspicua*	*Aspergillus* spp.
L. lactis spp. *lactis*	*S. bovis*	*L. brevis*	*L. pseudomesenteroides*	*P. freudenreichii*	*E. faecalis*	*Bacillus* spp.	*Acinetobacter* spp.	*Cryptococcus* spp. *C. curvatus* *C. carnescens* *C. victoriae*	*Fusarium* spp.
L. piscium	*S. gysgalactiae*	*L. buchneri*		*P. jensenii*	*E. faecium*	*Bifidobacterium*	*Aeromonas* spp.	*Debaryomyces hansenii*	*Geotrichum* spp.
L. raffinolactis	*S. macedonicus*	*L. casei*		*P. thoenii*	*E. italicus*	*Brevibacterium*	*Alcaligenes* spp.	*Geotrichum* spp. *G. candidum* *G. catenulate*	*Mucor* spp.
	S. thermophilus	*L. crispatus*			*E. mundtii*	*Chlostridium* spp.	*Chryseobacterium*	*Kluyveromyces* spp. *K. marxianus* *K. lactis*	*Penicillium* spp.
	S. uberis	*L. curvatus*				*Corynebacterium* spp.	*Enterobacter* spp.	*Pichia*	*Rhizomucor*
		L. fermentum				*Microbacterium*	*Flavobacterium*	*Rhodotorula mucilaginosa*	*Rhizopus*
		L. gasseri				*Micrococcus*	*Pseudomonas* spp.	*Torrubiella*	
		L. johnsonii					*Serratia*	*Trichosporon* spp. *T. cutaneum* *T. lactis*	
		L. paracasei							
		L. pentosus							
		L. plantarum							
		L. reuteri							
		L. rhamnosus							
		L. sake							

[1] The table was prepared on the basis of the information gathered in [14,16,27].

3.2.2. Raw Milk Pathogenic Microorganisms

Raw milk can harbor also a great number of pathogens (Table 2), even when it is sourced from clinically healthy animals and they can represent a serious health threat for humans. They can originate from feed and drinking water (*Toxoplasma gondii*) [33], dairy farm environment (*Salmonella* spp., *L. monocytogenes*, Shiga toxin-producing *E. coli*, *Campylobacter jejuni*, *Y. enterocolitica* and *Clostridium* spp.), mammary gland, cow diseases or infections (*Staphilococcus aureus* and *Brucella* spp.) but also from equipment, raw milk tanks and personnel. Transmission to raw milk can, in fact, occur either from animals (zoonotic pathogens), or from contaminated environment (exogenous pathogens).

Salmonella spp., *Listeria* spp., *E. coli*, *Campylobacter* spp., *Brucella* spp., *Clostridium* spp. and/or *Shigella* spp. are the most common milk-borne pathogens and also the main causative agents of microbial food-borne diseases, specifically, milk-borne infections, milk-borne intoxications and milk-borne toxico-infections [14].

Generally speaking, typical symptoms from drinking raw milk contaminated by the above-mentioned pathogens are fever, nausea, vomiting, diarrhea and abdominal pains. However, they can potentially affect also the cardiovascular, cutaneous, neurological, ocular and pulmonary system, and only in some cases they cause death, as it is the case for *Listeria* spp. (30–35%) and *Streptococcus* spp. (up to 29%) [34].

Salmonella spp. are natural inhabitants of the gastrointestinal trait of animals. Milk contamination by them can generally occur at harvest and only in rare cases they determine sub-clinical mastitis which will cause the milk-borne disease at its turn. They are mesophilic microorganisms with an optimum growth temperature of 35–37 °C [14] but can also grow at a wider temperature range, i.e., 5–46 °C. According to the USA Centre for Disease Control and Prevention (CDC), 38% of raw milk outbreaks involving children from 2007 to 2012 in USA were determined by *Salmonella* spp. [6]. The gastroenteric form of non-typhoid salmonellosis is frequently related to the consumption of raw milk. *Salmonella* spp. have, however, a poor thermal tolerance and are thus sensitive to pasteurization.

Table 2. Main raw milk pathogens and related zoonoses [1].

Pathogen	Taxonomy	Morphology	Disease	Transmission Route	System Potentially Affected					
					Cardio Vascular	***Cutaneous***	***Gastro Intestinal***	***Neurological***	***Ocular***	***Pulmonary***
Brucella spp. *B. abortus* *B. melitensis*	Bacteria	Gram (−) coccobacilli	Brucellosis	Cutaneous Ingestion Inhalation	x	x	x	x	x	x
Campylobacter spp. *C. fetus* *C. jejuni*	Bacteria	Gram (−) corkscrew	Campylobacteriosis	Ingestion	x		x	x		
C. burnetii	Bacteria	Gram (−) coccobacilli	Q fever	Ingestion Inhalation	x		x	x		x
E. coli	Bacteria	Gram (−) bacilli	Hemolytic uremic syndrome Hemorrhagiccolitis	Ingestion Inhalation		x	x	x		
L. monocytogenes	Bacteria	Gram (+) bacilli	Listeriosis	Ingestion Cutaneous	x	x	x	x		x
Mycobacterium spp. *M. tubercolosis* *M. bovis*	Bacteria	No Gram classification bacilli	Tubercolosis	Cutaneous Inhalation Ingestion		x	x	x		x
Salmonella spp.	Bacteria	Gram (−) bacilli	Salmonellosis	Ingestion			x			
Shigella spp.	Bacteria	Gram (−) bacilli	Shigellosis	Ingestion		x	x	x		
Staphylococcus spp.	Bacteria	Gram (+) staphylococci	Staphylococcal disease	Cutaneous Inhalation Ingestion	x	x	x	x		x
Streptococcus spp.	Bacteria	Gram (+) streptococci	Toxic shock syndrome	Cutaneous Inhalation Ingestion	x	x	x	x		x
Yersinia spp. *Y. pseudotubercolosis* *Y. enterocolotica*	Bacteria	Gram (−) bacilli	Yersiniosis	Cutaneous Inhalation	x	x	x	x		x

[1] The table was prepared on the basis of the information gathered in [34].

L. monocytogenes is one more example of foodborne pathogen possibly contaminating raw milk. Feces dirtying milking equipment are a source of contamination. In humans, it causes large outbreaks of listeriosis, a serious invasive disease-causing abortion in pregnant women, meningitis, encephalitis, and septicemia in neonates and immune-compromised adults, with quite a general high mortality rate [35]. The threat is due to the fact that it can grow and multiply during raw milk storage also at low temperatures (0–4 °C), that implying that even the application of a correct cold chain would not completely eliminate the microorganism. Its occurrence in raw farm milk and bulk tank milk is reportedly frequent. It can also grow on steel and rubber surfaces and plays an important role in the formation of biofilms in vending machines.

E. coli has been recognized as an indicator of fecal contamination. The most pathogenic strains are referred to as verocyto-toxigenic *E. coli* (VTEC), Shiga toxin-producing *E. coli* (STEC) and enterohemorrhagic *E. coli* (EHEC), also known as *E. coli* serotype O157:H7. Cattle feces are the major reservoir of EHEC which commonly contaminates bulk tank milk. Milk contamination is hence a result of direct exposure to fecal material or environmental contamination. Raw milk poses a risk for STEC, and a number of outbreaks has been recently reported for this pathogen [33,36]. In 2013, 3% of 860 tested raw milk samples were found positive for STEC in Europe [33], whereas in USA, according to CDC, Shiga toxin-producing *E. coli* caused 17% of the outbreaks that occurred 2007 to 2012. VTEC serotypes have also been detected in cow's mastitic milk, that implying that an additional contamination route may be sub-clinical mammary infections. Most strains are not heat-resistant, pasteurization thus destroys them.

Campylobacter spp. belong to the family of *Campylobacteraceae* and are an etiological agent of human gastroenteritis. Among them, the most detected isolate in raw milk is *C. jejuni* which is acid- and heat-sensitive and is hence killed by pasteurization. Outbreaks of campylobacteriosis, following the consumption of raw milk, have been reported in the USA, the Netherlands and Hungary, for instance [14]. Specifically, van Asselt and colleagues (2017) report that raw milk consumption accounts for a relatively high number of *Campylobacter* outbreaks: in 2013, 32 strong-evidence *Campylobacter* spp. outbreaks were reported in the EU, of which between 9% in 2013 and up to 20% in 2012 could be attributed to it [33].

Brucella spp. are the main causative agents of the bacterial zoonosis brucellosis. They are very infectious microorganisms which can cause disease in both animals and humans. The most pathogenic strains which have been associated with disease in humans are *Brucella abortus* and *Brucella melitensis*. The former is more often associated with cattle, whereas *B. melitensis* is especially associated with sheep and goats. Most cases of food-borne brucellosis in humans are contracted via consumption of raw milk and derivatives. Among the milk-borne pathogens, *Brucella* spp. are in fact able to survive and multiply at refrigeration temperatures, together with *L. monocytogenes* and *Y. enterocolitica*. *Brucella* spp. are not particularly resistant to thermal processing and standard pasteurization can sufficiently destroy them. However, the issue with it is that it can survive and multiply in milk also upon contamination after pasteurization.

S. aureus is a Gram-positive bacterium, which causes mastitis in cows and other domestic dairy ruminants. It can come to contaminate milk via the teat canal, when there is infection of the mammary gland, via the environment, or by bad hygiene habits during or after milking, such as, not washing hands when handling milk storage equipment [16]. *S. aureus* may cause diseases through the production of heat-stable enterotoxins. The latter, in fact, are very resistant to heating and pasteurization. For that reason, boiling milk for 1 h may decrease the quantity of toxin present in milk, but autoclaving at 15 psi for 20 min seems to be the main treatment able to completely destroy the toxins [37].

Two more zoonotic bacteria of concern are *Mycobacterium avium* subsp. *Paratubercolosis* (MAP) and *Mycobacterium bovis*. MAP causes para-tuberculosis or Johne's disease, which mainly infect domestic animals. It survives and multiplies in the animal intestinal tract mucosa. In recent times, some evidence has been provided about a relationship between MAP and Crohn's disease in humans [15], though the association remains controversial. High prevalence of MAP has been reported in raw milk. It is however relatively heat-resistant. Dairy processors affirm that it may survive pasteurization at 72 °C for 15 s and trials on its resistance to heat have so far reported controversial results [24]. In 2002, researchers of the Queen's University of Belfast [38] screened 567 samples of commercial pasteurized milk and found that 1.8% were contaminated with *M. avium* subsp. *tuberculosis*. This microorganism can survive HTST pasteurization and can also be found in pasteurized milk due to post-processing contamination.

M. bovis causes bovine tuberculosis in animals but it can also spread to humans via the consumption of raw milk. It causes zoonotic tuberculosis, which is indistinguishable from human tuberculosis. Upon consumption of infected milk, extra pulmonary lesions may develop [36]. However, pasteurization removes it. Moreover, countries like the Netherlands have an official bovine tuberculosis-free status [36] which potentially implies the elimination of the pathogen from the food chain.

Y. enterocolitica is the causative agent of acute gastroenteritis, whose symptoms are abdominal pain, diarrhea and fever. It may however mimic appendicitis and occasionally leads to misdiagnosis. Pasteurization can kill this bacterium; however, it happens sometimes that the heat-treatment is not strong enough or recontamination may occur; the bacterium can thus multiply also under refrigeration temperatures [16]. However, *Y. enterocolitica* incidence in raw milk and low-heat-treated milk products is reportedly low and only a few positive results have been recently reported within the EU [16].

Coxiella burnetii is the causative agent of Q fever. It can infect several animal species, such as cows, sheep, goats, but it is by far the main infectious agent of humans. In them, *C. burnetii* shows up with flu-like symptoms leading to endocarditis and hepatitis. It is relatively heat-resistant but is killed by regular pasteurization treatments.

Ensuring the safety of raw drinking milk can hence be very difficult. The control of handling and storage temperatures can be an approach to maintain the microbiological stability and shelf-life of milk, because for some bacteria present in raw milk higher temperatures are required to grow; however, also when milk is cooled and stored properly at <4 °C, bacterial multiplication is not limited for all bacteria. The growth limitation, for example, is not applicable to psychrotrophic bacterial pathogens which may multiply at these temperatures.

3.3. Assessment of Public Health Risks upon Raw Milk Consumption

3.3.1. Quantitative Microbial Risk Assessment

In situations like this, when public health risks related to consumption of raw milk are claimed by the scientific community and, on the other hand, raw milk is consumed because perceived as more natural than heat-treated milk, the establishment of approaches based on risk analysis is to recommend.

Generally speaking, risk analysis is a process comprising three components: risk assessment, risk management and risk communication [39]. Within a framework of microbiological hazards in foods, risk assessment is the tool by which the quantitative probability of illness cases in population can be expressed.

Quantitative microbial risk assessment (QMRA) provides, in fact, the magnitude of the prevailing risk on the basis of outputs (e.g., epidemiological data) and is the means which enables real public health risks to be assessed by health authorities, so that risk intervention scenarios can be designed and risk management options for implementation of intervention actions can be eventually identified and selected.

Over the last ten years, QMRA models have been developed in Australia [40] and New Zealand [41], in the United States [42,43] and in Europe, specifically in Greece [44], Italy [45–50] and the United Kingdom [51], to evaluate the real risk associated with consumption of raw and/or pasteurized milk.

In details, the predicting models developed to estimate the risk of disease after consumption of raw drinking milk regarded the risk of campylobacteriosis [40,41,45,47], listeriosis [41,43,46], hemolytic uremic syndrome (HUS) [41,45,48], salmonellosis [41,46] and staphylococcal disease [42,50] (Table 3).

Table 3. Microbiological hazards upon raw milk consumption analyzed in the currently available QMRA models.

Reference	Country	Scenarios under Consideration	Hazards				
			Campylobacter spp.	*L. monocytogenes*	*Salmonella* spp.	*S. aureus Staphylococcus enterotoxin A*	STEC
[40]	Australia	Farm gate consumption Off-farm sale Sale at retail outlets	√	√	√	-	√
[41]	New Zealand	Farm gate consumption Farm gate sale Off-farm sale Sale at retail outlets	√	√	√	-	√
[42]	United States	Pathogen growth and staphilococcal enterotoxin A production scenarios Storage conditions (various times and temperatures)	-	-	-	√	-
[43]	United States	Farm gate consumption Off-farm sale Sale at retail outlets	-	√	-	-	-
[45]	Italy	Storage scenario (best and worst storage conditions)	√ (*C. jejuni*)	-	-	-	√
[46]	Italy	Storage scenario (best and worst storage conditions)	-	√	√	-	-
[47]	Italy	Storage scenario (best and worst storage conditions) Boiling and not boiling milk	√ (*C. jejuni*)	-	-	-	-
[48]	Italy	Storage scenario (best and worst storage conditions) Boiling and not boiling milk	-	-	-	-	√
[50]	Italy	Pathogenity of multiple strains of a single pathogen Consumer behavior at household level	-	-	-	√	-

The models developed by Koutsoumanis and colleagues (2010) [44] and Barker and colleagues (2013) [51] investigated the risk of listeriosis and staphylococcal disease, respectively, in pasteurized milk.

Crotta and colleagues (2016a) assume that storage duration and temperature before consumption might have a critical influence in the final outcomes of the predicting model and thus lead to an overestimation of the risk. They recognize the importance of assessing the risk from farm to table, with a focus on consumer behavior at the household level when milk is no longer under the control of professionals and no enforcement by law can be applied. However, they highlight that the model outputs do have a dependence on the likelihood of a raw milk serving actually being consumed for any storage time-temperature combination. They thus drew the conclusion that ignoring spoilage of raw milk in QMRA models and, hence, assuming that milk is always consumed, regardless of any occurring organoleptic modifications during storage, is not realistic and strongly influences the model outputs [49].

3.3.2. Developed Models

So far, the developed models estimate the risk of illness per serving and/or per year upon consumption of raw milk. All of them were elaborated on the basis of data on prevalence of hazards in raw milk, obtained in previous monitoring actions, as well as on the basis of data on pathogen dose per serving size, dose response and consumption habits. They also take into consideration different scenarios. Consumers can, in fact, obtain raw milk from several sources, and the related pathways are key elements to consider when assessing and managing risk. In some countries, raw milk sale is allowed only on the farm premises, where consumers either bring their own containers and have them filled directly from the bulk tank; and/or in other cases consumers can purchase bottled raw milk from on-farm stores and/or they do it from retail stores. The risk can be also assessed depending on the demographics of the consuming population, e.g., in children, in the intermediate-age population, in perinatal or elderly people, because of a supposed higher susceptibility of some consumers.

These models show a likely link between raw milk consumption and public health risks, especially in some cases, e.g., when some bad storage conditions are applied and/or when the "to boil" indication is neglected. However, due to a lack of epidemiological data, the burden of disease cannot be completely and ever assessed. Further shortcomings in the elaborated predictive models have also emerged—data on the proportion of raw milk sold directly to consumers are limited or lacking; data on the prevalence of hazards in raw milk are lacking; servings are sometimes estimated and not measured; data on the incidence of specific pathogens are based on passive surveillance and underestimate their true incidence.

Moreover, the magnitude of estimates differs by several orders among models, hence any comparison among results might be difficult to be performed.

However, the currently available predicting models can allow to identify the main sources of contamination, highlight the critical points along the milk production and supply chain where contamination is likely to occur the most, and last but not least they can enable the identification of data gaps and control options [4]. By far, improving on-farm hygiene, developing educational programs for consumers might contribute to decreasing the number of predicted cases of illness.

3.4. Heat Treatment of Raw Milk

Milk is exceptionally rich in macronutrients, namely amino acids, lipids and sugar and micronutrients, such as vitamins and minerals. Due to this richness in nutritive components, it is a fertile medium for the growth of microorganisms that may cause milk spoilage and also provoke food-borne diseases in humans. Moreover, enzymes also occur in milk that contribute to the onset of undesirable changes during milk storage. Hence, milk commonly undergoes industrial processing in order to make it safe for human consumption and prolong its shelf-life.

Heat treatment is the most common way of preserving milk and make it safe. The main goals of heating are (i) killing pathogenic microorganisms, (ii) inactivating most (>95%) spoilage organisms and (iii) inactivating enzymes, native to milk or excreted by microorganisms, responsible for the reduction of milk keeping quality.

The most common heat treatments widely used in the dairy industry to achieve milk safety and preservation are pasteurization and UHT (ultra-high temperature) sterilization. Thermization and in-bottle sterilization are also performed on raw milk.

Basically, the above-mentioned heating treatments differ in the heat loads, specifically in the temperature and duration of heating. The choice of the heat treatment to be applied mainly depends on a trade-off among milk safety, extent of milk shelf-life and changes in milk quality. The heat load necessary to achieve milk safety depends, at its turn, on the microbiological quality of raw milk and on the growth potential of spore-forming bacteria after heating. Consumer preferences and target market should be also considered in choosing heating treatments.

Definitely, several combinations of treatment temperature and time can be used and different categories of milk are thus obtained (Table 4).

Table 4. Milk heat-treatments and effects on microbiological, organoleptic and nutritional quality.

Heating Treatment	Heating Conditions	Milk Category	Shelf-Life and Storage Conditions	Microbiological Effect	Nutritional Effect	Organoleptic Effect
HTST pasteurization	72 °C for 15 s (commonly 75 °C for 20 s)	Pasteurized milk	Refrigerated conditions (<7 °C for 3–21 days based on raw milk quality)	Inactivation of pathogens (included *M. tuberculosis*), molds, yeasts and most bacteria (not all vegetative bacteria are killed).	- Little impact on casein structure; - minor changes to whey protein structure; - loss of lysine; - no effect on fatty acid profile; - decrease of most vitamin content but little impact on total dietary intakes thereof; - no effect on milk mineral content and bioavailability.	No heating flavors
High-temperature pasteurization	≥85 °C for 20 s (usually 115–120 °C for 2–5 s)	High-pasteurized milk	Refrigerated conditions (<7 °C for 45–60 days based on raw milk quality)	- Inactivation of pathogens and all vegetative microorganisms; - Bacterial spores are not killed; - Milk enzymes are not fully inactivated.	- Little impact on casein structure; - denaturation of whey protein structure; - loss of lysine; - no effect on fatty acid profile; - decrease of most vitamin content but little impact on total dietary intakes thereof; - no effect on milk mineral content and bioavailability.	Cooked flavor

Table 4. *Cont.*

Heating Treatment	Heating Conditions	Milk Category	Shelf-Life and Storage Conditions	Microbiological Effect	Nutritional Effect	Organoleptic Effect
UHT treatment	135–150 °C for 1–4 s (commonly >140 °C for 5 s)	UHT milk	Non-refrigerated conditions (<32 °C) for 3–12 months	- All pathogenic and non-pathogenic microorganisms and spores are destroyed; - Milk enzymes are inactivated; - Some bacterial proteinases and lipases are inactivated.	- Denaturation of whey protein structure; - loss of lysine; - no effect on fatty acid profile; - decrease of most vitamin content but little impact on total dietary intakes thereof; - no effect on milk mineral content and bioavailability.	Cooked and ketone flavor, browning
In-bottle sterilization	105–120 °C for 20–40 min (commonly 110 °C for 30 min)	Sterilized milk	Non-refrigerated conditions (<32 °C) for 8–12 months	- All pathogenic and non-pathogenic microorganisms and spores are destroyed; - Milk enzymes are inactivated; - Some bacterial proteinases and lipases are inactivated.	- Denaturation of whey protein structure; - loss of lysine; - no effect on fatty acid profile; - decrease of most vitamin content but little impact on total dietary intakes thereof; - no effect on milk mineral content and bioavailability.	Sterilized-caramelized flavor and browning

3.4.1. Pasteurization

According to the Codex Alimentarius, pasteurization is a "microbiocidal heat treatment aimed at reducing the number of any pathogenic microorganisms in milk and liquid milk products, if present, to a level at which they do not constitute a significant health hazard. Pasteurization conditions are designed to effectively destroy the organism *Mycobacterium tuberculosis* and *C. burnettii*" [52].

On the basis of the temperature and the time applied, pasteurization can be classified as HTST (high-temperature short-time) pasteurization and LTLT (low-temperature long-time) pasteurization. The former is also referred to as "low pasteurization" and the condition commonly used for milk is 72 °C for 15 s, whereas the LTLT pasteurization is performed at 63 °C for 30 min or at 68 °C for 10 min [52]. Moreover, the HTST pasteurization is carried out as a continuous operation consisting on heating milk in a heat exchanger and holding it for the required time necessary to the destruction and/or inhibition of any hazardous microorganisms. The LTLT pasteurization is performed as a batch operation, that is, milk is placed in a container and then heated to a certain temperature for sufficiently long time to eliminate pathogens.

Heating conditions (temperature and time) depend on the raw milk microbiological quality, on milk fat or sugar content and also vary from country to country based on microorganism strain heat resistance. Thus, pasteurization can be performed also at temperatures higher than 85 °C for 30 s ("high pasteurization"). The increase in temperature and/or an extension of holding time is recommended to inactivate heat-resistant strains of *L. monocytogenes*, *E. coli* and *Campylobacter* spp. [53]. A more intensive heating is also applied to eliminate MAP.

However, a severe heating treatment may affect negatively the keeping quality of milk. For instance, the spores of *Bacillus* spp. may germinate and grow because of heat shocking and the keeping quality of pasteurized milk may be thus reduced [54]. The best keeping quality of pasteurized milk is achieved by using temperatures below 77 °C that do not inactivate the lactoperoxidase enzyme (LPO) and do not stimulate the growth of spores.

3.4.2. UHT Sterilization

The UHT treatment is a sterilization process that has been defined by the Codex Alimentarius as "the application of heat to a continuously flowing product using such high temperature conditions for such time that renders the product commercially sterile at the time of processing. When the UHT treatment is combined with aseptic packaging, it results in a commercially sterile product" [52].

The heating is commonly in the range 135–150 °C for 1 s up to 4 s, in order to achieve "commercial sterility", that is, low probability for microorganisms to grow in the product under the normal conditions of storage.

The UHT process can be performed by "direct" or "indirect" heat transfer. In the direct UHT treatment, superheated steam is mixed with milk. In detail, steam may be injected into milk (steam injection) or milk may be sprayed into steam (steam-infusion). In the indirect system, a heat exchanger transfers heat across a partition between milk and steam or hot water.

One drawback of the indirect method is the possibility of plant to fouling. In the lower-temperature section (<100 °C), whey proteins—mainly β-lactoglobulin—denaturate and deposit, while in the higher-temperature section (>100 °C) calcium phosphate deposits due to its reduced solubility. Thus, a decrease in heat transfer and an increase in pressure might be observed.

The shelf-life of UHT milk may be up to 12 months, despite it is usually consumed much earlier.

3.4.3. In-Bottle Sterilization

In-bottle sterilization is commonly performed at 110 °C for 30 min, however temperatures ranging from 105 °C to 120 °C for 20–40 min can be used [55]. All pathogens and non-pathogens microorganism are destroyed, as well spores. A 9-log reduction in the spores of thermophilic bacteria and 12-log reduction of *C. botulinum* are obtained. All milk enzymes are inactivated but not all bacterial lipases and proteinases.

Nevertheless, this processing shows some drawbacks such as slow product heating and cooling and limitation of temperatures, due to the generated internal pressures. It has also detrimental effect on organoleptic and nutritional quality of milk.

3.4.4. Thermization

Thermization is a heat treatment usually performed at 60 to 69 °C for 20 s. The main purpose is to kill bacteria, especially psychrotrophics, thus preventing the production of heat-resistant lipases and proteinases that may impair the milk keeping quality. Thermization thus enables to extend the storage time of raw milk before processing and to enhance the keeping quality of milk [56]. Nevertheless, it does not ensure milk safety, as it cannot completely eliminate pathogens—*L. monocytogenes* can grow in chilled-stored thermized milk [57] and the effect on *M. bovis* and *C. burnetii* is limited [55].

3.5. Heat Treatment and Milk Quality

Milk heat treatment mainly aims at achieving its safety for human consumption by killing pathogens and/or reducing microorganisms which may cause spoilage. However, changes also in organoleptic and nutritional properties of milk occur during heat treatments depending on the heat load. They are discussed in the following sections.

3.5.1. Microbiological Effect

The microbiota of raw and heat-processed milk deeply differs. As mentioned above, pasteurization was, in fact, conceived to destroy vegetative pathogenic microorganisms, that are the main causative agents of milk-borne diseases.

Salmonella spp., *C. jejuni*, *E. coli*, *L. monocytogenes*, *Y. enterocolitica*, *Brucella* spp. do not generally survive pasteurization (Table 5). Spores of pathogens such as *C. botulinum*, *Clostridium perfringens* and *B. cereus* are, however, not eliminated by heat treatment [56,58], although a very low disease incidence is reported. In particular, the spores of *C. perfringens* do not represent a health hazard in pasteurized milk because they are not able to germinate and grow at refrigeration temperatures. On the other hand, the spores of *B. cereus* can grow at low temperatures and cause milk-borne disease outbreaks.

S. aureus does not survive pasteurization but it may produce heat-stable enterotoxins which are very resistant to heating and pasteurization. In particular, the enterotoxin A can remain active upon heat-treatment at 121 °C for 28 min. Recently, Rall and colleagues (2008) [59] screened raw and pasteurized milk samples for *S. aureus* and found it in 70.4% of raw milk samples, in eight samples of pasteurized milk before the expiration date and in 11 samples analyzed on the expiration date.

The effect of pasteurization on MAP is controversial [24]. According to Ryser (2012), it can survive HTST pasteurization (72 °C for 15 s) and can be present as a post-process contaminant [60]. *M. bovis* is, on the other hand, killed by pasteurization.

Despite *C. burnetii* is the most heat-resistant non-sporulating pathogen present in milk, it does not survive regular pasteurization that was designed to achieve at least a 5-log reduction of *C. burnettii* in whole milk [54].

Milk that has undergone a correct pasteurization treatment is, therefore, unlikely to cause disease [61]. However, in case inadequate heat treatments were applied or recontamination events occurred after pasteurization, *Salmonella* spp., *L. monocytogenes*, *C. jejuni*, *Y. enterocolitica*, STEC, *B. cereus*, *Mycobacterium* spp., *S. aureus*, or *C. botulinum* may be present in milk and dairy products [62,63].

As regards spoilage microorganisms, thermolabile psychrotrophs are killed by pasteurization but post-process contamination and/or heat resistance can occur. For instance, during the filling process pasteurized milk may be contaminated by Gram-negative psychrotrophs. The presence and count of psychrotrophs in pasteurized milk depends on the initial count before the heat-treatment. *Pseudomonas* spp. have been long considered heat-sensitive and unable to survive pasteurization;

however, new analytical methods (culture-independent) have revealed that the *Pseudomonas* population is reduced, rather than eliminated, by pasteurization [15]. This implies that damaged but potentially metabolically active cells are present after the heat treatment. They are hence the most dominant microorganisms present in pasteurized milk, together with *Flavobacterium* which are also present but to a lesser extent. *P. fluorescens* is the main causative agent of off-flavors in milk, e.g., stale, cheesy, sour and bitter [24]. *Lactobacillus* and *Lactococcus* are only rarely found in pasteurized milk. Acidification occurs only when milk is left at room temperature. Low pasteurization also ensures killing of all yeasts and molds that can be in raw milk.

The UHT process destroys all vegetative bacteria (both pathogenic and non-pathogenic) and most spore-formers. However, raw milk quality is a key factor affecting the quality of UHT milk. If a relatively high population of sporeforming bacteria is present in raw milk, a low amount thereof may survive the UHT treatment. For instance, the bacterium *B. sporothermodurans* produces highly-resistant spores [28]. They do not cause spoilage, except a slight discoloration of the milk. However, as reported in a bulletin by the International Dairy Federation, it is very tough to remove them from equipment, and contamination thereof has often been the reason of shutting some UHT plants [24].

Raw milk destined for UHT treatment should be stored at less than 5 °C for no more than 48 h after milking. In the case that raw milk is stored at higher temperatures and/or for longer time, psychrotrophic bacteria may grow as well, and produce lactic acid, which causes a reduction of milk pH and a flat sour defect [24]. Enzymes, such as proteases and lipases, may alter the organoleptic properties of milk, in terms of bitter flavor, gelation and rancid flavor. The UHT process does not inactivate some of the enzymes produced by psychrotrophic bacteria, such as *Pseudomonas* spp. [54].

Heat-resistant thermophiles, like *Geobacillus stearothermophilus* and *B. licheniformis*, might be also encountered in UHT milk; nevertheless, they do not grow in milk stored at less than 30 °C. *Bacillus* spp. can be also found in UHT milk, although it is controversial if their presence is due to post-sterilization contamination or heat-resistance. The most detected species are *B. licheniformis*, *Bacillus coagulans*, *Bacillus badius* and *B. cereus*. The latter is, however, unlikely able to survive UHT treatment [24,64], that implying post-sterilization contamination.

Table 5. Survival of microorganisms to heat treatments.

Microorganisms	Survival to Pasteurization	Survival to UHT
S. aureus	√ (enterotoxins)	√ (enterotoxins)
C. jejuni	×	×
Salmonella spp.	×	×
E. coli	×	×
L. monocytogenes	×	×
Y. enterocolitica	×	×
Mycobacterium avium subsp. *paratubercolosis*	√/×	×
M. bovis	×	×
B. cereus	√ (spores)	×
Clostridium spp.	√ (spores)	√ (spores)

√ = survive; × = not survive; √/× = controversial.

3.5.2. Nutritional Effect

Milk contains nearly all the nutrients necessary to sustain life and because of their balance, milk nutritional value is particularly high. The composition of milk varies, depending on the mammal species, the animal status and health and the feed. Heat treatments also influence the nutritional profile of milk.

In the following sections, the effect of milk processing by heating on nutrients is discussed.

Proteins and Enzymes

Milk contains caseins and whey (or serum) proteins. The former represent 80% of milk proteins, and they are precursors of bioactive compounds with antimicrobial activity. They form micelles, containing calcium and phosphorus. Caseins are not heat labile and they do not undergo heat denaturation (in contrast to whey proteins). However, very severe heat treatments may dephosphorylate, hydrolyze or aggregate them. In detail, they can aggregate, and coagulation can occur. Other factors, such as low milk pH and the Ca^{2+} activity, may determine their coagulation [65].

Whey proteins include α-lactalbumin, β-lactoglobulin, serum albumin, immunoglobulins and bioactive peptides, and have important physiological properties. Heat treatments cause their denaturation, as a consequence serine, serine phosphate, glycosylated serine, cysteine and cysteine residues are formed. These compounds may undergo β-elimination and form dehydroalanine, which can react with several amino acids producing proteins that are not hydrolyzed by the intestinal tract. The nutritional value of milk is thus decreased.

Generally speaking, pasteurization little affects casein structure and causes minor changes to the structure of whey proteins [61,66]. However, no significant changes in the nutritional quality of milk protein due to pasteurization were observed in animal and human studies [67,68]. In contrast, Lacroix and colleagues (2008) observed in a human study that UHT treatment modifies the digestive kinetics and hence the metabolism of dietary proteins [68].

As far as amino acids are concerned, the main essential amino acid in milk is lysine. Heating determines lysine losses, ranging between 1% to 4%, while its effect on the other amino acids is negligible [61]. Lysine losses are caused by the extensive Maillard reaction that takes place during heat treatments, especially in in-bottle sterilization. A partial loss of lysine has been observed in UHT milk also during storage. Nevertheless, the loss of this amino acid is not serious, because in milk protein lysine is in excess [65].

Several indigenous enzymes are also present in milk, and the heating treatments it commonly undergoes can denaturate them. As a consequence, the activity of the enzymatic systems is used as an index of the thermal treatments milk undergoes. The activity of alkaline phosphatase is used to monitor the efficacy of pasteurization; therefore, the inactivation of the enzyme ensures that all non sporeforming pathogens have been killed. The activity of lactoperoxidase is used as an indicator for heat treatments more severe than low pasteurization. Gamma-glutamyl-transferase is also used to detect milk treatment above 77 °C.

Lipids

The fat content of marketed milk is standardized by the removal of cream or the addition of whole milk, semi-skimmed milk or skimmed milk.

During heating treatments, physicochemical and chemical changes of milk lipids may occur. The UHT sterilization may increase the amount of free fatty acids. When the indirect system is used, a higher concentration of free-fatty acids is observed compared to the direct method.

At high temperatures, polyunsaturated fatty acids may be converted into conjugated isomers. It has been observed that conjugated linoleic acid has anti-carcinogenic properties [69]. Recently, Pestana and colleagues (2015) investigated the effects of pasteurization and UHT treatments on milk lipids and found no changes in fat level nor in fatty acid profile [70].

Lactose

Lactose is the main milk carbohydrate. It has prebiotic properties and promotes the absorption of calcium and magnesium.

Pasteurization has no effect on lactose, while treatments at higher temperature, such as UHT sterilization, induce the isomerization to lactulose and the formation of acids and Maillard reaction compounds [61,71,72].

Commonly, the lactulose formation from lactose might be observed via the Lobry de Bruyn-Alberda van Ekestein transformation upon heating under slightly alkaline conditions. Since lactulose is not detectable in raw milk, it is used as a heat load indicator and then as an index of the severity of heat treatment that milk underwent.

Heat treatments above 100 °C also determine the degradation of lactose to acids, especially formic acid and lactic acid, and hence an increase in titratable acidity can be observed.

Lactose may also take part to the Maillard reaction that determines the formation of brown products and flavors.

Vitamins

It has been claimed that raw milk has a higher nutritional value than pasteurized milk since it provides a higher number of vitamins. Actually, the heat treatment conditions, in addition to the packaging type and storage conditions, may affect vitamin content in marketed milk.

Recently, Macdonald and colleagues (2011) performed a systematic review to evaluate the impact of pasteurization on vitamins in raw milk [73]. Forty studies assessing the effects of pasteurization on vitamin content were included, and it was found that vitamin B_{12}, vitamin E, vitamin C, folate and riboflavin (B_2) decreased upon pasteurization. In contrast, vitamin A increased and no significant effect of pasteurization on vitamin B_6 levels were found. Despite some vitamins are destroyed by heat treatments (e.g., vitamin C and folate), the contribution of vitamin content to the recommended daily intake (RDI) should be considered in order to compare the nutritional value of raw and heat-treated milk. For example, 20 L of raw milk per day should be consumed to achieve the vitamin C RDI, therefore the degradation thereof due to heat-treatment is not subject of matter. The same applies to vitamin B_{12} and vitamin E, thereof bovine milk is not an important source in occidental diets with a content of 2–5 μg/L and 10–30 μg/dL, respectively [74]. This implies that the effects of pasteurization on the adult daily intake of these vitamins cannot be a concern in diminishing the nutritive value of milk, just because milk is not a primary source thereof.

This explains also the establishment of food fortification programs for vitamins in milk as a public health intervention in Canada and many other countries to correct and/or prevent nutrition problems of public health significance [75,76].

However, vitamin C protects folic acid from oxidation and its breakdown is connected with that of vitamin B_{12}. As far as vitamin B_{12} is concerned, 250 mL of raw milk contribute to more than 80% to the RDI, while in UHT milk the contribution decreases to about 70% [48].

Minerals

Milk is a good source of some minerals, especially calcium and phosphorous, and no significant differences between raw and heat-treated milk in the content thereof have been reported. Moreover, heat-treatments have no effects on the bioavailability of these nutrients [61].

3.5.3. Organoleptic Effect

Milk of good quality has a slightly sweet taste, a very little odor and a smooth and rich feel in the mouth. It is characterized by whiteness and glossiness.

The heat treatments required to achieve milk safety may influence the organoleptic properties of this food, depending on the heating load. Specifically, they affect the flavor and color of milk.

Each heat treatment causes a distinctive flavor profile. Some flavors are induced by heat treatment, others (caused by microorganisms or enzymes) are reduced or annulled.

The typical "cowy" flavor of fresh milk is reduced or masked due to the formation of flavor compounds, such as cooked flavor, UHT ketone flavor and sterilized-caramelized flavors.

Cooked flavor is mainly caused by the Sulphur compounds originating from denaturation of whey protein. As a matter of fact, the denaturation of whey protein exposes sulfhydryl groups that may form sulfhydrylic acid and dimethyl sulphide. The latter are responsible for the cooked flavor of milk undergone to severe heat treatment, such as high pasteurization and UHT treatment [65]. Nevertheless, freshly processed UHT milk has a "cooked" and "cabbage" flavor that however partly disappears during the first week after processing, due to the oxidation of Sulphur compounds. Indirectly, processed milk has a more intense cooked flavor. In addition, stale flavors may develop due to the higher level of dissolved oxygen [54].

Ketone flavor originating in the lipid fraction is also present in UHT milk.

The caramel-like flavor is also called "sterilized flavor", since it is distinctive of sterilized milk. This flavor is caused by the Maillard reaction that also leads to browning.

Moreover, the organoleptic properties of milk are influenced by storage conditions and by the microbial ecology of heat-treated milk. For instance, microbial growth may cause off-flavors. Psychrotrophic bacteria cause putrid flavors, lactic acid bacteria cause sour flavor, *Bacillus circulans* causes a phenolic flavor in in-bottle sterilized milk, *B. cereus* leads to very unclean flavor [65].

Milk enzymes may contribute to the development of milk flavor. The proteolysis of plasmin in UHT milk leads to a bitter flavor and the lipolysis by lipoprotein lipase causes a rancid flavor in low-pasteurized milk.

3.6. Scientific Evidence behind Claimed Health Benefits of Raw Milk Consumption

The consumption of raw milk has been associated to benefits on human health, such as a higher nutritional value and protection against the lactose intolerance, and asthma and allergy diseases. In contrast, the heating treatment is reported to have detrimental effects on these benefits. The nutritional value of raw milk has been compared to the heat-treated milk in the above sections. Following paragraphs report the role of raw milk in the management of lactose intolerance, and asthma and allergy disorders.

3.6.1. Raw Milk and Lactose Intolerance

Lactose is the main carbohydrate in mammal milk and milk products. The inability to digest lactose is referred to as lactose intolerance and it is due to the lack of the enzyme lactase. The main symptoms include flatulence, bloating, diarrhea and abdominal pain.

The incidence of lactose intolerance increases with age and varies by community and ethnic group [77]. It has been estimated that lactose intolerance affects 65% or more of the total human population. In Asia and in North and South America the percentage of adults unable to tolerate lactose in their diet is very high, while in Ireland and Northern European countries lactose intolerance is rare with 74% to more than 90% of population being lactose tolerant [78].

Recently, raw milk consumption has been claimed to reduce lactose intolerance. It has been suggested that raw milk contains natural lactase enzymes that are not found in heated milk, as they are destroyed by heating. However, a lack of scientific evidence supporting this claim exists. Claeys and colleagues (2013) report that both raw and heated milk contain no lactase, and the production thereof by lactic acid bacteria in raw milk is limited, since raw milk must be stored at refrigerated condition due to safety reasons [61]. In contrast, yogurts are tolerated better than milk, as they contain bacteria having lactase enzyme.

A pilot study on adults positive for lactose malabsorption was recently performed by Mummah and colleagues (2014) in order to assess whether raw milk consumption can reduce lactose intolerance symptoms. No significant differences of intolerance symptoms emerged when subjects consumed raw vs pasteurized milk [79]. Additional studies, possibly with larger study groups, are needed in order to support or refuse the claimed protection of raw milk consumption against lactose intolerance.

3.6.2. Raw Milk and Protection against Asthma and Allergies

Beneficial effects of raw milk consumption on human health have been claimed. Among them, an inverse association between raw milk consumption in childhood and the development of asthma, allergies and atopy has been reported [80].

Asthma and allergies have dramatically increased in last decades, especially in Westernized countries [81]. The raise in the incidence and prevalence of atopic disorders that has been observed over the last 30–40 years has occurred within a time span too short to be explained by a genetic shift in the population, thus environmental and/or lifestyle changes might have significantly contributed to this trend. An increase of asthma prevalence by 50% every decade is reported by Braman (2006) [82] and, as a consequence, the morbidity and mortality rates and economic burden associated with asthma management has raised, as well.

The "hygiene hypothesis" formulated by Strachan (1989) [83] reports an inverse relationship between family size and development of atopic disorders and suggests that a lower incidence of infections in early life could boost the rise in allergic diseases.

Within this hypothesis, it has been supposed that a lifestyle enabling exposure during the childhood to microbes, such as farm-living, may have a protective effect against the onset of allergies. Several European cohort studies focused on the association between farm-living and allergy and asthma in children, namely the European Allergy and Endotoxin (ALEX), the Prevention of Allergy Risk Factors of Sensitization in Children Related to Farming and Anthroposophic Lifestyle (PARSIFAL), and the multidisciplinary study to identify the genetic and environmental causes of asthma in the European community (GABRIEL). Evidence of lower incidence and prevalence of asthma, hay fever and atopic sensitization in children exposed to farming lifestyle has been extensively reported [84–92].

In contrast to the above-mentioned cohort studies, other Authors found farming to be not protective against the development of atopic respiratory disorders [93,94].

The term "farming" actually includes several habits, namely exposure to farm animals, to barns and stables, to endotoxins and to the consumption of farm milk (that is unpasteurized milk). The association between each "farm-factor" and allergy disease risk was evaluated, in order to identify the aspects of farming lifestyle that explain the inverse association. As far as farm milk consumption is concerned, Riedler and colleagues (2001) showed, within the ALEX study, that the consumption of raw milk reduced the development of asthma, hay fever and atopic sensitization and the protection was higher in children younger than one year than in those aged 1–5 years [87]. Perkin and Strachan (2006) investigated the association between different farming factors and the prevalence of allergic disorders in children living in English rural farming and non-farming areas. They found that farmers' children had less current asthma symptoms and seasonal allergic rhinitis but non-eczema symptoms. In contrast, the consumption of unpasteurized milk was associated with less eczema symptoms [95]. Hence, the protective effect was associated with the consumption of unpasteurized milk and was independent of farming status.

Ege and colleagues (2007) also found that farm milk consumption was inversely related with asthma prevalence in children, but pig keeping and frequent stay in sheds also acted as protective factors [96]. Waser and colleagues (2007) showed an inverse association between farm milk consumption and childhood asthma, rhinoconjunctivitis and sensitization to pollen, while other farm produced foods were not related to asthma and allergy prevalence [90]. Data were collected within the PARSIFAL study, a cross-sectional multicenter study including almost 15,000 children aged 5–13 years from five European countries and with different lifestyles: some lived in rural areas, others in (sub)urban area, other had an anthroposophical lifestyle, including restrictive use of antibiotics, antipyretics and vaccinations. As previously observed by Riedler and colleagues (2001) [87], they found that the association between farm milk consumption and development of asthma/allergy was most evident in children consuming farm milk since their first year of life. Unfortunately, results from this survey are based on questionnaire data, and no objective confirmation of the raw milk status of the farm milk is available. Some parents explained they boiled milk prior to consumption, others consumed milk as raw milk.

More recently, Loss and colleagues (2015) studied the effect of consuming raw, boiled and industrially processed milk on common infections in the first year of life, in a prospective cohort study including about 1000 children from rural areas of 5 European countries. It emerged an inverse association between the consumption of raw cow's milk and rhinitis, infections of the respiratory tract, otitis and fever. Boiled farm milk showed a similar but milder effect. Heat-processed milk, except UHT, was found to protect against fever [97].

The timing of exposures also appears to deeply affect the possible protection against allergy disorders. In detail, an early-life exposure was found protective. Besides the evidence in the ALEX study [82], Radon and colleagues (2004) also observed a greater protection from allergy risks by exposure to animal buildings during the first year of life or between ages 3 and 5 [98]. Even the prenatal exposure to farming environment was reported to affect the atopic sensitization at birth. Ege and colleagues (2007) evaluated data from PASTURE cohort study and highlighted that maternal lifestyle during pregnancy, included the use of boiled or un-boiled farm milk, affects the production of fetal IgE, determined in cord blood at birth [99]. In contrast, later life exposures of children do not provide any protection or may exacerbate symptoms [89].

Despite the scientific evidence about protection against allergy disorders, the consumption of raw milk still remains to be discouraged.

3.7. Novel Milk Processing Technologies

Thermal processing of milk is the oldest and most common treatment of raw milk before it is deemed fit for human consumption. However, heat treatments may have some drawbacks, such as changes in the organoleptic properties and lower nutritional value thereof. The increasing demand among consumers for fresh-like products, which are more nutritious and of higher organoleptic quality than heat-treated milk, has led to the emergence of alternative thermal and non-thermal milk processing technologies.

Some of the new technologies can meet these demands. Hence, they have captured the attention of the scientific community, governments, as well as food industries endeavoring to stay one step ahead in terms of technology.

These technologies include among others: ohmic and microwave heating, pulsed electric fields, high hydrostatic pressure, microfiltration and ultrasound.

In novel thermal technologies, such as ohmic heating and microwave heating, rise in temperature in the product is mainly responsible for the effect on milk microbial safety, as in conventional methods. In contrast, non-thermal technologies do not involve heat to kill microorganisms. As a consequence, the detrimental effect that conventional thermal treatment has on milk quality is reduced.

3.7.1. Ohmic Heating

The application of ohmic heating (OH) to milk was known since the 19th century [100], but it fell into disuse due to the high cost of electricity, lack of materials suitable to electrode production, and to difficulties to control the process. Recently, improvements were made and it is currently used to blanch, pasteurize and sterilize milk, vegetable products, fruit preparations and meat products [101–104].

When OH technology is applied to milk, heat is generated directly within milk, by using electrodes contacting the food matrix. The latter actually act as an electrical resistor, thus converting the electrical energy into thermal energy.

All food matrices with an electrical conductivity in the range 0.1–10 S cm^{-1} can always be heated by using OH. The food matrix electrical conductivity increases with temperature; hence the effect of the treatment becomes more effective at higher temperatures [105]. Factors such as, food properties (conductivity, viscosity and specific heat capacity), the design of the treatment equipment and the output of the power supply influence the heating rates.

Dispersed systems show differences in conductivity that may cause a non-uniformity of heating with the formation of hot and cold spots, that are local high and low temperature peaks, respectively. As far as milk is concerned, the liquid phase is the most abundant, and it is also the fraction with the highest electrical conductivity. This results in a faster heating and in a heat dissipation towards the particulate fraction, thus compensating possible non-homogeneities during the heating [106]. Therefore, OH promotes fast and more uniform heating in the food matrix.

The rapid heating has a double advantage: the impact on food quality is reduced and the energy necessary for the treatment is lowered [106], thus resulting a more sustainable technology. A uniform heating also prevents the formation of regions at high or low temperatures that represent a critical point for food quality and food safety, respectively. Hot spots are a food quality problem due to their over processing [107], while cold spots are a food safety issue.

The OH has an additional advantage over the conventional heating: it reduces fouling that reduces the heat transfer rates and promotes the formation of biofilm on surfaces, thus compromising the microbial safety of the final product [108].

Despite the above-mentioned advantages over conventional heating, some issues remain on the way, namely the effects of the OH process on the physical and chemical properties of milk, the cost and difficulties in controlling the process parameters, and the effect thereof on fouling. In addition, so far, the impact of OH process on the allergenicity of milk and dairy products has not been investigated yet [108].

3.7.2. Microwave Heating

Microwave heating (MWH) consists on the use of electromagnetic waves of certain frequencies (300 MHz–300 GHz) to generate heat within products [109]. The heating is caused by the ability of materials to absorb microwave energy and then to convert it into heat.

Microwave heating application derives from the establishment of conditions which, on the one hand, provide the desired degree of safety and, on the other hand, guarantee a minimum product quality degradation.

The application of microwave heating to pasteurize milk has been well studied [110–118] and has been a commercial practice for quite a long time. The industrial setting up of microwave heating processes, nevertheless, faces two major issues. There is a non-uniform temperature distribution inside food product, creating temperature gradients within the product and resulting in hot and cold spots within the food matrix, moreover energy costs are high [119].

One of the key issues in assuring milk safety is, however, how effective the treatment is in inactivating microorganisms of public health concern yet preserving the quality of the product. Overall, pasteurization of milk by MWH can increase milk shelf-life over conventional pasteurized milk due to destruction of psychrotrophic bacteria [120].

According to some studies [110,111,121] heating of milk in a microwave oven at a temperature and time used in normal pasteurization are not successful in inactivating pathogens, such as *Salmonella typhimurium*. Outputs from the studies of Stearns and Vasavada (1986) and Galuska and colleagues (1989) showed that MWH causes sub-lethal injuries to milk-borne pathogens, such as, *L. monocytogenes*, *S. aureus* and *E. coli* [112,116]. Variations in the volume of milk treated by MWH can influence the inactivation of *L. monocytogenes* [111].

Insignificant loss of vitamin A, β-carotene, vitamin B_1 or B_2 also occurs [122]. In detail, Sierra and colleagues (1999) compared the heat stability of vitamins B_1 and B_2 in milk treated by continuous microwave heating and conventional system, and observed no significant losses in the vitamins during microwave heating at 90 °C without holding period; for vitamin B_2 a decrease by 3–5% during 30–60 s of holding was found [123].

A loss of approximately 17% for vitamin E and 36% for vitamin C can be observed [122]. Bai and colleagues (2015) have recently shown that milk layer thickness, microwave time and microwave power can be a significant factor affecting vitamin C concentration with milk layer thickness being the most influencing factor [124].

Microwave heating of milk does not affect fat components. As regards protein compounds, Lopez-Fandino and colleagues (1996) investigated the denaturation of β-lactoglobulin and the inactivation of alkaline phosphatase and lactoperoxidase using a modified microwave oven at

2450 MHz [114]. Upon comparison of the main outcomes of this study with results obtained by conventional thermal treatment in a plate-type heat exchanger, it emerged that the degree of inactivation caused by the thermal treatment was similar in the two cases. More recently, Raman (2007) found that denaturation of whey proteins was lower in milk pasteurized by MWH than by a conventional thermal process, whereas the denaturation of β-lactoglobulin was almost similar in both processes [122].

Contrasting opinions are reported on the influence of MWH on milk sensory profile. According to some studies, volatile components in milk conventionally treated and milk treated by MWH in continuous flow differ significantly [122]; Lopez-Fandino and colleagues (1996), on the other hand, maintained that the sensory characteristics of microwave-pasteurized milk were comparable to those achieved by traditional pasteurization after 15-day storage [114].

3.7.3. Pulsed Electric Field

The application of pulsed electric field (PEF) technology in food processing consists on the treatment of a food matrix, placed between two electrodes, with high voltage (5–20 kV) short (1–10 μs) electric pulses. The use of PEF to milk processing has been reported capable of inactivating unwanted pathogenic and spoilage bacteria while keeping sensory and nutritional attributes unaffected [125]. The inactivation of vegetative forms of microorganisms is due to the formation of hydrophilic pores in the cell membrane and to the opening of protein channels causing the loss of cell membrane functionality.

PEF has been found effective in the inactivation of *Pseudomonas* spp. that constitute the predominant microorganisms limiting the raw milk shelf-life under refrigerated conditions and also of *Listeria* spp., *Salmonella* spp., *E. coli*, *B. cereus*, *S. aureus*, *Brucella* spp., *Coxiella* spp. and *Enterococcus* [126] that can occur in raw milk.

This technology was found to be effective alone or in combination with mild heat treatment. Bermúdez-Aguirre and colleagues (2011) found that mesophilic and psychrophilic bacteria in raw skim milk were inactivated by PEF at 20–40 °C and a synergistic effect of the two treatments was suggested [127]. It also emerged that milk fat content possibly protects the mesophilic and psychrophilic bacteria from inactivation during combined PEF-heat treatment. More recently, McAuley and colleagues (2016) compared the impact of PEF at 53 and 63 °C and conventional heating at 63 °C and 72 °C on raw milk microbiological and physicochemical stability [128]. It emerged that PEF processing (22 μs at 30 kV/cm) at 63 °C achieves microbial stability in whole milk similar to thermal pasteurization (72 °C for 15 s). Compared to latter, PEF combined with heat treatment at 63 °C had no adverse effect on milk physicochemical properties. Moreover, raw milk processing by PEF (22 μs at 30 kV cm^{-1}) at 53 °C extends the shelf-life thereof by 3–4 days in refrigerated conditions (4 °C).

As far as the effect of PEF on milk enzymes is concerned, several studies were performed in order to test the enzyme stability in different dairy products. Buckow and colleagues (2012) investigated the effect of combined PEF/thermal treatments on lactoperoxidase (LPO) dissolved in simulated milk ultra filtrate, and found that LPO inactivation was mainly due to thermal effects; nevertheless, 5–12% inactivation may be related to electro-chemical effects [129]. More recently, Sharma and colleagues (2017) assessed the effect of PEF and thermal treatments both on whole bovine milk enzymes, such as alkaline phosphatase, xanthine oxidase, lipase and plasmin, and on microorganisms count over 21 days of storage at 4 °C [130]. It emerged that the effect of PEF on microorganisms and alkaline phosphatase activity immediately after the treatment and after 21-day storage at 4 °C was comparable to the effect of heat treatment. As far as xanthine oxidase and plasmin are concerned, their activities were reduced after PEF treatment, but at the end of storage period they were similar to raw milk. In addition, the lipolytic activity increased over storage. Hence, it emerged that PEF is suitable to process milk intended for cheese making, since enzymes involved in the development of flavor and aroma are retained.

From a nutritional point of view, it was assessed that the application of PEF 400 μs at 18.3–27.1 kV cm^{-1} did not affect the content of fat soluble vitamins (cholecalciferol and tocopherol) and water-soluble vitamins, except ascorbic acid [125].

Actually, the scale-up of PEF treatment was and still is an engineering challenge, since most studies were performed on small volume samples. Moreover, the scale up should ensure electric field uniformity and consider flow behavior, heat conduction and residence times [128].

3.7.4. High-Pressure Processing

High-Pressure Processing (HPP), also known as High Hydrostatic Pressure (HHP) or Ultra High Pressure (UHP), is a non-thermal treatment representing a clear alternative to traditional heat treatments. It involves application of high pressures at room temperature. Its main advantage is the retention of the food original freshness, color, flavor, taste and nutritional quality, and the non-thermal induction of cooked off-flavors, along with the inactivation of microorganisms. HP treatments are usually performed in the range of 100–1000 MPa at room temperature, or higher when spore inactivation is required (up to 60–80 °C) for up to 30 min [131].

Depending on the microbiological quality of milk, the effect of HPP application at 400–600 MPa may be comparable to pasteurization (72.8 °C, 15 s) [132], whereas it is not to sterilization because of the resistance of spores to HPP.

Application of HPP for microbial inactivation has been extensively studied and reviewed [133]. Overall, pressures ranging between 300 and 600 MPa can be effective in inactivating microorganisms, including foodborne pathogens, without damaging the nutritional and sensory characteristics of food.

Most vegetative forms of microorganisms can be destroyed at 600 MPa for 15 min and at 20–30 °C. Bacteria spores are more resistant to HP than vegetative cells and can survive at a pressure of 1000 MPa. *E. coli* and *L. monocytogenes* are reportedly the most pressure-resistant species at room temperature. Gram-positive microorganisms are more pressure resistant than gram-negative microorganisms. Yeasts and molds are the most sensitive to pressure [134]; most of them are inactivated within a few minutes by 300–400 MPa at 25 °C.

Endospores are far more resistant against HP, requiring treatment at pressures exceeding 1000 MPa and temperature higher than 80 °C for full inactivation [132].

Some authors have demonstrated some difficulties for HP to inactivate microorganisms [134], hence possible combinations of HP have been figured out, such as with mild temperatures (30–50 °C) and/or bacteriocins (nisin, pediocin, lacticin) which sometimes improve the inhibition of foodborne bacteria and spores.

As to milk quality, HPP can have a disruptive effect on milk casein micelles and the structure of whey proteins. β-lactoglobulin are easily denatured under pressure treatments of up to 500 MPa at 25 °C. Denaturation of immunoglobulins and α-lactalbumin occurs at much higher pressures and particularly at 50 °C [114].

Enzyme inactivation by HP is more difficult, as they are more resistant [135]. Resistance to pressures lower than 400 MPa at 25 °C is reported for alkaline phosphatase, lactoperoxidase, phosphohexose-isomerase and γ-glutamyltransferase [114,132].

Small molecules, such as, amino acids, vitamins, simple sugars and flavor components, are not affected by HPP and remain unaltered.

3.7.5. Microfiltration

Microfiltration (MF) is a non-thermal treatment method with the specific advantage of being very effective in the removal of bacterial spores in comparison with conventional pasteurization.

A major drawback of MF is fouling at the membrane surface which affects selectivity in an adverse way and requires frequent rinsing and cleaning procedures which can have a detrimental effect on the cost-effectiveness of the technology [136].

MF offers several opportunities to the dairy chain, as it allows milk products to keep organoleptic characteristics which are similar to fresh milk with improved shelf-life. A good number of micro-filtered milk is available on the market and its success is due to a perceived freshness and the abovementioned extended shelf-life.

3.7.6. Ultrasound

Ultrasounds are waves with a frequency higher than 20 kHz, with a distinction between low- and high-intensity ultrasounds, which have a power level of ≤0.1 MHz and 10–1000 W cm^{-2}, respectively [137].

In ultrasonic treatments, ultrasound waves travel though a liquid, alternating compression and expansion cycles. During the expansion cycle, high-intensity ultrasound causes the growth of existing bubbles which implode violently when they attain a volume at which they do not absorb more energy (cavitation phenomenon). At the implosion phase, locally very high temperatures (up to 5500 °C) and pressures (50 MPa) are reached inside the bubbles. This has a detrimental effect on microorganisms.

The main applications of ultrasound in milk and dairy products are due to its effect in inactivating bacteria and enzymes, homogenizing milk, extracting enzymes and lactose hydrolysis [134]. However, it has been stated that the energy consumption required in ultrasound application to kill microorganisms is higher than for conventional methods.

Moreover, it has been demonstrated that ultrasound on its own is not very effective for inactivation of microorganisms and enzymes in milk, hence combinations of ultrasound with heat (thermos-sonication) and pressure (mano-sonication) have been developed [137].

Effects on fat, whey proteins, caseins, alkaline phosphatase, lactoperoxidase and γ-glutamyltransferase have been so far evaluated. Ultrasound continuous-flow system has proved to be an adequate method for preservation and homogenization of milk [138].

Due to the slight effect on microorganisms and enzymes, it has been difficult for ultrasonic treatment to become a commercial process; however, it has a good potential as a minimal processing method in combination with other treatments.

Further investigations are, nevertheless, required to improve the processing equipment and to gain more insights into the effect of ultrasound treatments on milk main components.

4. Conclusions

Raw milk consumption represents a realistic threat for human health and a public risk, because it can act as a vector of pathogens and spoilage microorganisms. Milk processing via heat treatments ensures to achieve milk safety, however it does not completely allow the retention of raw milk primary organoleptic and nutritional characteristics. Good agricultural practices (GAP), good hygienic practices (GHPs) and good animal husbandry practices at the farm level enable to obtain a high quality raw milk, which at its turn allows the application of less severe heat-treatments and thus the preservation of the primary quality of raw milk.

Further investigations are required to explain the claimed protective effect that raw milk has on the onset of asthma and allergy disorders in children. Novel and alternative technologies should be optimized for possible production of industrial milk which is safe and perceived as fresh.

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