




Review

Trends and Opportunities in the Dairy Industry: A2 Milk and Processing Methods

Klara Żbik *, Anna Onopiuk , Elżbieta Górską-Horczyzak * and Agnieszka Wierzbicka

Department of Technique and Food Development, Institute of Human Nutrition Sciences, Warsaw University of Life Sciences—SGGW, Nowoursynowska 159c Street, 32, 02-776 Warsaw, Poland

* Correspondence: klara_zbik@sggw.edu.pl (K.Ż.); elzbieta_gorska-horczyzak@sggw.edu.pl (E.G.-H.)

Abstract: Milk is a valuable raw material with incomparable nutritional and technological properties. The dairy market is a fast-growing economical area with more and more innovations emerging recently. The review identifies contemporary trends in the dairy industry, focusing on specific types of A1 and A2 milk and their applications. The A2-type milk is a promising innovation with the potential to alleviate the problem of milk consumption associated with the BCM-7 peptide which is important for many consumers. An increase in its production could also positively impact on biodiversity. A1 and A2 milk have different properties which should be analyzed in future studies. An important topic is newly developed processing methods that allow obtaining safe dairy products without a significant impact on their nutritional value and functional and sensory properties. Thermal, non-thermal, and combined processing technologies are continuously developed. However, there is currently insufficient information on the impact of processing technology on A2 milk. This will likely change in the near future. The combination of the latest technological advances will soon make it possible to provide new, increased quality of dairy products to consumers.

Keywords: milk A1 and A2; beta-caseins; milk proteins; food processing; shelf-life; dairy products; non-thermal treatments



Citation: Żbik, K.; Onopiuk, A.; Górską-Horczyzak, E.; Wierzbicka, A. Trends and Opportunities in the Dairy Industry: A2 Milk and Processing Methods. *Appl. Sci.* **2024**, *14*, 6513. <https://doi.org/10.3390/app14156513>

Academic Editor: Anna Lante

Received: 22 May 2024

Revised: 14 June 2024

Accepted: 19 July 2024

Published: 25 July 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Mammals produce milk, a liquid food, to ensure that their offspring meet their nutritional needs in the first period of life. Milk proteins provide the essential amino acids, and amino groups enable the newborn to biosynthesize proteins necessary for growth. Milk also contains immunoglobulins, enzymes, enzyme inhibitors, and hormones, which increase newborn's chances of survival. An essential role of milk is also to provide energy. It is carried by lipids and carbohydrates, mainly lactose and unused proteins. Essential fatty acids, vitamins and minerals, and water are other groups of nutrients in milk [1,2]. Each species has a specific milk composition. Humans began consuming milk from other species over 8000 years ago, which influenced the evolution of our species and the expression of various genes (lactose digestion/lack of enzyme). This depends on the region from which the population group originated and whether they have traditionally consumed milk for generations [3,4].

Today, the milk market is growing every year. Fresh milk products (cheeses, yogurts, whey, powder), and also selected fractions of individual milk components (proteins, lipids, lactose) are used by the industry to produce and design advanced functional foods. Apart from the use of milk itself, the separation of its fractions to synthesize new products is also a meaningful trend that responds to contemporary consumer needs.

One trend currently gaining popularity in the dairy industry that could significantly impact human health and production economics is the differentiation of milk types and attention to this A2 type. During years of selection, the gene responsible for A1 casein in milk, rather than A2, the main protein, has been fixed. Recent studies indicate that A2-type

milk has different technological properties, e.g., longer rennet coagulation, higher curd firmness, and lower gel strength than A1-type milk [5–7]. More importantly, type A2 milk seems to cause fewer health consequences after consumption for people hypersensitive to A1 casein. Therefore, studying both types of milk and comparing their properties seems a reasonable direction for research. In the next years, the dairy industry may notice an increase in consumer interest in A2-type milk and A2 milk products. This trend also offers a choice of dairy products to people who, until now, have had health problems caused by the consumption of A1-type casein. Some researchers see the growing interest in A2-type milk as a factor that will modernize the dairy industry, which, needing to provide people with safe and high-quality milk, will be forced to move away from the current homogeneous status quo [8–13].

Apart from milk as a raw material, the second area of innovation is the way of its processing. It is directly linked to the previous trend as any change in the parameters of a long-known raw material requires a potential redesign of all processes to ensure high-quality products. In addition to optimizing thermal processes that have been successfully used in industries for years, attempts are also made to develop and industrially implement innovative non-thermal methods that could ensure the microbiological safety of milk and have minimal impact on its nutritional and sensory parameters. Reducing the population of pathogenic micro-organisms in milk is crucial for its health value and storability. Some scientists have pointed out that a switch from traditional methods could also, with appropriate optimization, reduce energy consumption [14].

The goal of this review is to present the latest trends in the dairy industry. First, the market and currently used milk preservation methods will be described, taking into account the available information on bovine milk types (A1 and A2) and indicating the gaps in this area of current knowledge. So far, most literature reviews on A2 milk have focused on the dietary aspects without summarizing the technological area. Therefore, this review presents dietary issues as a secondary consideration in this.

2. Global Market and General Trends

The current world-produced milk comes predominantly from cows (83%) [15]. According to the latest 2023 data, the largest producer of cow's milk in the world was the European Union (the sum of 27 countries in the community), with a production of 143 million metric tons, followed by the United States, with 104.1 million metric tons, and in third place was India with a production of 99.5 million metric tons [16]. From 2015 to 2023, the total world production of cow's milk increased from 496.84 million metric tons to 549.48 million metric tons, and the overall global production trend has been rising for years, which is likely to continue in the next years [17–19]. However, it is essential to note the developing trends in the dairy industry: growing consumer awareness and expectations of ethics and sustainable production practices, voices denying the desirability of sourcing milk in the face of the growing availability of plant-based products, and, in the future, possibly synthetic milk [20].

Milk is a valuable commodity not only as an unprocessed product, with the highest consumption in liquid form in 2023 reported in India at 87,450 metric tons, the European Union at 23,650 metric tons, and the United States at 20,900 metric tons, but also as a base for the production of highly nutritious food and intermediate products [21,22]. Milk powders with versatile properties and functionality (milk protein concentrates—MPCs and milk protein isolates—MPIs) are valuable to the industry for their pleasant taste and high nutritional value. They are increasingly used to enrich dairy products not only for technological reasons, but also for consumer expectations. This is possible thanks to the physicochemical characteristics of milk proteins (caseins) and the essential amino acids that make them complete proteins (and their peptides). They also provide the amino acids and amino groups necessary for the biosynthesis of endogenous amino acids [23,24].

In the past, milk production was carried out on small farms and animals remained in contact with humans. Since industrial revolution technology allowed for relentless increases

in production efficiency, dairying has become an industry dominated by corporations, intensifying possible profits [20]. Increasing interest in A2 milk may have economic and environmental benefits, causing a rebalancing between the relevance of corporate and smaller producers. The mere stimulation of farmers and producers to diversify their offerings counteracts the phenomenon of dairy market homologation, which translates into increased income for small farms, often located in unfavorable places, and allows them to compete with larger suppliers. This allows diversification and provides stability in the milk market, making it possible to guarantee a decent living for farmers and respond to the needs of consumers and their right to access quality and safe food. This will enable more sustainable production that ensures animal's well-being on the formerly prevailing terms of human–animal relations. The implication is that A2 milk is produced by cows from “traditional” breeds, most of which are found in areas not affected by industrialization [25]. Such an approach would also enable the spread of milk from pasture-grazed cows (this is more popular on small and medium-sized farms), which is beneficial for ethical and environmental reasons, and there are reports of changes in the nutritional value of milk and its flavor in favor of that from pasture-grazed cows [26].

It is necessary to consider the limitations of A2-type milk production volume. Cows from breeds that produce it have a slightly lower yield than the most popular current breeds and are not commonly bred for this reason. Their maintenance is thus associated with higher costs. However, it allows an increase in biodiversity among the animals [27]. In addition, it should be noted that no thesis on the impact of A2-type milk on human health is confirmed and accepted as correct. In the EU, the EFSA's opinion, formed in 2009, still applies, stating that at that point, it could not be determined whether the bioactive peptides in milk containing both A1 and A2 proteins have adverse health effects. However, since the issuance of the EFSA opinion, several new studies in the field of dietetics and nutrition have emerged, leading to the opinion that there are indications that A2-type casein has benefits for human health caused by the lack of release in the gastrointestinal tract of the biopeptide BCM-7 leading to inflammation, having a.o., a negative impact on intestinal microflora, which is associated with short and long-term health consequences [25,28–30].

Bojovic and McGregor [20] presented an analysis of the key trends in the dairy industry over the coming years. In their analysis, they took into account the social context of dairy product consumption (80% of people consume milk daily). Their findings indicate that dairy milk production and consumption are shifting from the Global North to the Global South. Additionally, there has been a notable increase in mechanized, standardized, and corporate dairy farms. Nevertheless, there is growing awareness of the environmental impact of intensive dairying. Furthermore, the sector is facing disruption due to plant-based and potentially synthetic milks. The identified trends related to the intensification of production and, at the same time, sustainability appear contradictory, but appropriate efforts in the long term can contribute to their realization. The market variation between regions with different goals and needs is also significant, which does not allow a clear thesis for the industry. It is necessary to address the challenges of megatrends in a way that allows this industry to develop harmoniously for all geo-locations.

3. Important Milk Components

Milk is often referred to as the “ideal food” because of its composition, ingredients, and their bioavailability. Humans can be considered the only species that consumes milk from other species and, in many cases, continue to consume it throughout their lives, which is unique in the animal world [15]. This is due to the technological utility of milk, its attractive sensory properties, and its high nutritional value, which is also crucial for adult humans. The composition of proteins, fats, and carbohydrates is even, which, including other nutrients, makes the nutritional value of milk proportional to its caloric content. Along with proteins, essential micronutrients (especially Ca, Mg, K, and P, as well as Na, Cl, Cu, Zn, Mn, Se, I, Cr, Co, Mb, F, As, Ni, Si, and B) are supplied in an easily digestible form, which is made possible by the interactions between milk components and the formation of

“vehicles” for micronutrients from proteins. Iron is the only element considered deficient, although it is also found in milk. This characteristic means that milk can be recommended as a daily dietary supplement containing the most of essential elements [31,32].

The beneficial effects of cow’s milk consumption on human health have been repeatedly proven, especially during the early years of life, when the skeleton is formed, and the supply of calcium and phosphorus from milk is essential for preventing osteoporosis in old age. It is also worth noting that milk and dairy products effectively prevent and treat malnutrition in seniors, in whom the progression of sarcopenia is reduced when they are included in the diet. Bioactive peptides are also present in dairy products, released by the action of digestive enzymes in the digestive system and fermented products, thanks to proteolytic enzymes derived from starter cultures [31–36]. Regularly consumed milk can also be a water-soluble vitamin for children and adults. The RDA of vitamins B1, B2, and B12 and a large portion of vitamins A, C, and pantothenic acid can be covered just by drinking 1 L [31].

3.1. Proteins

About 95% of the nitrogen in milk is of protein origin. Milk proteins are a valuable source of amino acids essential for producing raw materials. More than 200 milk proteins have been identified in cattle milk. Milk proteins are divided into two main groups: caseins and whey proteins [37]. They are 91% digestible relative to the reference protein, which allows them to be described as proteins of high biological value, and the high content of lysine, which is the limiting amino acid, makes milk proteins suitable for improving the amino acid profile of other products. Another argument for treating them as a functional ingredient is the regulatory effect on the delivery of satiety signals that regulate food intake [31].

The composition of milk in terms of the percentage of protein, fat, and carbohydrates between A1 and A2 types differs according to variation among breeds of cows and the conditions in which they live, which is natural even between bovines of the A1A1 genotype. The genes responsible for casein polymorphism do not determine the properties of other milk components but can be linked to differences in quantity or yield [38].

Some people exclude milk from their diets because of the gastrointestinal discomfort, allergies, and intolerances they cause after consumption—caused by abnormal immune reactions, non-immune reactions, and mechanisms not yet understood. Milk that does not contain casein type A1 targets people in the group whose problems are caused by non-immune mechanisms and may be caused by sensitivity to BCM-7 [39]. Researchers also pay attention to the long-term potential consequences of adverse effects of BCM-7 on the receptors of the digestive system. Milk and its products labeled commercially as “A2”, “A2 β -casein protein”, “A1 casein free”, and “A2 milk” may fill a need for consumers and introduce a new life-enhancing choice into daily life, as has happened with “lactose-free” products [40].

3.2. Fats

Milk fat is a carrier for fat-soluble vitamins (A, D, E, K), is responsible for a significant portion of milk’s energy value (9 kcal/g fat), and one of its fractions is essential fatty acids (EFAs), such as conjugated linoleic acid (CLA), which is a valuable nutrient with health-promoting properties, as well as other short-chain TCs. Fatty acids in milk are diverse and have chain lengths ranging from 4 to more than 20 carbon atoms. The fat fraction in milk is characterized by low levels of mono- and diglycerides.

Milk fat forms milk fat globules (MFGs) that range from 0.1 μ m to more than 22 μ m in diameter (usually 4–6 μ m). They consist of a non-polar nucleus of triglycerides and cholesterol esters. It maintains stability and is protected from lipase action by a nanometer-thick membrane on its surface—the milk fat globule membrane (MFGM). The MFGM ensures the stability of the milk emulsion and contains various bioactive components such

as glycoproteins, phospholipids, sphingolipids, cholesterol, and free fatty acids, which are crucial for brain development [41].

For the industry, its sensory and technological aspects are also important—fat is known as a flavor carrier, and, similarly, in this case, it imparts flavor to milk and dairy products. It is responsible for the soft, smooth texture of cheeses and the rich flavor of other products. In addition, fat fractions are separated from the milk matrix and used in the preparation of the raw material to increase the nutritional value of the food, as is the case with protein [37,41].

The attention paid by the industry and consumers to the value of individual milk components has led to the intensive development of food products that can be used independently. The current focus on milk proteins and, in particular, the distinction between their types (A1/A2 β -casein) allows new products to be designed for people with specific nutritional needs. The fractionation of milk and the use of its components also allows for more precise product design due to the considerable variation in the chemical composition of milk batches depending on the breed of cattle, geographical location, living conditions, and many other factors.

4. A2 Milk

There are two main classes of proteins in milk (Figure 1), which can be separated based on their solubility at an isoelectric pH of 4.6–4.8 and a solution temperature of 20–30 °C [42–44]. Phosphorylated proteins belonging to the casein group precipitate in such an environment, forming clots that are built up by spherical structures, micelles, while whey/serum proteins remain dissolved [45]. There is also a release of calcium ions from the casein structures, which remain with the remaining soluble proteins dispersed in the liquid part of the solution. Caseins make up an average of 75–80% of all proteins in bovine milk (about 29.5 g/L), making them milk's primary nitrogen source. They owe their name to the precipitation phenomenon and thus the production possibilities it provides—Latin *caseus* means cheese [46–48]. The difference in the structure of beta casein makes it possible to distinguish between A1- and A2-type milk.

4.1. β -Casomorphin 7

It is known that A1 and A2 milk in the human digestive system are digested differently, yielding different biopeptides as products due to differences in β -casein structure [49]. The digestion of A1 milk releases β -casein 7 (BCM-7), a very active opioid peptide. One theory is that its presence in the gut causes activation of opioid receptors, which can alter the stagnant composition of the intestinal microflora, resulting in impaired intestinal barrier integrity and bile acid metabolism, leading to further effects. BCM-7 is believed to be responsible for potential adverse human effects, such as the increased risk of type I diabetes, skin lesions, and inflammatory changes affecting the endocrine, neurological, and cardiovascular systems [48,50,51]. Daily, consuming A2 milk may benefit those experiencing discomfort after consuming A1 milk unrelated to lactose, including reducing gastrointestinal symptoms [43,52].

4.2. β -Caseins A1 and A2—The Differences

Among the caseins in cow's milk, A1 and A2 β -caseins comprise the most significant portion. These are peptides consisting of 207 amino acids. The difference in structure between A1 and A2 β -caseins lies in an amino acid change at the 67th position (Figure 2).

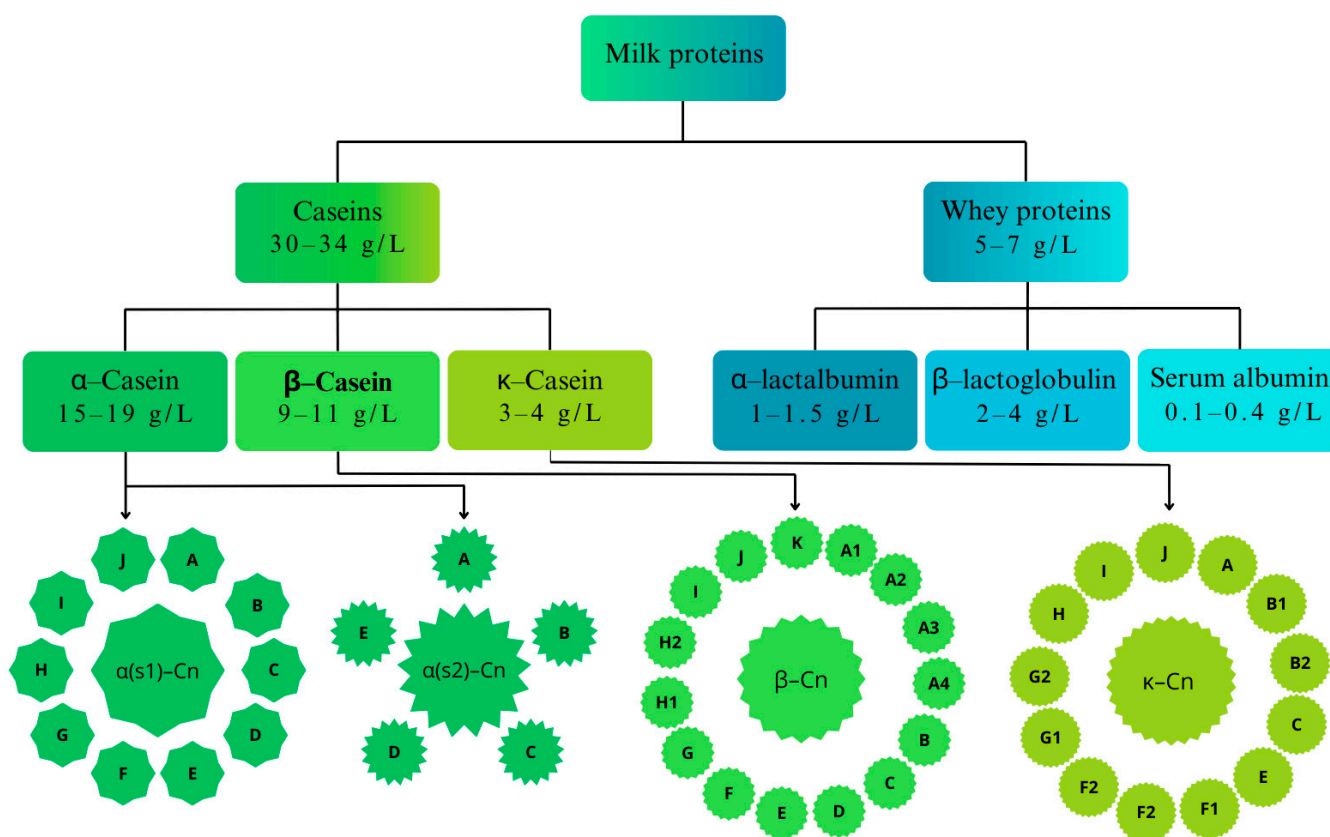


Figure 1. Scheme of the content of the different protein fractions in milk and casein types [53].

A1-type casein has the amino acid proline at the 67th position, while A2 has histidine at the same position. The binding of histidine to the preceding amino acid Isoleucine is easily broken by pancreatic elastase, resulting in the release in the gastrointestinal tract of the biologically active peptide BCM 7, which comprises seven amino acids. This does not occur with A2-type casein with proline at the histidine position, whose binding to Isoleucine is not hydrolyzed by pancreatic elastase. A2-type casein is generally more susceptible to enzymatic hydrolysis by gastric enzymes such as pancreatic elastase, pepsin, and leucine aminopeptidase. As a result of the different binding hydrolysis sites—in the case of A2 casein—a non-bonded BCM-9 is formed [40,54].

Even before human domestication of cattle, A2 casein was the predominant casein variant. Following a natural mutation 5000–10,000 years ago, individuals emerged whose milk began to be dominated by A1-type casein. Over the following years, as a result of crossbreeding of individuals giving the most milk, which was beneficial from a production point of view, the breeds currently dominant in industrial milk production—Holstein, British Shorthorn, Ayrshire, Friesian, and Sahiwal—were separated. These are breeds with the A2A2 genotype, i.e., predominantly producing A2-type casein. The reduction in cattle biodiversity due to selective breeding has displaced A1-type milk. The percentage of A1 β -casein is higher in black and white breeds than in yellow and brown breeds, such as Pezzata Rossa and Bruna. A1 β -casein is absent in the milk of pure Asian and African cattle [25,40,55].

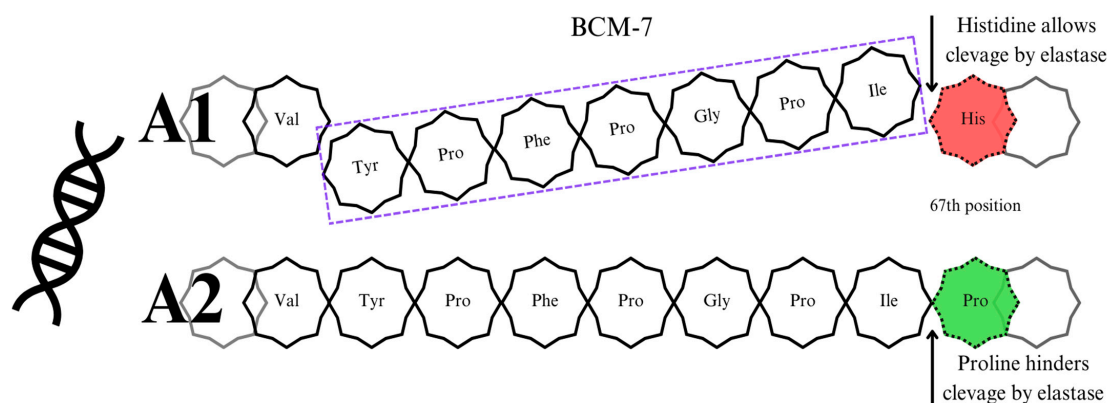


Figure 2. The difference between the amino acid chain of A1 and A2 casein [56].

According to the A1/A2 milk hypothesis, there is evidence of limited adverse health effects of A2 milk compared to A1. This is caused by BCM-7’s structural similarity to endogenous opioid peptides, which causes it to attach to opioid receptors found in humans’ central nervous system and gastrointestinal tract. At the same time, this structural change also causes another potential effect on human health, like the hydrophobicity of β -casein. Similarly, these two forms affect casein’s stability and emulsifying and coagulating properties (Table 1) [48,54,55,57].

Table 1. Properties of A1 vs. A2 milk.

Study Subject	Results A1	Results A2	Ref.
Acid and rennet coagulation	<ul style="list-style-type: none"> Less firm curd 	<ul style="list-style-type: none"> Slightly higher acid gel density, rennet aggregation rate to control milk (A1A1 + A1A2 blend) 	[9]
	<ul style="list-style-type: none"> Acid and enzymatic coagulation times were the same for A1 and A2 No differences in the fermentation curves No significant differences in the water holding capacity Comparable acid and rennet coagulation properties—creating dairy products in a similar way with both milk types where possible 		
Rennet coagulation and cheese making	<ul style="list-style-type: none"> Lower protein and higher fat content in prepared Cheddar cheese Cheese was softest, most fracturable than A2A2 (hardest, last fracturable) and A1A2 samples 	<ul style="list-style-type: none"> Poorer rennet coagulation Higher protein and lower fat content in prepared cheese Slower cheese curd formation 	[5]
	<ul style="list-style-type: none"> Proteolysis was not found to differ Cohesiveness difference was not found in cheese ripened for 180 days 		
Impact of heating	<ul style="list-style-type: none"> Higher presence of aggregated structures after heating Histidine present in A1 is involved in the formation of dehydroalanine 	<ul style="list-style-type: none"> Composed of larger casein micelle particles 	[11]
Cheese yield, curd nutrient recovery, whey composition, and cheese composition	<ul style="list-style-type: none"> The efficiency of nutrient recovery from whey was higher for samples with mainly A1 beta-casein 	<ul style="list-style-type: none"> An increase in the β-casein A2 content of milk has been linked to lower cheese yields 	[58]
	<ul style="list-style-type: none"> No differences in the final cheese composition 		
Calcium distribution, acid gelation, foaming properties, and microstructure of acid gels	<ul style="list-style-type: none"> Shorter time for gelation 	<ul style="list-style-type: none"> Higher proportion of free ionic calcium, enhanced foam formation capability More porous microstructure and thinner protein strands in the gel than in A1, which resulted in lower gel strength for the A2 milk case 	[59]

Table 1. Cont.

Study Subject	Results A1	Results A2	Ref.
Coagulation, fatty acids composition, and sensory characteristics	<ul style="list-style-type: none"> Higher levels of monounsaturated fatty acids and lower levels of saturated fatty acids 	<ul style="list-style-type: none"> Curd-firming rates were fastest and rennet coagulation times were shorter Curd firmness was highest Higher level of polyunsaturated fatty acids 	[60]
	<ul style="list-style-type: none"> Comparable sensory characteristics 		
Main components, fatty acids composition, amino acids composition, and sensory characteristics	<ul style="list-style-type: none"> Higher monounsaturated fatty acids, and lower of saturated fatty acids levels 	<ul style="list-style-type: none"> Higher omega-3 and omega-6 levels Results suggest that A2 milk was more appealing to consumers 	[61]
Milk composition, rennet coagulation properties, and cheese-making properties	<ul style="list-style-type: none"> More firmness in curd Better performance in cheese production, but worse digestive properties 	<ul style="list-style-type: none"> Longer rennet coagulation time Less efficient cheese-making process Higher whey protein concentrations 	[6]
	<ul style="list-style-type: none"> No significant differences in the cheese-making abilities No difference between total caseins content, but in proportions 		
Sensory characteristics and consumers opinions	<ul style="list-style-type: none"> A1 cheese Minas Frescal was considered more consistent, rubbery, and drier 	<ul style="list-style-type: none"> A2 cheese Minas Frescal characterized as softer and creamier 	[7]
	<ul style="list-style-type: none"> No difference in sensory properties in Petit Suisse cheese case Both products showed good sensory acceptances—A2 milk can be dairy innovation 		

The technological differences between A1 and A2 milk indicate that A2 milk proteins may form less compact curds than those found in conventional milk products, which is associated with the finer structure of the cheeses and curds obtained in this way. In addition, differences in coagulation time are apparent between A1 and A2 milk (longer in the case of A2), which, for technological reasons, must be thoroughly investigated. The impact of heating showed that A1 milk had a higher presence of aggregated structures and histidine involved in dehydroalanine formation, while A2 milk consisted of larger casein micelle particles. However, the available database of publications does not allow firm conclusions to be drawn due to the significant variation in milk samples, the characteristics of which depend on many factors (among others, breed of cows, housing conditions, and season) and from which some of the differences noted to date may result. It is important to note that sensory characteristics showed no negative perception of A2 milk.

5. Milk Processing

Processing milk aims to ensure its microbiological safety (reducing the population of micro-organisms responsible for its spoilage and pathogenic for humans) and reducing enzyme activity (reducing the course of chemical reactions), thereby extending its storage life. It makes it possible to provide consumers with a product that retains a high quality for the indicated period and to secure the raw material for further processing—production of powders, cheeses, yogurts, and fermented beverages [62].

Due to the composition of milk and the sensitivity of its valuable components, treatment using elevated temperatures can cause changes in sensory and nutritional value and contribute to the formation of toxic products due to temperature-induced reactions. Non-thermal methods in the treatment of milk can contribute to preserving its valuable characteristics and, at the same time, be as effective in ensuring the safety of milk as conventional methods.

Currently, A2 milk is just gaining popularity, and there needs to be more research on the impact of technological processes (Table 2) on its physicochemical characteristics, which is necessary for proper planning in the industry [62]. Due to the difference in protein structure and amino acid binding strength, it seems fitting to approach the fact that in addition to affecting the behavior of milk in the digestive system, it also affects milk during

processing, as indicated by emerging scientific articles. Therefore, in the context of emerging new, potentially more sustainable milk processing technologies, conducting studies on both A1 and A2 casein-containing milk is advisable to fill in the missing knowledge [59,63].

5.1. Thermal Methods

5.1.1. Thermization

Thermization refers to subpasteurization, which involves sanitizing raw milk using lower temperatures than pasteurization and sterilization to avoid thermal damage. It is used to prepare milk for cheese production to create conditions suitable for the proliferation of lactic acid bacteria, to extend the storage time of raw milk, to preheat milk before evaporation, and to produce powder [62]. It is carried out in the temperature range of 57 to 68 °C for 15–30 s. A temperature of 62–68 °C for 15 s is the most common temperature range for milk. This process is possible with the same equipment in which pasteurization is carried out. It makes it possible to extend the shelf life of raw milk by 24–72 h if stored at 4–7 °C [62,64–66].

Performed before pasteurization of the milk, thermization also reduces the formation of flocs from the fat; these flocs are caused by the phospholipase from *B. cereus*, which damages the membrane that holds the fat globules in their form, and thermization reduces its activity. It destroys most of the non-spore-forming psychrotrophic bacteria that cause spoilage. It does not inactivate milk alkaline phosphatase, lipase, lactoperoxidase, plasmin, or milk bacterial proteases/lipases. Although thermization is not a substitute for more effective methods of ensuring milk microbial spoilage, its advantages include minimal impact on the properties of milk induced by elevated temperatures [62,65].

5.1.2. Pasteurization and Sterilization

The conditions used during pasteurization are designed to inactivate the most heat-resistant, non-spore-forming pathogenic bacteria in milk—*Mycobacterium tuberculosis* and *Coxiella burnetii*. It is a standard among methods for extending the storage life of milk and dairy products and ensuring their safety. However, it has limitations due to its high energy consumption, cost versus efficiency, and impact on the physicochemical properties of milk. Different types of pasteurization and sterilization are used depending on production needs: Low Temperature Long Time (LTLT: 30 min/63 °C), High Temperature Short Time/Continuous (HTST: 15 s/72 °C), Flash/Ultra Pasteurization (2 s/138 °C), and Ultra High Temperature (UHT: 2 s/150 °C) sterilization [62,67,68]. The combination of temperature and time will adequately reduce the bacterial population, which varies depending on the type and conditions (such as viscosity, percentage of protein and fat, and solids content) of the pasteurized product. Other types of pasteurization are also being designed to optimize the process and its cost, like Low Temperature Short Time (LTST: 0.02 s/≤72.7 °C). In this process, milk is dispersed as droplets in a process chamber and heated for a fraction of a second at pasteurization temperature or lower [68,69].

Due to the temperatures used in these processes, changes occur in the milk: denaturation of proteins, the occurrence of Maillard reactions and the appearance of their products, and loss and changes of aroma, so attempts are being made to develop better methods for preserving milk quality [70].

5.1.3. Ohmic Heating (OH)

OH enables a volumetric increase in temperature inside food through the flow of an alternating electric current generated by electrodes placed in the food matrix (Joule effect). Compared to conventional heat transfer, the temperature rise is faster and more uniform, which can result in better decontamination of the raw material, which is not exposed to overheating. The most crucial factor that can be manipulated is the strength of the electric field, and it should be optimized for each product. Some studies indicate that OH applied to conventional milk (A1) may be at least as effective in inactivating enzymes and killing micro-organisms as pasteurization at temperatures above 65 °C [14].

Balthazar et al. proved that using OH to decontaminate sheep milk consumed 73% less energy than conventional pasteurization using an electric field strength of 8.33. The reduction in bacterial populations in all samples was >3.9 CFU/mL [71].

Such treatment changes the protein's functional properties and structure, affecting the products' subsequent functional and technological properties, so different effects on milk depending on its types are possible, but no studies are available on this topic. For both A1 and A2 milk, further research is needed to establish a consensus [72].

5.2. Non-Thermal Methods

Non-thermal methods (Table 2) of milk processing have the same goal as thermal methods: extending shelf-life and ensuring microbiological safety. However, they attempt to reduce the adverse effects of elevated temperatures on milk and reduce energy consumption. Non-thermal methods make it possible to inactivate micro-organisms and enzymes with minimal impact on the nutritional value of milk and its sensory properties [73]. Their influence on the physicochemical characteristics of milk is also an important aspect that can be regulated, and this can be used in the design of new products. To be considered competitive with pasteurization, the method must meet criteria such as the same level of microbiological safety; satisfactory sensory quality and shelf-life; no significant changes in technological properties; no formation of toxic substances; the processing must be as short as possible, and maintainable; the equipment must be safe for personnel and easy to maintain in hygiene; and the cost of the process must not increase the price of the product. Nevertheless, there are regulatory, technological, and economic obstacles to overcome before they can be scaled up for industrial use. However, for example, the application of high hydrostatic pressure (HHP) as an alternative to traditional pasteurization has already been successfully implemented [74,75].

Table 2. Non-thermal milk processing methods.

Method	Parameters	Study Subject	Impact	Ref.
Pulsed electric field (PEF)	10 kV/cm Pulse width 30 μ s	Microbial inactivation and the physical properties of low-fat milk	<ul style="list-style-type: none"> • 4.5 log reduction in <i>E. coli</i> • 4.4 log reduction in <i>L. brevis</i> • 6.0 log reduction in <i>S. cerevisiae</i> • Slow growth of surviving micro-organisms during storage for 15 days at 4 °C • No changes in pH, color, and particle-size distribution 	[76]
	55 kV/cm 90 Hz Pulse width 900 μ s 100 s	Curd properties prepared with PEF-treated raw milk	<ul style="list-style-type: none"> • Higher acidity of curd treated only with PEF than samples treated with heat method and mixed method • Higher microbial load (6.65 log) for PEF samples • Greater syneresis and softer texture; poorer sensory experience for PEF samples • PEF mixed with heat treatment improved texture and antimicrobial effect • No significant difference occurred during shelf-life 	[77]
	20–26 kV Pulse width 34 μ s	Physicochemical properties of whole milk-treated PEF (fat, xanthine oxidase, caseins, and whey proteins)	<ul style="list-style-type: none"> • PEF does not affect the final temperatures of fat melting and xanthine oxidase denaturation • Denaturation of whey proteins decreased in the PEF-treated milk • The formation of complexes by interaction between MFGM (milk fat globule membrane) proteins and skimmed milk proteins has been observed in PEF-treated milk 	[78]
Supercritical fluid technology	Liquid CO ₂ 100–300 bar 50 min 60–70 °C	Mitigating β -LG antigenicity by supercritical fluid extraction in whole milk powder	<ul style="list-style-type: none"> • Reduction antigenicity of β-LG of $42.9 \pm 2.83\%$ and $54.75 \pm 2.43\%$ at 63 °C/200 bar and 75 °C/300 • Temperature and pressure had a significant effect on the antigenic response of β-LG • Thermal treatment at 63 and 75 °C had no effect on the antigenicity 	[79]

Table 2. Cont.

Method	Parameters	Study Subject	Impact	Ref.
	Liquid CO ₂ 15–25 MPa Co-solvent ethanol 10–50 mL 40–80 °C	Supercritical fluid extraction of cholesterol from whole milk powder	<ul style="list-style-type: none"> 46% cholesterol could be removed without affecting its physicochemical properties at 48 °C, 17 MPa, and 31 mL ethanol Decrease in the extraction rate was observed with an increase in temperature Physical parameters, like milk powder color, vary significantly with variation of the process parameters 	[80]
	Liquid CO ₂ 80–180 bar 10–30 min 30–70 °C	Inactivation of alkaline phosphatase and <i>Escherichia coli</i> in raw whole milk	<ul style="list-style-type: none"> Inactivation is accelerated with increasing pressure, temperature, and time Inactivation of alkaline phosphatase around 94.5% when the process was operated at CO₂ to milk mass ratio of 0.05, 70 °C, 80 bar, and time of 30 min Decrease in the microbial count for treatment time higher than 20 min 	[81]
UV radiation	UV-C lamp with total output power 18 W Flow rate 5–18 mL/min 4–25 °C	Raw milk bacterial load	<ul style="list-style-type: none"> Approx. 2 log decrease in total mesophilic aerobic bacteria Approx. 4 log decrease in yeast-mold count Mixing of UV-C treatment and pasteurization techniques can lead to a more effective reduction in bacterial load 	[82]
	UV-C lamp with total output power 19 W Flow rate 50–150 mL/min 20 °C	Effect of combined UV and heat to inactivate <i>B. subtilis</i> spores in skimmed and whole milk	<ul style="list-style-type: none"> UV pretreatment with treatment at 110 °C for 30 s resulted in a reduction of approximately 6 log CFU/mL in bovine skim milk and 2.90 log CFU/mL in whole bovine milk UV alone is not sufficient to obtain necessary spore inactivation 	[83]
	UV-C lamp with total output power 144 W Flow rate 3 L/min	Enhancing the quality of raw bovine milk	<ul style="list-style-type: none"> pH remained stable after UV treatment Reduction in lipid peroxidation occurred in UV-treated samples during 72 h—lower TBARS values than for raw and pasteurized milk No changes in natural characteristics and sensory qualities High inactivation efficiency for aerobic bacterial counts 	[84]
	Gamma irradiation Dose 2–10 kGy Dose rate of 1.19 Gy/s	pH, acidity, and microbial contamination of raw milk	<ul style="list-style-type: none"> The highest dose of gamma radiation (10 kGy) did not have an effect on acidity and pH at 15 days of storage Radiation combined with the addition of ascorbic acid reduced the number of micro-organisms in the samples 	[85]
Ionizing radiation	Electron beam (e-beam) irradiation 5–20 kGy Conveyor speed 80–400 cm/min	Antiproliferative, antidiabetic, and antioxidant activities of defatted cow milk	<ul style="list-style-type: none"> E-beam increased total phenolic content of whole milk, sweet whey, and acid whey of cow milk Irradiation preserved (or enhanced) the DPPH radical scavenging activity of most cow milk fractions β-casein of cow milk showed anti-proliferative activity against the A549 lung cancer cells proliferation 	[86]
	Gamma irradiation 1–3 kGy Dose rate 45 Gy/min	Bacteriological and sensory quality of raw whole milk	<ul style="list-style-type: none"> Psychrotrophic bacteria did not proliferate in the gamma radiation-treated milks in each of the study groups At the end of 60-days storage, all of the irradiated samples showed a lower mesophilic bacterial count than the control The majority of panelists noted a difference in the sensory characteristics of the milk from the control vs. irradiated samples More than 21% of panelists pointed out that samples irradiated (3 kGy) characterized rancid odors and flavors; almost 19% of panelists responded that the irradiated milk was tastier Irradiation dose of 2 kGy was effective for preserving a raw whole milk 	[87]

Table 2. Cont.

Method	Parameters	Study Subject	Impact	Ref.
Nonthermal plasma (NTP)	Resonant frequency of 52 kHz 32 kV discharge and frequency 1 kHz	Reducing activity of <i>Pseudomonas</i> -secreted proteases in milk	<ul style="list-style-type: none"> • Lower treatment time resulted in lower degree of hydrolysis during shelf-life • Reduced the activity of <i>Pseudomonas</i>-secreted proteases by more than 60% with a 10 min • pH was not affected 	[88]
	9 kV AC power supply <35 °C 0–20 min	Changes in protein, free fatty acids, and volatile profiles of whole raw milk	<ul style="list-style-type: none"> • No changes to the lipid composition • Increased the total aldehyde content during 20 min treatment • No changes to the total ketone and alcohol levels • Significant changes occurred in volatile compounds profile 	[89]
	N ₂ -O ₂ plasma O ₂ plasma	Acid gelation properties of skim milk	<ul style="list-style-type: none"> • Sulfhydryl content decreased with treatment time—fast for N₂-O₂ and gradual for O₂ treatment • Increased skim milk viscosity over time • Longer exposure times led to lower gel firmness • Syneresis in acid gels decreased below 60% after 2 min but increase to 70% after 4 min for N₂-O₂ treatment 	[90]
High-pressure processing (HPP)	100–150 MPa 25 °C	<i>Pseudomonas fluorescens</i> protease inactivation in milk	<ul style="list-style-type: none"> • 150 MPa was efficient in reducing the proteolytic rate • Clotting formation was faster by 29% after 100 MPa treatment and 51% after 150 MPa • No reduction in proteolysis was observed • Inactivation of the <i>Pseudomonas fluorescens</i> protease was not effective 	[91]
	200–600 MPa 15 min 20 °C	Coagulation of protein and fat globules in whole and skim milk	<ul style="list-style-type: none"> • Pressures over 400 MPa caused looser and more fragmented gastric clot structures • Weight of the dried clots milk treated at 600 MPa were significantly lower than in untreated milk samples • Moisture content in the clots milk treated at 600 MPa were significantly higher than in untreated milk samples 	[92]
	250–550 MPa 3–15 min 20 °C	Microbiological quality of skim milk	<ul style="list-style-type: none"> • HPP treatment at 400 MPa ensures the same bacterial load reduction as pasteurization • For shelf-life elongation, pressure > 400 MPa and a holding time of at least 15 min are necessary • 250 MPa/3 min/room temperature—1.51 log CFU/mL reduction 	[93]
Ultrasounds (US)	Frequency 24 kHz 200–400 W 2.5–10 min 20–55 °C	Chemical composition and sensory properties of the milk	<ul style="list-style-type: none"> • Samples treated with ultrasound power 200 W at 20 °C for max. 7.5 min had best sensory characteristic • Milk pH slightly dropped at 400 W and 20 °C • No changes in total solids, solids-non-fat, fat, and protein mass fractions • Samples treated with 200 and 400 W US had a small increase in fat content 	[94]
	106–375 W Energy density 190.4, 570.7, 674.3, 2016.9 J/g 3–9 min 4 °C	Rheological and textural properties of rennet-coagulated skim milk	<ul style="list-style-type: none"> • Firmness gel of non-sonicated samples was higher, but when highest energy density was used, gel firmness started to increase • Rennet coagulation time doubled when 674.3 J/g was applied • Coagulant strength and curd fixation rate decreased when the energy density was increased to 2016.9 J/g • US and energy density manipulations can be used to prolong the renneting time of milk 	[95]
	22.5 kHz 28 W 1–30 min	Protein changes in fresh skim milk	<ul style="list-style-type: none"> • Integrity of casein micelles was not damaged by 30 min US, but turbidity started to decrease after 5 min • Secondary structure of protein in skim milk changed after 1 min • pH of fresh skim milk was unaltered by sonication up to 30 min • Excessive sonication (>20 min) could disrupt the aggregates of whey–whey and whey–casein 	[96]

Table 2. Cont.

Method	Parameters	Study Subject	Impact	Ref.
Membrane technologies	Ultrafiltration	Dairy wastewater filtration effectiveness	<ul style="list-style-type: none"> Combination of an integrated three-dimensional printed turbulence promoter and high stirring speeds can effectively reduce membrane fouling in a dairy wastewater treatment module 	[97]
	Polyethersulfone membrane			
	pore size 0.07 μm			
Membrane technologies	Microfiltration	Technology to isolate MFGM from raw and pausterized milk	<ul style="list-style-type: none"> MFGM isolates from milk pasteurized before microfiltration had a significantly higher content of β-lactoglobulin compared with MFGM isolated from milk pasteurized after microfiltration β-lactoglobulin content in MFGM material increased progressively with pasteurization temperatures Casein content of MFGM isolates was significantly reduced by filtration 	[98]
	Polyvinylidene fluoride membrane			
	Pore size 0.65 μm			
Membrane technologies	Microfiltration	Fat separation from skim and raw milk	<ul style="list-style-type: none"> Fat globules (MFGs) from raw milk showed highest 95% fat separation at 15 °C and 0.34% for skim milk Separation at 50 °C showed a higher distribution of larger MFGs Membrane showing no irreversible fouling Ceramic silicon carbide membranes are suitable for fat separation as alternative to centrifugation 	[99]
	Silicon carbide ceramic membrane			
	Pore size 1.4 μm			

5.2.1. Pulsed Electric Field (PEF)

PEF aims to inactivate pathogenic micro-organisms by treating them with short (μs and ms) high-voltage pulses (10–80 kV/cm²) of food placed between electrodes [100]. This is a promising technology for application to liquid products such as milk and juice. Industrial systems consist of a high-voltage pulse generator and a treatment chamber. In the generator, pulses are formed by rapid discharges of electrical energy over a short period. The pulse preparation and formation occur in a particular part of the impulse generator: the pulse-forming network. Pulses pass into the raw material through electrodes, which are located in the central processing chambers. The chamber should be uniformly filled with the treated material to ensure good contact with the electrodes without air bubbles, allowing the electric field to be evenly distributed throughout the milk batch [101].

The effectiveness of PEF depends mainly on the duration of the treatment, the strength of the applied electric field, and the pulses' shape, number, width, and frequency [100]. In industry, monopolar and bipolar pulses are most commonly used. Changes in the activity and inactivation of enzymes and micro-organisms are thought to be due to conformational modifications in their structure, as well as electroporation (electropermeabilization) and dielectric breakdown of the cell membrane, in which pores of induced PEFs are formed and stabilized [73,100,101].

For milk processing, the most favorable results may be obtained by combining PEF with other technologies, such as gentle heating (below pasteurization temperature), which will not overheat the raw material but will provide a synergistic effect. Disadvantages of this method include the relatively high cost, the failure to destroy pathogen spores, only their vegetative forms, and the passage of metal ions (Fe, Zn, Mn, and Cr) from the electrodes into the milk; no information is currently available on the effect of this method on A2 milk [73].

5.2.2. Supercritical Fluid Technology

A supercritical state is a state in which, when a critical value of temperature and pressure is exceeded, a liquid exhibits the properties of a gas and becomes a supercritical fluid (SCF). Various supercritical substances, including ethylene, water, and ammonia, can be employed in the food industry. Nevertheless, the majority of research utilizing supercritical fluid technology employs carbon dioxide. Carbon dioxide is considered as a

chemically inert, non-corrosive, non-flammable, non-toxic, inexpensive, readily available, and generally recognized as a safe (GRAS) solvent. Supercritical carbon dioxide technology employs pressure with carbon dioxide to eliminate micro-organisms without compromising the material's nutritional value or organoleptic properties.

Furthermore, the low critical temperature (31.04 °C) allows for application at near-room temperature, which prevents the degradation of thermosensitive and volatile compounds, thereby minimizing changes in the food's physicochemical, sensory, and nutritional properties, thus obtaining high-quality products. The conditions during the process allow for the reduction of aerobic mesophilic bacteria and *E. coli*. The killing of micro-organisms depends on temperature, pressure, and time [83]. The distinction between SCF and HHP is that SCF can occur at lower pressures (10–20 MPa) and shorter times with comparable efficacy in inactivating micro-organisms and enzymes. This translates into higher efficiencies [102,103].

5.2.3. UV Radiation

The EFSA has endorsed using UV radiation to treat milk as a safe method of preserving milk that dairy producers can use [104]. In addition, consumers have no objection to consuming such treated foods and associate this treatment with high product quality [105,106]. Ultraviolet light covers the wavelength range from 100 to 400 nm, which produces four main types of UV light: UVA (315–400 nm), UVB (280–315 nm), UVC (200–280 nm), and vacuum UV (100–200 nm). UV-C rays are described as germicidal because they are the most effective in killing various micro-organisms, such as bacteria, viruses, fungi, and algae [107]. The treatment process involves the application of UV light at short wavelengths, in the 200–280 nm range, which is able to disrupt the DNA of micro-organisms, altering their metabolism and reproduction, leading to cell death. UV light applications are performed using different devices for solids or liquids; therefore, it is necessary to develop a UV light irradiation device with the appropriate lamps and size to achieve the desired effect. Reactors are used to irradiate liquids with UV light, while UV chambers are developed to irradiate the surface of solids [105].

Atik and Gumus demonstrated that using UV-C radiation reduces about 2 log in the total number of mesophilic aerobic bacteria and about 4 log in the number of yeasts and molds in raw milk. The lamp radiation treatment parameters they used were 18 W at 253.7 nm. They concluded that it is necessary to integrate UV-C treatment with existing pasteurization techniques to protect milk more effectively [82]. However, UV treatment can affect the sensory characteristics of the raw material—in one study, a 'tallowy' aftertaste was perceptible in milk treated in this way [108]. Until now, virtually no studies have considered the separation of milk types regarding casein distribution.

5.2.4. Ionizing Radiation (e-Beam, Gamma)

The electron beam emitter (e-beam) directs the electron beam toward the targeted sample, which leads to the interaction of electrons with food. It leads to the inactivation of micro-organisms through both direct and post-mediated mechanisms. Direct radiation includes ionizing radiation that damages DNA and consequently inhibits cell division. In addition, it generates free radicals in the interior of microbial cells, which cause DNA strand breaks and physical damage, leading to cell death. The indirect action of e-beam causes the formation of reactive hydroxyl radicals. Their accumulation leads to lysis of the cell. Products subjected to such treatment can change their sensory properties, the perception of which (negative/positive) depends on the evaluator [109,110].

Gamma radiation works on the same principle, contributing to the indirect and direct inactivation of micro-organisms and viruses [111]. Like e-beam, it can be used at lower doses (0.25–2.25 kGy) to increase the quality of vegetables and fruits without affecting ripening speed or loss of firmness. Higher doses up to 30 kGy are used to extend the shelf life of animal products and disinfect surfaces and packaging [86].

Milk can also be exposed to electromagnetic radiation. This process must be carried out for a short time, which allows it to maintain higher nutritional values than in traditional processing. In many European countries, the irradiation could be applied at 3 kGy to casein up to 30 kGy to dried milk products [86]. Harizi and his team (2023) recommend using e-beam treatment at 5–10 kGy to preserve cow's milk and 20 kGy to obtain a functional product with health-promoting properties. Lower doses for dairy products appear less effective, as demonstrated by the Santos team (2017), which could not extend the shelf life of butter made from sheep's milk to which a dose of 1 kGy of gamma radiation was applied [86,112].

5.2.5. Cold Plasma—Nonthermal Plasma (NTP)

Cold plasma or nonthermal plasma is a quasi-neutral ionized gas (partially or entirely) consisting of charged particles—ions, free radicals, atoms, molecules, and some radiation, exhibiting a resultant neutral charge. Its most active elements are reactive oxygen species (ROS) and reactive nitrogen species (RNS) [113]. Furthermore, in concert with free radicals, the generation of ultraviolet radiation by plasma results in the oxidation of phospholipids and proteins located on the plasma membrane, thereby damaging nucleic acids. This ultimately leads to the death of micro-organisms. Conversely, reactive oxygen species can lower the pH of foods and modify the properties—viscosity, solubility, and water absorption—of polysaccharides and proteins [114]. Atmospheric cold plasma (ACP) is a state of partially ionized gas in which ionization is about 5% or less of the gas and is maintained at approximately room temperature. Exposing food to ionizing radiation is an innovative way to inactivate micro-organisms in the food industry [113,115]. Its benefits include short processing times (from a few seconds to minutes), efficiency at room temperature, which is essential for heat-sensitive products—such as milk—and low energy consumption, which makes it an environmentally friendly method. A processing time of at most 5 min is recommended due to color changes associated with oxygen in the raw material and oxidation of proteins and fats [115].

Lee et al. [116] extended the shelf life of milk to 15 days using NTP for 10 min, where TPC was above 6 CFU/mL and titratable acidity was below 0.18% according to standards. However, the treatment released free lipids into the milk, which underwent oxidation reactions. No significant effect on nutritional value or xanthine oxidase activity was observed, but the milk changed color more strongly than the control samples. The team noted that this is a worthwhile technology to develop—data on different types of milk are not available.

5.2.6. High-Pressure Processing (HPP)

HPP is an alternative to traditional thermal treatments, yielding similar results in inactivating food pathogens but simultaneously preventing the loss of temperature-sensitive nutrients. The entire process is carried out in a chamber in the pressure range of 300–1000 MPa for several minutes at room temperature [117]. Processing milk at 20–60 MPa to 620 MPa results in the formation of aftertastes and volatile compounds, such as hexanal from the oxidation of monounsaturated fatty acids (MUFAs) or methyl ketones from saturated fatty acids (SFAs), so it seems appropriate to add antioxidants during processing [118].

The milk temperature during the process may rise as compression sub-raises the temperature of the food matrices by about 3 °C for every 100 MPa. Milk subjected to this process regarding sensory characteristics can be compared to raw milk when microbial content is reduced by at least 5 log CFU/mL. However, HPP can affect milk's physico-chemical and technological properties due to the modification of protein structures. This is important in terms of possible different effects on A2 milk than A1 milk and changes in BCM-7 release [117,119]. Rodríguez-Alcalá et al. showed that processing different batches of cow's milk with HPP up to 900 MPa did not change the composition of lipid compounds

or fatty acids, which may indicate that potentially, in A2 milk, such changes should not occur either [118].

Although the process is very effective in eliminating vegetative micro-organisms and practically has no effect on spore forms if carried out at room temperature, that is, among other things, *Bacillus* spp. and other forming micro-organisms will not be inactivated, posing microbiological risks and causing spoilage of milk and products. Therefore, a sensible approach is to combine HPP with heat treatment, which allows manipulation of the process's time and temperature, allowing the consumption of a safe, high-quality raw material with the shelf life expected by consumers [119].

5.2.7. Ultrasounds (USs)

Ultrasound is sound waves that exceed a frequency of 20 kHz. In the food industry, low-intensity and high-frequency USs (intensity: $<1 \text{ W/cm}^2$; frequency: 100 kHz) are used in the non-invasive analysis of structure or composition, while high-intensity and low-frequency USs ($10\text{--}1000 \text{ W/cm}^2$; 20–100 kHz) have found application in, among other things, microstructure adjustment, support of emulsification, extraction, and processes to extend the shelf life of raw materials. Food preservation with ultrasound is based on the propagation of US waves through the raw material, causing alternate compression and expansion of matrix particles—acoustic cavitation. This leads to the formation of bubbles, which, when they reach a critical point, suddenly implode, releasing a significant amount of energy. This increases the temperature (almost $5000 \text{ }^\circ\text{C}$) and pressure (more than 1000 atm.) at the implosion site, resulting in free radicals, physical damage to micro-organisms, and enzyme inactivation due to free radical activity and protein denaturation. In addition, damage to macromolecular chains is possible. It should be considered that this can also cause damage to the processed raw material [120].

The US is also used to control the microstructure, modify the texture properties of fat-containing products, and manipulate the properties of proteins and enzymes, which, with adequately selected parameters, are not destroyed but change their activity level due to conformational changes [73].

Using the US method, it is possible, in addition to reducing the population of micro-organisms, to achieve a better degree of homogenization of milk compared to conventional homogenization methods. It was also reported to reduce the size of milk protein parts and increase their solubility in aqueous solutions after US treatment. However, it did not disrupt the primary structure of many proteins and, significantly, the structure of casein micelles. In general, the properties of milk are affected by breaking down fat molecules, so despite the lack of research, it can be assumed that their effect on A1 and A2 milk should be the same. It can remove gases, which translates into excellent oxidative stability in milk. The downside of this treatment, mentioned earlier, is the formation of free radicals, which can damage proteins, amino acids, and fats, catalyzing undesirable reactions [73,121].

5.2.8. Membrane Technologies

Membrane-based processes are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). The capacity to regulate the permeability of ceramic filter membranes enables the utilization of these membranes for the separation of milk components, as well as for the separation of bacteria and spores. MF can fractionate fat globules ($10 \text{ }\mu\text{m}$) and remove bacteria and spores ($1 \text{ }\mu\text{m}$). UF can separate casein micelles (100 nm) or serum proteins (10 nm). When NF and RO are utilized, lactose (1 nm), salt (0.1 nm), and water can be recovered [122]. Filtering out micro-organism spores is very effective and, compared to other non-thermal methods, gives the best results, which can be compared to the effects of pasteurization [123].

The industry uses multi-stage membrane filtration to extract separated protein or fat fractions (for example, MFGM), which is common in commercial dairy plants. These do not affect the components of the milk but only allow them to be purified or separated. This

offers the possibility of producing functional intermediates, such as protein-rich permeate streams, and the mentioned separation of micro-organisms [124,125].

Membrane separation technology is a revolutionary approach to isolating and purifying milk fat globules (MFGMs). The most common membrane separation techniques for MFGM isolation are microfiltration (0.1–1 µm), ultrafiltration (0.001–0.1 µm), and diafiltration. The diameter of the whey protein (3–6 nm) is significantly smaller than that of the MFGM fragments (100–400 nm), allowing for their removal by filtration. Compared to traditional methods, membrane separation technology is highly effective in increasing the relative abundance of MFGM fractions and MFGM protein while reducing the presence of other molecules in the product [126].

6. Conclusions and Future Opportunities

The global milk market is experiencing a progression of supply and demand, with the introduction of novel products and an intensification of consumer expectations regarding health and environmental concerns. A2 milk is a promising trend in the dairy industry to provide consumers with new health products with limited adverse health effects due to the lack of release of BCM-7 during its digestion. There is a need for further development of technology that will maintain the highest nutritional value of milk and its sensory attractiveness. Non-thermal methods for extending the shelf life of milk and ensuring its safety, such as PEF, supercritical fluid technology, radiation, NTP, HPP, US, and membrane technologies, are a promising direction.

The A2-type milk entering the market poses new technological challenges, which include, among others, assessing the impact of previously developed processes on the physicochemical properties of A2 milk.

Author Contributions: Conceptualization, K.Ż.; methodology, K.Ż.; investigation, K.Ż.; resources, K.Ż.; data curation, K.Ż.; writing—original draft preparation, K.Ż., A.O., E.G.-H. and A.W.; writing—review and editing, K.Ż., A.O. and E.G.-H.; visualization, K.Ż.; supervision, A.O. and A.W. All authors have read and agreed to the published version of the manuscript.

Funding: Research financed by the Polish Ministry of Science and Higher Education within funds of Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences (WULS), for scientific research.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Silva, B.Q.; Smetana, S. Review on Milk Substitutes from an Environmental and Nutritional Point of View. *Appl. Food Res.* **2022**, *2*, 100105. [[CrossRef](#)]
2. Chalupa-Krebsdak, S.; Long, C.J.; Bohrer, B.M. Nutrient Density and Nutritional Value of Milk and Plant-Based Milk Alternatives. *Int. Dairy J.* **2018**, *87*, 84–92. [[CrossRef](#)]
3. Curry, A. Archaeology: The Milk Revolution. *Nature* **2013**, *500*, 20–22. [[CrossRef](#)] [[PubMed](#)]
4. Walker, C.; Thomas, M.G. The Evolution of Lactose Digestion. In *Lactose*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 1–48.
5. Gai, N.; Waldron, D.S.; Uniacke-Lowe, T.; Li, B.; O'Regan, J.; Goulding, D.A.; Kelly, A.L. Influence of β -Casein Genotype on Cheddar Cheese Making and Ripening. *Int. Dairy J.* **2024**, *149*, 105824. [[CrossRef](#)]
6. Bisutti, V.; Pegolo, S.; Giannuzzi, D.; Mota, L.F.M.; Vanzin, A.; Toscano, A.; Trevisi, E.; Ajmone Marsan, P.; Brasca, M.; Cecchinato, A. The β -Casein (CSN2) A2 Allelic Variant Alters Milk Protein Profile and Slightly Worsens Coagulation Properties in Holstein Cows. *J. Dairy Sci.* **2022**, *105*, 3794–3809. [[CrossRef](#)] [[PubMed](#)]
7. Oliveira Mendes, M.; Ferreira de Moraes, M.; Ferreira Rodrigues, J. A2A2 Milk: Brazilian Consumers' Opinions and Effect on Sensory Characteristics of Petit Suisse and Minas Cheeses. *LWT* **2019**, *108*, 207–213. [[CrossRef](#)]

8. Dantas, A.; Kumar, H.; Prudencio, E.S.; de Avila, L.B.; Orellana-Palma, P.; Dosoky, N.S.; Nepovimova, E.; Kuča, K.; Cruz-Martins, N.; Verma, R.; et al. An Approach on Detection, Quantification, Technological Properties, and Trends Market of A2 Cow Milk. *Food Res. Int.* **2023**, *167*, 112690. [CrossRef]
9. Juan, B.; Trujillo, A.-J. Acid and Rennet Coagulation Properties of A2 Milk. *Foods* **2022**, *11*, 3648. [CrossRef]
10. Hemar, Y.; Banjar, W.; Otter, D.; Yang, Z. Viscosity, Size, Structural and Interfacial Properties of Sodium Caseinate Obtained from A2 Milk. *Colloids Surf. A Physicochem. Eng. Asp.* **2021**, *614*, 126163. [CrossRef]
11. Daniloski, D.; McCarthy, N.A.; Vasiljevic, T. Impact of Heating on the Properties of A1/A1, A1/A2, and A2/A2 β -Casein Milk Phenotypes. *Food Hydrocoll.* **2022**, *128*, 107604. [CrossRef]
12. Daniloski, D.; McCarthy, N.A.; Gazi, I.; Vasiljevic, T. Rheological and Structural Properties of Acid-Induced Milk Gels as a Function of β -Casein Phenotype. *Food Hydrocoll.* **2022**, *131*, 107846. [CrossRef]
13. Kaskous, S. A1- and A2-Milk and Their Effect on Human Health. *J. Food Eng. Technol.* **2020**, *9*, 15–21. [CrossRef]
14. D’Incecco, P.; Limbo, S.; Hogenboom, J.A.; Pellegrino, L. Novel Technologies for Extending the Shelf Life of Drinking Milk: Concepts, Research Trends and Current Applications. *LWT* **2021**, *148*, 111746. [CrossRef]
15. Foroutan, A.; Guo, A.C.; Vazquez-Fresno, R.; Lipfert, M.; Zhang, L.; Zheng, J.; Badran, H.; Budinski, Z.; Mandal, R.; Ametaj, B.N.; et al. Chemical Composition of Commercial Cow’s Milk. *J. Agric. Food Chem.* **2019**, *67*, 4897–4914. [CrossRef] [PubMed]
16. US Department of Agriculture. *Major Producers of Cow Milk Worldwide in 2023, by Country (in Million Metric Tons)*; US Department of Agriculture: Washington, DC, USA, 2023.
17. US Department of Agriculture. *Total Milk Production in the United States from 1999 to 2024 (in Million Pounds)**; US Department of Agriculture: Washington, DC, USA, 2024.
18. USDA Foreign Agricultural Service. *Cow Milk Production Worldwide from 2015 to 2023 (in Million Metric Tons)*; USDA Foreign Agricultural Service: Washington, DC, USA, 2023.
19. Dg Agri Dashboard: Dairy Products. 2024. Available online: https://agriculture.ec.europa.eu/document/download/c9474932-d061-4169-80f1-38951aa8615e_en?filename=dashboard-dairy_en.pdf (accessed on 25 March 2024).
20. Bojovic, M.; McGregor, A. A Review of Megatrends in the Global Dairy Sector: What Are the Socioecological Implications? *Agric. Hum. Values* **2023**, *40*, 373–394. [CrossRef]
21. USDA Foreign Agricultural Service. *Annual Consumption of Fluid Cow Milk Worldwide in 2023, by Country (in 1,000 Metric Tons)*; USDA Foreign Agricultural Service: Washington, DC, USA, 2022.
22. Shahbandeh, M. *Global Consumption of Milk per Year by Country 2023*; Statista: Hamburg, Germany, 2023; Available online: <https://www.statista.com/statistics/272003/global-annual-consumption-of-milk-by-region/> (accessed on 2 April 2024).
23. Agarwal, S.; Beausire, R.L.W.; Patel, S.; Patel, H. Innovative Uses of Milk Protein Concentrates in Product Development. *J. Food Sci.* **2015**, *80*, A23–A29. [CrossRef] [PubMed]
24. Goulding, D.A.; Fox, P.F.; O’Mahony, J.A. Milk Proteins: An Overview. In *Milk Proteins*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 21–98.
25. Bentivoglio, D.; Finco, A.; Bucci, G.; Staffolani, G. Is There a Promising Market for the A2 Milk? Analysis of Italian Consumer Preferences. *Sustainability* **2020**, *12*, 6763. [CrossRef]
26. Magan, J.B.; O’Callaghan, T.F.; Kelly, A.L.; McCarthy, N.A. Compositional and Functional Properties of Milk and Dairy Products Derived from Cows Fed Pasture or Concentrate-based Diets. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20*, 2769–2800. [CrossRef]
27. Dhillon, B.; Singh Sodhi, K.; Chaudhary, R. Is A2 Milk a Healthier Choice than A1 Milk? A Review. *Adv. Bio. Res.* **2021**, *12*, 253–259.
28. O’callaghan, T.F. An Overview of the A1/A2 Milk Hypothesis. In *Dairy Nutrition Forum*; The National Dairy Council: Dublin, Ireland, 2020; Volume 12, pp. 1–4.
29. European Food Safety Authority (EFSA). Review of the Potential Health Impact of β -Casomorphins and Related Peptides. *EFSA J.* **2009**, *7*, 231r. [CrossRef]
30. Küllenberg de Gaudry, D.; Lohner, S.; Schmucker, C.; Kapp, P.; Motschall, E.; Hörrlein, S.; Röger, C.; Meerpohl, J.J. Milk A1 β -Casein and Health-Related Outcomes in Humans: A Systematic Review. *Nutr. Rev.* **2019**, *77*, 278–306. [CrossRef]
31. de la Fuente, M.A.; Juárez, M. Milk and Dairy Products. In *Handbook of Mineral Elements in Food*; Wiley: Hoboken, NJ, USA, 2015; pp. 645–668.
32. Górska-Warsewicz, H.; Rejman, K.; Laskowski, W.; Czacotko, M. Milk and Dairy Products and Their Nutritional Contribution to the Average Polish Diet. *Nutrients* **2019**, *11*, 1771. [CrossRef] [PubMed]
33. Dor, C.; Stark, A.H.; Dichtiar, R.; Keinan-Boker, L.; Shimony, T.; Sinai, T. Milk and Dairy Consumption Is Positively Associated with Height in Adolescents: Results from the Israeli National Youth Health and Nutrition Survey. *Eur. J. Nutr.* **2022**, *61*, 429–438. [CrossRef]
34. Givens, D.I. MILK Symposium Review: The Importance of Milk and Dairy Foods in the Diets of Infants, Adolescents, Pregnant Women, Adults, and the Elderly. *J. Dairy Sci.* **2020**, *103*, 9681–9699. [CrossRef]
35. Franzoi, M.; Niero, G.; Penasa, M.; Cassandro, M.; De Marchi, M. Technical Note: Development and Validation of a New Method for the Quantification of Soluble and Micellar Calcium, Magnesium, and Potassium in Milk. *J. Dairy Sci.* **2018**, *101*, 1883–1888. [CrossRef] [PubMed]

36. Brick, T.; Hettinga, K.; Kirchner, B.; Pfaffl, M.W.; Ege, M.J. The Beneficial Effect of Farm Milk Consumption on Asthma, Allergies, and Infections: From Meta-Analysis of Evidence to Clinical Trial. *J. Allergy Clin. Immunol. Pract.* **2020**, *8*, 878–889.e3. [[CrossRef](#)] [[PubMed](#)]
37. Mehta, B.M. Chemical Composition of Milk and Milk Products. In *Handbook of Food Chemistry*; Springer: Berlin/Heidelberg, Germany, 2015; pp. 1–34.
38. Boro, P.; Debnath, J.; Das, T.; Naha, B.; Debbarma, P.; Debbarma, C.; Suinti, L.; Devi, B.; Gynashwari, T. Milk Composition and Factors Affecting It in Dairy Buffaloes: A Review. *J. Entomol. Zool. Stud.* **2018**, *6*, 340–343.
39. Manuyakorn, W.; Tanpowpong, P. Cow Milk Protein Allergy and Other Common Food Allergies and Intolerances. *Paediatr. Int. Child Health* **2019**, *39*, 32–40. [[CrossRef](#)]
40. Jiménez-Montenegro, L.; Alfonso, L.; Mendizabal, J.A.; Urrutia, O. Worldwide Research Trends on Milk Containing Only A2 β -Casein: A Bibliometric Study. *Animals* **2022**, *12*, 1909. [[CrossRef](#)]
41. Nie, C.; Zhao, Y.; Wang, X.; Li, Y.; Fang, B.; Wang, R.; Wang, X.; Liao, H.; Li, G.; Wang, P.; et al. Structure, Biological Functions, Separation, Properties, and Potential Applications of Milk Fat Globule Membrane (MFGM): A Review. *Nutrients* **2024**, *16*, 587. [[CrossRef](#)]
42. Sadiq, U.; Gill, H.; Chandrapala, J. Casein Micelles as an Emerging Delivery System for Bioactive Food Components. *Foods* **2021**, *10*, 1965. [[CrossRef](#)]
43. Tosif, M.M.; Najda, A.; Bains, A.; Krishna, T.C.; Chawla, P.; Dyduch-Siemińska, M.; Klepacka, J.; Kaushik, R. A Comprehensive Review on the Interaction of Milk Protein Concentrates with Plant-Based Polyphenolics. *Int. J. Mol. Sci.* **2021**, *22*, 13548. [[CrossRef](#)] [[PubMed](#)]
44. Bhat, M.Y.; Dar, T.A.; Singh, L.R. Casein Proteins: Structural and Functional Aspects. In *Milk Proteins—From Structure to Biological Properties and Health Aspects*; InTech: London, UK, 2016.
45. Huppertz, T.; Fox, P.F.; Kelly, A.L. The Caseins: Structure, Stability, and Functionality. In *Proteins in Food Processing*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 49–92.
46. Davoodi, S.H.; Shahbazi, R.; Esmaeili, S.; Sohrabvandi, S.; Mortazavian, A.; Jazayeri, S.; Taslimi, A. Health-Related Aspects of Milk Proteins. *Iran. J. Pharm. Res.* **2016**, *15*, 573–591. [[PubMed](#)]
47. Runthala, A.; Mbye, M.; Ayyash, M.; Xu, Y.; Kamal-Eldin, A. Caseins: Versatility of Their Micellar Organization in Relation to the Functional and Nutritional Properties of Milk. *Molecules* **2023**, *28*, 2023. [[CrossRef](#)] [[PubMed](#)]
48. Giribaldi, M.; Lamberti, C.; Cirrincione, S.; Giuffrida, M.G.; Cavallarin, L. A2 Milk and BCM-7 Peptide as Emerging Parameters of Milk Quality. *Front. Nutr.* **2022**, *9*, 842375. [[CrossRef](#)] [[PubMed](#)]
49. Gonzales-Malca, J.A.; Tirado-Kulieva, V.A.; Abanto-López, M.S.; Aldana-Juárez, W.L.; Palacios-Zapata, C.M. Worldwide Research on the Health Effects of Bovine Milk Containing A1 and A2 β -Casein: Unraveling the Current Scenario and Future Trends through Bibliometrics and Text Mining. *Curr. Res. Food Sci.* **2023**, *7*, 100602. [[CrossRef](#)] [[PubMed](#)]
50. Pal, S.; Woodford, K.; Kukuljan, S.; Ho, S. Milk Intolerance, Beta-Casein and Lactose. *Nutrients* **2015**, *7*, 7285–7297. [[CrossRef](#)] [[PubMed](#)]
51. Chia, J.S.J.; McRae, J.L.; Kukuljan, S.; Woodford, K.; Elliott, R.B.; Swinburn, B.; Dwyer, K.M. A1 Beta-Casein Milk Protein and Other Environmental Pre-Disposing Factors for Type 1 Diabetes. *Nutr. Diabetes* **2017**, *7*, e274. [[CrossRef](#)] [[PubMed](#)]
52. Kuellenberg de Gaudry, D.; Lohner, S.; Bischoff, K.; Schmucker, C.; Hoerrlein, S.; Roeger, C.; Schwingshackl, L.; Meerpohl, J.J. A1- and A2 Beta-Casein on Health-Related Outcomes: A Scoping Review of Animal Studies. *Eur. J. Nutr.* **2022**, *61*, 1–21. [[CrossRef](#)]
53. Yildirim-Elikoglu, S.; Erdem, Y.K. Interactions between Milk Proteins and Polyphenols: Binding Mechanisms, Related Changes, and the Future Trends in the Dairy Industry. *Food Rev. Int.* **2018**, *34*, 665–697. [[CrossRef](#)]
54. Edwards, T.S.; Dawson, K.L.; Keenan, J.I.; Day, A.S. A Simple Method to Generate β -Casomorphin-7 by in Vitro Digestion of Casein from Bovine Milk. *J. Funct. Foods* **2021**, *85*, 104631. [[CrossRef](#)]
55. MG, P.; Gourkhede, D.P.; HB, V.; Shinde, B.; Mishra, B.P.; Wankhade, P.R.; Belore, B.; Lalthanmawii, J.; Koneti, P.B. Delving into the A1/A2 Milk Hypothesis: A Comprehensive Analysis of Milk Proteins and Their Impact on Human Health. *Int. J. Vet. Sci. Anim. Husb.* **2024**, *9*, 594–605. [[CrossRef](#)]
56. Kumar, A.; Rao, B.; De, A.K. Milk Proteins, Health Issues and Its Implications on National Livestock Breeding Policy of India. *Curr. Sci.* **2018**, *115*, 1393. [[CrossRef](#)]
57. Jeong, H.; Park, Y.-S.; Yoon, S.-S. A2 Milk Consumption and Its Health Benefits: An Update. *Food Sci. Biotechnol.* **2024**, *33*, 491–503. [[CrossRef](#)] [[PubMed](#)]
58. Vigolo, V.; Visentin, E.; Ballancin, E.; Lopez-Villalobos, N.; Penasa, M.; De Marchi, M. β -Casein A1 and A2: Effects of Polymorphism on the Cheese-Making Process. *J. Dairy Sci.* **2023**, *106*, 5276–5287. [[CrossRef](#)] [[PubMed](#)]
59. Nguyen, H.T.H.; Schwendel, H.; Harland, D.; Day, L. Differences in the Yoghurt Gel Microstructure and Physicochemical Properties of Bovine Milk Containing A1A1 and A2A2 β -Casein Phenotypes. *Food Res. Int.* **2018**, *112*, 217–224. [[CrossRef](#)] [[PubMed](#)]
60. de Vitte, K.; Kerziene, S.; Klementavičiūtė, J.; de Vitte, M.; Dilbiene, V.; Stankevičius, R. Relationship between β -Casein Genotypes (A1A1, A1A2, and A2A2) and Coagulation Properties of Milk and the Fatty Acid Composition and Sensory Characteristics of Dairy Products (Soft Cheese, Sour Cream, and Butter). *Acta Agric. Scand. A Anim. Sci.* **2022**, *71*, 21–32. [[CrossRef](#)]

61. de Vitte, K.; Kerziene, S.; Klementavičiūtė, J.; de Vitte, M.; Mišeikienė, R.; Kudlinskienė, I.; Čepaitė, J.; Dilbiene, V.; Stankevičius, R. Relationship of β -Casein Genotypes (A1A1, A1A2 and A2A2) to the Physicochemical Composition and Sensory Characteristics of Cows' Milk. *J. Appl. Anim. Res.* **2022**, *50*, 161–166. [[CrossRef](#)]
62. Deeth, H.C.; Lewis, M.J. Heat Treatments of Milk—Thermisation and Pasteurisation. In *High Temperature Processing of Milk and Milk Products*; Wiley: Hoboken, NJ, USA, 2017; pp. 15–39.
63. Albarella, S.; Selvaggi, M.; D'Anza, E.; Cosenza, G.; Caira, S.; Scaloni, A.; Fontana, A.; Peretti, V.; Ciotola, F. Influence of the Casein Composite Genotype on Milk Quality and Coagulation Properties in the Endangered Agerolese Cattle Breed. *Animals* **2020**, *10*, 892. [[CrossRef](#)]
64. Sempira, E.J.; Mugisa, D.J.; Galiwango, J.; Kisaalita, W.S. Combining Thermization and Evaporative Cooling toward Milk Freshness Preservation at the Smallholder Farm Level. *J. Food Process Eng.* **2020**, *43*, e13529. [[CrossRef](#)]
65. Rukke, E.O.; Sørhaug, T.; Stepaniak, L. HEAT TREATMENT OF MILK | Thermization of Milk. In *Encyclopedia of Dairy Sciences*; Elsevier: Amsterdam, The Netherlands, 2011; pp. 693–698.
66. Panthi, R.R.; Jordan, K.N.; Kelly, A.L.; (Diarmuid) Sheehan, J.J. Selection and Treatment of Milk for Cheesemaking. In *Cheese*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 23–50.
67. Dhotre, A.V. Milk Pasteurization and Equipment. In *Animal Products Technology*; Mandal, P.K., Biswas, A.K., Eds.; Studium Press (India) Pvt.Ltd.: New Delhi, India, 2014; pp. 51–78.
68. Myer, P.R.; Parker, K.R.; Kanach, A.T.; Zhu, T.; Morgan, M.T.; Applegate, B.M. The Effect of a Novel Low Temperature-Short Time (LTST) Process to Extend the Shelf-Life of Fluid Milk. *Springerplus* **2016**, *5*, 660. [[CrossRef](#)] [[PubMed](#)]
69. Indumathy, M.; Sobana, S.; Panda, B.; Panda, R.C. Modelling and Control of Plate Heat Exchanger with Continuous High-Temperature Short Time Milk Pasteurization Process—A Review. *Chem. Eng. J. Adv.* **2022**, *11*, 100305. [[CrossRef](#)]
70. Zhang, Y.; Yi, S.; Lu, J.; Pang, X.; Xu, X.; Lv, J.; Zhang, S. Effect of Different Heat Treatments on the Maillard Reaction Products, Volatile Compounds and Glycation Level of Milk. *Int. Dairy J.* **2021**, *123*, 105182. [[CrossRef](#)]
71. Balthazar, C.F.; Cabral, L.; Guimarães, J.T.; Noronha, M.F.; Cappato, L.P.; Cruz, A.G.; Sant'Ana, A.S. Conventional and Ohmic Heating Pasteurization of Fresh and Thawed Sheep Milk: Energy Consumption and Assessment of Bacterial Microbiota during Refrigerated Storage. *Innov. Food Sci. Emerg. Technol.* **2022**, *76*, 102947. [[CrossRef](#)]
72. Rocha, R.S.; Silva, R.; Ramos, G.L.P.; Cabral, L.A.; Pimentel, T.C.; Campelo, P.H.; Blumer Zacarchenco, P.; Freitas, M.Q.; Esmerino Erick, A.; Silva, M.C.; et al. Ohmic Heating Treatment in High-Protein Vanilla Flavored Milk: Quality, Processing Factors, and Biological Activity. *Food Res. Int.* **2022**, *161*, 111827. [[CrossRef](#)]
73. Ahmad, T.; Butt, M.Z.; Aadil, R.M.; Inam-ur-Raheem, M.; Abdullah; Bekhit, A.E.; Guimarães, J.T.; Balthazar, C.F.; Rocha, R.S.; Esmerino, E.A.; et al. Impact of Nonthermal Processing on Different Milk Enzymes. *Int. J. Dairy Technol.* **2019**, *72*, 481–495. [[CrossRef](#)]
74. Abrahamsen, R.K.; Narvhus, J.A. Can Ultrasound Treatment Replace Conventional High Temperature Short Time Pasteurization of Milk? A Critical Review. *Int. Dairy J.* **2022**, *131*, 105375. [[CrossRef](#)]
75. Masotti, F.; Cattaneo, S.; Stuknytė, M.; De Noni, I. Current Insights into Non-Thermal Preservation Technologies Alternative to Conventional High-Temperature Short-Time Pasteurization of Drinking Milk. *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 5643–5660. [[CrossRef](#)] [[PubMed](#)]
76. Lee, G.J.; Han, B.K.; Choi, H.J.; Kang, S.H.; Baick, S.C.; Lee, D.-U. Inactivation of Escherichia Coli, Saccharomyces Cerevisiae, and Lactobacillus Brevis in Low-Fat Milk by Pulsed Electric Field Treatment: A Pilot-Scale Study. *Korean J. Food Sci. Anim. Resour.* **2015**, *35*, 800–806. [[CrossRef](#)]
77. Preeti, B.; Ravindra, M.R.; Shivaram, M.; Gajanan, D.P.; Singh, A.M. Effect of Pulsed Electric Field Treated on Quality of Curd. *Food Sci. Technol. Int.* **2023**, *29*, 598–609. [[CrossRef](#)]
78. Sharma, P.; Oey, I.; Everett, D.W. Thermal Properties of Milk Fat, Xanthine Oxidase, Caseins and Whey Proteins in Pulsed Electric Field-Treated Bovine Whole Milk. *Food Chem.* **2016**, *207*, 34–42. [[CrossRef](#)] [[PubMed](#)]
79. Venkatram, R.; García-Cano, I.; Jiménez-Flores, R. Reduction in the Antigenicity of Beta-Lactoglobulin in Whole Milk Powder via Supercritical CO₂ Treatment. *J. Dairy Sci.* **2024**, *107*, 4216–4234. [[CrossRef](#)] [[PubMed](#)]
80. Dey Paul, I.; Jayakumar, C.; Niwas Mishra, H. Optimization of Process Parameters for Supercritical Fluid Extraction of Cholesterol from Whole Milk Powder Using Ethanol as Co-solvent. *J. Sci. Food Agric.* **2016**, *96*, 4885–4895. [[CrossRef](#)] [[PubMed](#)]
81. Ceni, G.; Fernandes Silva, M.; Valério, C., Jr.; Cansian, R.L.; Oliveira, J.V.; Dalla Rosa, C.; Mazutti, M.A. Continuous Inactivation of Alkaline Phosphatase and Escherichia Coli in Milk Using Compressed Carbon Dioxide as Inactivating Agent. *J. CO₂ Util.* **2016**, *13*, 24–28. [[CrossRef](#)]
82. Atik, A.; Gumus, T. The Effect of Different Doses of UV-C Treatment on Microbiological Quality of Bovine Milk. *LWT* **2021**, *136*, 110322. [[CrossRef](#)]
83. Ansari, J.A.; Ismail, M.; Farid, M. Investigate the Efficacy of UV Pretreatment on Thermal Inactivation of Bacillus Subtilis Spores in Different Types of Milk. *Innov. Food Sci. Emerg. Technol.* **2019**, *52*, 387–393. [[CrossRef](#)]
84. Makararpong, D.; Tantayanon, S.; Gowanit, C.; Jareonsawat, J.; Samgnamnim, S.; Wataradee, S.; Hogeveen, H.; Inchaisri, C. Enhancing Raw Bovine Milk Quality Using Ultraviolet-C (UV-C) Irradiation: A Microbial and Lipid Peroxidation Study. *Food Sci. Anim. Resour.* **2024**, *44*, 372–389. [[CrossRef](#)] [[PubMed](#)]

85. Sadeghzadeh, M.; Ghorbani-HasanSaraei, A.; Sadeghzadeh, N. Evaluation of the Effect of Gamma Irradiation in Combination with Ascorbic Acid on the Chemical Parameters and the Decontamination of Raw Milk. *Radiat. Phys. Chem.* **2022**, *201*, 110462. [[CrossRef](#)]
86. Harizi, N.; Madureira, J.; Haffani, Y.Z.; Zouari, A.; Ayadi, M.A.; Verde, S.C.; Boudhrioua, N. E-Beam Irradiation of Defatted Liquid Camel and Cow Milk Fractions: Antiproliferative, Antidiabetic and Antioxidant Activities. *Innov. Food Sci. Emerg. Technol.* **2023**, *89*, 103457. [[CrossRef](#)]
87. de Oliveira Silva, A.C.; de Oliveira, L.A.T.; de Jesus, E.F.O.; Cortez, M.A.S.; Alves, C.C.C.; Monteiro, M.L.G.; Conte Junior, C.A. Effect of Gamma Irradiation on the Bacteriological and Sensory Analysis of Raw Whole Milk under Refrigeration. *J. Food Process Preserv.* **2015**, *39*, 2404–2411. [[CrossRef](#)]
88. Mohammadpour, H.; Zarei, M.; Cullen, P.J.; Valtchev, P.; Schindeler, A.; Dehghani, F. Potential Application of Non-Thermal Atmospheric Plasma in Reducing the Activity of Pseudomonas-Secreted Proteases in Milk. *Int. Dairy J.* **2021**, *120*, 105078. [[CrossRef](#)]
89. Korachi, M.; Ozen, F.; Aslan, N.; Vannini, L.; Guerzoni, M.E.; Gottardi, D.; Ekinci, F.Y. Biochemical Changes to Milk Following Treatment by a Novel, Cold Atmospheric Plasma System. *Int. Dairy J.* **2015**, *42*, 64–69. [[CrossRef](#)]
90. Sharma, S.; Singh, R.K. Effect of Atmospheric Cold Plasma Treatment on Acid Gelation Properties of Skim Milk: Rheology and Textural Studies. *Food Res. Int.* **2023**, *172*, 113212. [[CrossRef](#)]
91. de Oliveira, M.M.; de Castro Leite Júnior, B.R.; Tribst, A.A.L.; Cristianini, M. Use of High Pressure Homogenization to Reduce Milk Proteolysis Caused by Pseudomonas Fluorescens Protease. *LWT* **2018**, *92*, 272–275. [[CrossRef](#)]
92. He, X.; Yang, M.; Yuan, F.; Singh, H.; Ye, A. High-Pressure Processing of Bovine Milk: Effects on the Coagulation of Protein and Fat Globules during Dynamic in Vitro Gastric Digestion. *Curr. Res. Food Sci.* **2022**, *5*, 1530–1538. [[CrossRef](#)] [[PubMed](#)]
93. Liepa, M.; Zagorska, J.; Galoburda, R.; Kostascuka, S. Effect of High-Pressure Processing on Microbial Quality of Skimmed Milk. *Proc. Latv. Acad. Sci. Sect. B Nat. Exact Appl. Sci.* **2018**, *72*, 118–122. [[CrossRef](#)]
94. Juraga, E.; Vukušić Pavičić, T.; Gajdoš Kljusurić, J.; Brnčić, M.; Juraga, T.; Herceg, Z. Properties of Milk Treated with High-Power Ultrasound and Bactofugation. *Food Technol. Biotechnol.* **2021**, *59*, 92–102. [[CrossRef](#)]
95. Hammam, A.R.A.; Martinez-Monteagudo, S.I.; Metzger, L.E.; Alsalem, K.A. Effect of Ultrasound Intensity on the Functional Characteristics of Rennet-coagulated Skim Milk. *J. Food Process Eng.* **2021**, *44*, e13800. [[CrossRef](#)]
96. Yang, J.; Yang, M.; Qin, J.; Zeng, Q.; Wang, Y.; Han, N. Effect of Ultrasound on the Structural Characteristics of Fresh Skim Milk. *Food Sci. Technol. Int.* **2020**, *26*, 222–230. [[CrossRef](#)]
97. Al-Tayawi, A.N.; Gulyás, N.S.; Gergely, G.; Fazekas, Á.F.; Szegedi, B.; Hodúr, C.; Lennert, J.R.; Kertész, S. Enhancing Ultrafiltration Performance for Dairy Wastewater Treatment Using a 3D Printed Turbulence Promoter. *Environ. Sci. Pollut. Res.* **2023**, *30*, 108907–108916. [[CrossRef](#)]
98. Hansen, S.F.; Petrat-Melin, B.; Rasmussen, J.T.; Larsen, L.B.; Ostenfeld, M.S.; Wiking, L. Placing Pasteurisation before or after Microfiltration Impacts the Protein Composition of Milk Fat Globule Membrane Material. *Int. Dairy J.* **2018**, *81*, 35–41. [[CrossRef](#)]
99. Dons, T.; Candelario, V.; Andersen, U.; Ahmé, L.M. Gentle Milk Fat Separation Using Silicon Carbide Ceramic Membranes. *Innov. Food Sci. Emerg. Technol.* **2023**, *84*, 103299. [[CrossRef](#)]
100. Shabbir, M.A.; Ahmed, H.; Maan, A.A.; Rehman, A.; Afraz, M.T.; Iqbal, M.W.; Khan, I.M.; Amir, R.M.; Ashraf, W.; Khan, M.R.; et al. Effect of Non-Thermal Processing Techniques on Pathogenic and Spoilage Microorganisms of Milk and Milk Products. *Food Sci. Technol.* **2021**, *41*, 279–294. [[CrossRef](#)]
101. Alirezalu, K.; Munekata, P.E.S.; Parniakov, O.; Barba, F.J.; Witt, J.; Toepfl, S.; Wiktor, A.; Lorenzo, J.M. Pulsed Electric Field and Mild Heating for Milk Processing: A Review on Recent Advances. *J. Sci. Food Agric.* **2020**, *100*, 16–24. [[CrossRef](#)] [[PubMed](#)]
102. Singh, S.K.; Pavan, M.S.; SaiPrasanna, N.; Kant, R. Applications of Super Critical Fluid Extraction in Milk and Dairy Industry: A Review. *J. Food Process. Technol.* **2018**, *9*, 12. [[CrossRef](#)]
103. Amaral, G.V.; Silva, E.K.; Cavalcanti, R.N.; Cappato, L.P.; Guimaraes, J.T.; Alvarenga, V.O.; Esmerino, E.A.; Portela, J.B.; Sant' Ana, A.S.; Freitas, M.Q.; et al. Dairy Processing Using Supercritical Carbon Dioxide Technology: Theoretical Fundamentals, Quality and Safety Aspects. *Trends Food Sci. Technol.* **2017**, *64*, 94–101. [[CrossRef](#)]
104. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Safety of UV-treated Milk as a Novel Food Pursuant to Regulation (EC) No 258/97. *EFSA J.* **2016**, *14*, 4370. [[CrossRef](#)]
105. Delorme, M.M.; Guimarães, J.T.; Coutinho, N.M.; Balthazar, C.F.; Rocha, R.S.; Silva, R.; Margalho, L.P.; Pimentel, T.C.; Silva, M.C.; Freitas, M.Q.; et al. Ultraviolet Radiation: An Interesting Technology to Preserve Quality and Safety of Milk and Dairy Foods. *Trends Food Sci. Technol.* **2020**, *102*, 146–154. [[CrossRef](#)]
106. Jermann, C.; Koutchma, T.; Margas, E.; Leadley, C.; Ros-Polski, V. Mapping Trends in Novel and Emerging Food Processing Technologies around the World. *Innov. Food Sci. Emerg. Technol.* **2015**, *31*, 14–27. [[CrossRef](#)]
107. Bhullar, M.S.; Patras, A.; Kilanzo-Nthenge, A.; Pokharel, B.; Yannam, S.K.; Rakariyatham, K.; Pan, C.; Xiao, H.; Sasges, M. Microbial Inactivation and Cytotoxicity Evaluation of UV Irradiated Coconut Water in a Novel Continuous Flow Spiral Reactor. *Food Res. Int.* **2018**, *103*, 59–67. [[CrossRef](#)]
108. Cilliers, F.P.; Gouws, P.A.; Koutchma, T.; Engelbrecht, Y.; Adriaanse, C.; Swart, P. A Microbiological, Biochemical and Sensory Characterisation of Bovine Milk Treated by Heat and Ultraviolet (UV) Light for Manufacturing Cheddar Cheese. *Innov. Food Sci. Emerg. Technol.* **2014**, *23*, 94–106. [[CrossRef](#)]

109. Wen, C.; Peng, Y.; Zhang, L.; Chen, Y.; Yu, J.; Bai, J.; Yang, K.; Ding, W. Effect of Electron Beam Irradiation on Raw Goat Milk: Microbiological, Physicochemical and Protein Structural Analysis. *J. Sci. Food Agric.* **2024**. *Online ahead of print*. [[CrossRef](#)] [[PubMed](#)]
110. Lung, H.-M.; Cheng, Y.-C.; Chang, Y.-H.; Huang, H.-W.; Yang, B.B.; Wang, C.-Y. Microbial Decontamination of Food by Electron Beam Irradiation. *Trends Food Sci. Technol.* **2015**, *44*, 66–78. [[CrossRef](#)]
111. Odueke, O.B.; Chadd, S.A.; Baines, R.N.; Farag, K.W.; Jansson, J. Effects of Gamma Irradiation on the Shelf-Life of a Dairy-like Product. *Radiat. Phys. Chem.* **2018**, *143*, 63–71. [[CrossRef](#)]
112. Carvalho Santos, I.; Pinto, J.; Pimenta, A.I.; Madureira, J.; Matos, P.; Viegas, C.; Raposo, A.; Margaça, F.M.A.; Cabo Verde, S. Use of Gamma Radiation in Sheep Butter Manufacturing Process for Shelf-Life Extension. *Int. Dairy J.* **2017**, *71*, 43–49. [[CrossRef](#)]
113. Rathod, N.B.; Kahar, S.P.; Ranveer, R.C.; Annapure, U.S. Cold Plasma an Emerging Nonthermal Technology for Milk and Milk Products: A Review. *Int. J. Dairy Technol.* **2021**, *74*, 615–626. [[CrossRef](#)]
114. Marcinkowska-Lesiak, M.; Wojtasik-Kalinowska, I.; Onopiuk, A.; Stelmasiak, A.; Wierzbicka, A.; Poltorak, A. Green Technology for Pork Loin Wet Curing—Unconventional Use of Cow and Soy Milk Treated with Non-Thermal Atmospheric Plasma. *Foods* **2022**, *11*, 2523. [[CrossRef](#)] [[PubMed](#)]
115. Nikmaram, N.; Keener, K.M. The Effects of Cold Plasma Technology on Physical, Nutritional, and Sensory Properties of Milk and Milk Products. *LWT* **2022**, *154*, 112729. [[CrossRef](#)]
116. Lee, T.-A.; Lin, Y.-H.; Li, P.-H.; Ho, J.-H. The Effects of Corona Discharge from a Cold Plasma Source on the Physicochemical Properties and Shelf-Life of Milk. *Food Biosci.* **2024**, 103980. [[CrossRef](#)]
117. Lim, S.H.; Chin, N.L.; Sulaiman, A.; Tay, C.H.; Wong, T.H. Sensory Analysis for Cow Milk Product Development Using High Pressure Processing (HPP) in the Dairy Industry. *Foods* **2022**, *11*, 1233. [[CrossRef](#)]
118. Rodríguez-Alcalá, L.M.; Castro-Gómez, P.; Felipe, X.; Noriega, L.; Fontecha, J. Effect of Processing of Cow Milk by High Pressures under Conditions up to 900 MPa on the Composition of Neutral, Polar Lipids and Fatty Acids. *LWT Food Sci. Technol.* **2015**, *62*, 265–270. [[CrossRef](#)]
119. Stratakos, A.C.; Inguglia, E.S.; Linton, M.; Tollerton, J.; Murphy, L.; Corcionivoschi, N.; Koidis, A.; Tiwari, B.K. Effect of High Pressure Processing on the Safety, Shelf Life and Quality of Raw Milk. *Innov. Food Sci. Emerg. Technol.* **2019**, *52*, 325–333. [[CrossRef](#)]
120. Chávez-Martínez, A.; Reyes-Villagrana, R.A.; Rentería-Monterrubio, A.L.; Sánchez-Vega, R.; Tirado-Gallegos, J.M.; Bolívar-Jacobo, N.A. Low and High-Intensity Ultrasound in Dairy Products: Applications and Effects on Physicochemical and Microbiological Quality. *Foods* **2020**, *9*, 1688. [[CrossRef](#)] [[PubMed](#)]
121. Singh, P.K.; Huppertz, T. Effect of Nonthermal Processing on Milk Protein Interactions and Functionality. In *Milk Proteins*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 293–324.
122. Reig, M.; Vecino, X.; Cortina, J.L. Use of Membrane Technologies in Dairy Industry: An Overview. *Foods* **2021**, *10*, 2768. [[CrossRef](#)]
123. Mukhopadhyay, S.; Ukuku, D.O. The Role of Emerging Technologies to Ensure the Microbial Safety of Fresh Produce, Milk and Eggs. *Curr. Opin. Food Sci.* **2018**, *19*, 145–154. [[CrossRef](#)]
124. Steinhauer, T.; Marx, M.; Bogendörfer, K.; Kulozik, U. Membrane Fouling during Ultra- and Microfiltration of Whey and Whey Proteins at Different Environmental Conditions: The Role of Aggregated Whey Proteins as Fouling Initiators. *J. Membr. Sci.* **2015**, *489*, 20–27. [[CrossRef](#)]
125. Hansen, S.F.; Hogan, S.A.; Tobin, J.; Rasmussen, J.T.; Larsen, L.B.; Wiking, L. Microfiltration of Raw Milk for Production of High-Purity Milk Fat Globule Membrane Material. *J. Food Eng.* **2020**, *276*, 109887. [[CrossRef](#)]
126. Yao, D.; Ranadheera, C.S.; Shen, C.; Wei, W.; Cheong, L.-Z. Milk Fat Globule Membrane: Composition, Production and Its Potential as Encapsulant for Bioactives and Probiotics. *Crit. Rev. Food Sci. Nutr.* **2023**, 1–16. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.