

# Hard and Semi-Hard Cheeses Preservation

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Cheese is a dairy product with potential health benefits. Cheese consumption has increased due to the significant diversity of varieties, versatility of product presentation, and changes in consumers' lifestyles. Spoilage of hard and semi-hard cheeses can be promoted by their maturation period and/or by their long shelf-life. Therefore, preservation studies play a fundamental role in maintaining and/or increasing their shelf-life, and are of significant importance for the dairy sector.

ripened cheese

shelf-life

security

storage improvement

conservation

## 1. Introduction

Extending the shelf-life of food products has long been a significant concern for the dairy industry. Traditionally, this utilized natural atmospheric conditions, such as sun drying in summer, and cold and freezing in winter, and the advantages of natural fermentation for cheese preservation <sup>[1]</sup>. In recent years, active and intelligent packaging and non-thermal technologies have emerged to prevent the deterioration of perishable food products.

Cheeses can be made from different types of milk and processing technologies, and ripened for different periods, resulting in numerous varieties with a wide diversity in terms of texture, flavor, and shape. Hard and semi-hard cheeses are versatile nutrient-dense dairy products. Although these are highly valued, from a health perspective, significant controversy exists among consumers and in the scientific community. Cheese contains saturated fatty acids, cholesterol, and salt, which have been associated with cardiovascular disease (CVD) risk; however, cheeses also contain a range of nutrients that are potentially beneficial to health <sup>[2][3][4]</sup>. Recent studies indicate that not all saturated fatty acids raise the content of cholesterol in plasma in the same manner, and that some saturated and trans fatty acids in cheese may play a beneficial role in human health. Moreover, other healthy components present in cheeses are conjugated linoleic acids (CLA) and phospholipids from the fat globular membrane <sup>[2][3][5]</sup>. In this regard, fermented dairy products have been proposed as functional foods with a cholesterol-lowering effect and, therefore, with a protective effect against CVD compared to non-fermented dairy products. The so-called French paradox, in which low mortality from coronary heart disease has been observed despite the high cheese intake by consumers, is an important consideration in the nutritional assessment of cheese consumption <sup>[4][6]</sup>. A recent study reported that increased dairy consumption may contribute to a lifestyle associated with a reduction in CVD risk <sup>[7]</sup>. In addition, bioactive components are generated during cheese fermentation, such as gamma aminobutyric acid, which favors the survival of probiotic microorganisms <sup>[8]</sup>.

Cheese also contains digestible proteins of high biological value. During cheese ripening and food digestion, caseins are hydrolyzed and peptides with antioxidant capacity are generated. The addition of an adjunct culture and a long ripening time increases the formation of peptides and enhances the antioxidant capacity. Some of these peptides are also a prominent source for nutraceutical functional foods [2]. Many of the fat-soluble vitamins are held in cheese fat. Although some water-soluble vitamins may be lost during whey drainage, folate, niacin, B12, and riboflavin remain in sufficient quantities in the cheese matrix to have a significant effect on human nutrition. In addition, propionic acid bacteria synthesize considerable levels of vitamin B12 in hard cheeses [2]. Ripened cheeses are an important source of minerals, particularly calcium (Ca) and phosphorus (P). The calcium in cheese is highly bioavailable due to the formation of complexes with peptides and its high content promotes fat excretion and reduces blood pressure. Similarly, cheese is a good source of this mineral for lactose intolerant individuals [6]. The acid phosphatase enzymes aid in the generation of phosphorylated peptides, which also have beneficial health effects. In this regard, IDF [9] indicates that the elimination of dairy products from diets may result in a loss of calcium and other essential nutrients for part of the population. Sodium (Na) is a nutrient that should be reduced in the diet, but it is estimated that cheese adds only about 5–8% of the total Na intake [2]. A recent study reported that the intake of Na from cheese may prevent induced vessel alteration by reducing oxidative stress, rather than the intake of Na from non-dairy foods. Therefore, cheese intake may be an effective dietary strategy to reduce the risk of CVD in healthy older adults without salt-sensitive blood pressure [7]. Therefore, cheese is a highly valued product by consumers and its consumption has increased in recent years because, among other reasons, a significant proportion of consumers perceive it as a healthy food.

The European Union (EU) is the world's leading cheese producer, followed by the United States (USA). Combined, the two areas account for around 70% of the global production. In total, EU countries produced 8959 million metric tons of cheese in 2010, and in 2020 the EC production was 10,350 million metric tons [10]. Global cheese production is expected to increase progressively until 2027, with developed countries increasing milk production by 9%. Of this increased milk production, 37% is expected to be used to make cheese [11]. Cheese consumption has grown in all global regions; USA and, in particular, the EU, are the main cheese consuming areas. In 2010, around 17 kg of cheese per person/per year was consumed globally, and this amount increased to 18.44 kg cheese per person in 2020 [12]. The EU has recently expanded cheese exports to Canada and the Russian Federation, and it is expected that China and Egypt will double cheese imports by 2027. In addition, other regions, such as the Middle East and North Africa, will become key destinations, accounting for 19% of global cheese imports by 2027, because cheese has been progressively introduced into the diets of their consumers [11]. The increase in global cheese consumption is also due to changes in food habits, particularly in East Asia, where the use of cheese as an ingredient in snacks and processed meals has increased. Thus, cheese preservation methods are highly important for the dairy industry in order to increase cheese consumption.

Cow's milk cheeses are produced in the greatest quantity throughout the year, whereas the production of small ruminant milk cheeses is lower and seasonal [13][14][15]. Evidently, this seasonality affects the regularity of cheese manufacture during the year, in addition to the milk composition. In this respect, because the manufacturing time is not long, the shelf-life of these cheeses during which they retain their optimal sensory characteristics is short [16][17].

Therefore, an adequate preservation method is extremely important for hard and semi-hard cheeses, to increase the availability for a longer period without changing their sensory characteristics.

The *Codex Alimentarius* classifies cheese varieties according to their composition and consistency parameters and ripening times, taking into account the percentage of moisture without fat. The extra-hard specification refers to cheeses with a moisture content less than 51%. Cheeses with a moisture content between 49–56% and 54–69% are called hard and semi-hard cheeses, respectively. Soft cheeses have a moisture content higher than 67% [18]. The Spanish regulation indicates that semi-hard (semi-cured) cheeses must have a minimum ripening time of 20 days (cheese weight less than 1.5 kg) or 35 days (cheese weight greater than 1.5 kg), depending on the cheese weight when the cheeses are marketed. By comparison, hard (cured) cheeses must be ripened for at least 45 or 105 days, depending on whether the cheese weighs less than 1.5 kg [19].

Changes in protein and fat content in cheese during ripening are responsible for many important nutritional and sensory properties. Chemical reactions such as lipolysis may also occur during cheese storage. This is an important biochemical reaction responsible for generating the desired flavor of many cheese varieties. However, excessive amounts of short-chain fatty acids can lead to a rancid off-flavor in ripened cheeses [20]. In contrast, proteolysis breaks down proteins into peptides and amino acids, generally improving cheese texture and flavor. The hydrolysis of peptides and catabolism of amino acids, fatty acids, and lactic acid results in the formation of volatile compounds that strongly influences the cheese flavor. However, this hydrolytic process can also lead to an increase in the concentration of substances that are toxic to human health, such as biogenic amines [21]. Cheese ripening followed by a long storage period may cause economic losses to cheese makers if degradation processes occur due to inadequate storage conditions [22]. Therefore, it is highly important to avoid the deterioration of the dairy product at all stages. Although hard and semi-hard cheeses have a reasonably long shelf-life, this may be limited by several factors during the maturation and storage periods, so effective conservation techniques should be employed prior to commercialization [23]. Microbial lipases and proteases can generate off-flavors, strange colorings, and mycotoxins, which decrease the cheese quality and safety [17]. Moreover, the cost of preservation treatments to prevent or control the surface growth of molds and yeasts in cheeses is high [24]. These treatments aim to reduce spoilage microorganisms and eliminate pathogens without affecting the lactic bacteria responsible for the final characteristics of the cheese. Exposure of cheese to heat, oxygen, and light stimulates enzymatic oxidation reactions that can produce different degradation processes, such as discoloration, production of off-flavors, nutrient loss, and formation of toxic substances [23]. Light-induced oxidation of photosensitive substances in cheese, such as riboflavin and carotenoids, also requires the presence of oxygen [25][26]. The removal of oxygen in the atmosphere surrounding the cheese surface may prevent degradation processes during storage and marketing. The characteristics of whole cheeses are also susceptible to changes due to environmental factors, when sold unpackaged. Therefore, optional preservation methods to be applied during storage and marketing should be specifically investigated for each cheese variety.

Due to these reasons, cheese preservation plays a fundamental role in increasing the shelf-life of the product, and is of significant importance to the cheese industry. The aim of this review is to discuss the main contributions presented in the scientific literature on the methods to preserve hard and semi-hard cheeses, from the traditional to

the most innovative. For the different technologies, in addition to reviewing the information reported about the impact of the preservation methods on the cheese quality and safety, the main benefits and limitations for their industrial application is also discussed. Other aspects of interest that are taken into account in this review are the environmental impact, the contribution to the sustainability of the food chain, and the consumer preferences.

## 2. Freezing and Frozen Storage

Freezing allows food to be preserved for long periods, while also maintaining a high nutritional quality. This process consists of lowering the food temperature below its freezing point, which causes water crystallization, significantly inhibiting microbial growth and biochemical reactions. The formation of ice crystals can cause physical damage to the food structure [27]. This technique is widely used in the food industry, and authors have described the influence of freezing and frozen storage on the characteristics of hard and semi-hard cheeses [14][28].

This storage method may enable the accumulation of a long-term stock cheese reserve for the dairy industry, and this advantage is more important for seasonal production cheeses, such as ewe's and goat's milk cheeses [13][14][15]. Thus, to regulate the market for these seasonal cheeses, several frozen storage tests have been carried out, with little success. Freezing milk or freezing concentrated milk before cheesemaking have been described in the literature, but significant organoleptic defects have been observed in cheeses when freezing at  $-15$  and  $-27$  °C. Total viable counts and coliforms decline faster at  $-15$  °C than at  $-27$  °C [29]. However, some authors [30] reported that good quality cheese can be obtained from frozen sheep milk at  $-15$  and  $-25$  °C for up to 6 months, without influencing cheese yield or composition. Freezing the cheese curd has traditionally been considered as a useful option to regulate the seasonal cheese market. In this regard, it was found that freezing produced significant changes in the microstructure of Crottin de Chavignol goat cheese and reduced the total lactic acid bacteria (LAB) count by 2 log units [31]. Hispánico cheese of satisfactory texture and sensory properties was obtained by mixing frozen ewes' milk curd with fresh cows' milk curd with no significant differences in LAB count [32]. However, few authors have studied the impact of the freezing process on the characteristics of hard and semi-hard cheeses [14][28]. Freezing of ripened cheeses has also been attempted to slow the over-ripening process and extend the cheese shelf-life by inhibiting or reducing enzyme activities and chemical reactions [16]. Results reported for Motal cheese showed that the storage at  $-18$  °C reduced the formation of excessive amounts of free fatty acids (FFAs) and led to volatile compounds and little decline in LAB count, resulting in the maintenance of the product quality and extended shelf-life [28].

The optimization of the freezing method, storage conditions (temperature and time), and thawing are crucial factors, because the preservation should preserve the desirable qualities of the final product. In addition, the product quality depends on the type of cheese, its composition, and ripening time [33][34]. Depending on the transition from water to ice, the internal structure of the cheese matrix can be damaged, thus altering cheese texture properties [35]. Fat, moisture, and salt contents play a critical role in frozen cheese properties. Thus, low moisture cheeses resist frozen storage better than high moisture cheeses. In addition, a higher fat content helps cheese to resist structural changes during frozen storage. A high fat content in cheese was reported to maintain the ice crystal size below 50 µm in diameter [36]. By comparison, low protein hydration during the freezing process is

one of the main causes of defects in the texture, resulting in insufficient elasticity, and a crumbly and powdery product. It was reported that, at  $-20^{\circ}\text{C}$ , the water-holding capacity of proteins and the hydrophilic properties of curds were preserved in some Russian semi-hard cheese varieties. When using lower freezing temperatures, a transition of the micelle-bound water into ice occurred, and there were structural changes that led to the appearance of an additionally elastic and crumbly cheese consistency [34].

Fontecha et al. [37] studied the textural and microstructural characteristics of semi-hard sheep cheeses submitted to slow freezing at  $-35^{\circ}\text{C}$  (plate freezer, 1.55 cm/h) and fast freezing at  $-80^{\circ}\text{C}$  (liquid nitrogen vapor, 4.0 cm/h), and subsequent ripening after thawing. In both treatments, cheeses were frozen up to  $-20^{\circ}\text{C}$  and storage at  $-20^{\circ}\text{C}$  for four months. The results showed that a slow freezing rate together with a longer period of frozen storage increased the deformation of the cheese matrix and decreased the share strength and firmness of the thawed cheeses. Slowly frozen cheeses presented a more extensive breakdown in their microstructure with longer cracks than fast frozen cheeses, for which textural properties were closer to those of the unfrozen cheeses. Nevertheless, the subsequent ripening process tended to offset the changes in the cheese matrix and to equalize the characteristics of the final products, both frozen and non-frozen. Tejada et al. [13][38] investigated the effect of the freezing rate (slow at  $-20^{\circ}\text{C}$  and fast at  $-82^{\circ}\text{C}$ ) and frozen storage time (9 months at  $-20^{\circ}\text{C}$ ) on the properties of a ewe's milk cheese after 90 ripening days. No significant effect was observed for the two freezing treatments on the chemical and microbiological characteristics of the cheeses, and graininess of the cheese was only slightly greater in slowly frozen cheeses. There were no significant differences compared with control cheeses and between the two freezing rates for total viable counts and enterococci, *Enterobacteriaceae*, coliforms, staphylococci, molds/yeasts, and micrococci. *Leuconostoc* and *Lactobacilli* showed a gradual decrease that was more accentuated at the end of the storage period (9 months). This study concluded that chemical and microbiological composition, and sensory properties of the cheeses, did not change after six months storage at  $-20^{\circ}\text{C}$ . Similar results were published for a 180 day ripened Manchego-type cheese stored at  $-20^{\circ}\text{C}$  for six months. The microbiological results exhibited similar counts for total viable microorganisms, LAB, and molds and yeasts, in contrast to micrococci and staphylococci that decreased during the frozen storage [14].

Similarly, freezing at  $-20$  and  $-30^{\circ}\text{C}$  after 42 ripening days and frozen storage at  $-10$  and  $-20^{\circ}\text{C}$  did not affect the content of moisture, fat, and total nitrogen in semi-hard Serpa sheep cheeses. However, higher values of non-protein nitrogen and hardness were found, and some color parameters changed (more luminous and more yellow-green), in the frozen cheeses after 12 months of storage. In addition, the damage reflected in cheese properties was diminished using storage temperatures of  $-20^{\circ}\text{C}$  in comparison to  $-10^{\circ}\text{C}$ . In this case, slow or fast freezing did not affect the physico-chemical cheese properties [39].

The usefulness of the freezing process for the conservation of some goat's milk cheeses with different ripening times has also been reported [40][41]. Minimal flavor effects were observed in a goat cheese variety after six months storage at  $-20^{\circ}\text{C}$ . Five years of frozen storage at  $-20^{\circ}\text{C}$  had minimal effects on cheese flavor, and only a more granular and pasty texture was observed [15]. The protein bound water does not crystallize at  $-20^{\circ}\text{C}$ , and its physical properties remain unchanged [34]. Thus, this temperature suits the maximum level of maintenance of the

protein structure, whereas other freezing treatments result in low quality cheeses due to the chemical reactions caused by the presence of a high quantity of the unfrozen solution and by the freezing of the bound water.

The low-temperature processing and storage ensure longer cheese preservation of up to a year or more, and can be beneficial for the profitability of the dairy sector and beneficial for the environment (lower energy consumption) [34]. Additionally, it was described that storage at freezing temperatures of Motal semi-hard cheese hindered the formation of biogenic amines after 180 days of storage, which contributed to healthier aged cheeses [28]. However, this conservation technique can have several drawbacks. To avoid freezing burns, cheeses must be packaged, and this pre-treatment involves the use of waterproof materials such as plastics, which is not a preferred option due to environmental and sustainability concerns, in addition to current legislative restrictions. Furthermore, the cold chain may break down during storage or transport and cause alterations in the cheese.

In general, the flavor and nutritional characteristics of the cheeses are not altered during frozen storage. In order to preserve cheese texture, several studies propose the use of a storage temperature of  $-20^{\circ}\text{C}$  rather than lower temperatures. Although freezing of milk, curd, or cheese has been proposed as an interesting option to regulate the market of seasonal products, this preservation technology is not currently used industrially for ripened cheeses.

Different innovative freezing processes are currently being tested to improve the quality of frozen foods. Johnston [42] investigated the potential of pressure-shift freezing at  $-20^{\circ}\text{C}$  at 200 MPa followed by pressure thawing of the cheese, with the aim of maintaining, as much as possible, the rheological characteristics of some cheese varieties. Although this innovative treatment can partially counteract changes in the rheological properties of Cheddar cheese, the frozen cheeses were still distinguishable from fresh cheeses in terms of texture parameters, such as deformation and compression at fracture. Many of these particular freezing techniques are still in the industrial development phase, and involve a high capital cost. For this reason, it is important to consider the product quality versus cost for the application of these preservative techniques to the dairy industry [27].

### 3. High Hydrostatic Pressure (HHP) Processing

HHP processing is probably the most advanced non-thermal emerging technology used for food processing at present time. Equipment for large-scale production with HHP is now commercially available, demonstrating the fast development that is taking place in the food industry sector [43]. During HHP treatment, the product is subjected for a short time period (10–20 min) to a very high pressure level (400–600 MPa is normally used at the industrial scale) and a temperature below  $45^{\circ}\text{C}$ . Based on the isostatic principle, pressure applied in HHP treatments is transmitted instantaneously and uniformly throughout food, regardless of size, shape, and composition [44]. This conservation treatment extends food shelf-life, and preserves nutritional characteristics and sensory attributes [45] [46].

HHP treatments have been described as being effective in reducing pathogenic and spoilage microorganisms in cheeses, and can produce biochemical changes due to alteration of proteolysis and lipolysis activities. Therefore, the shelf-life varies because ripening can be accelerated using HHP treatments with low to moderate pressures



(200–400 MPa), and storage cost can also be reduced [47][48]. In addition, it has been reported that pressures higher than 500 MPa cause a proteolysis reduction that prevents over-ripening of fresh, soft, and semi-hard cheeses, and slows chemical and enzymatic reactions that continue during refrigerated storage, at retail locations and at home [49][50][51].

Inactivation of microorganisms is due to morphological, biochemical, and genetic alterations that take place under high pressures. Gram positive (+) bacteria are more resistant to high pressure than Gram negative (–) bacteria, because the former are inactivated with treatments of 500–600 MPa, whereas 300–400 MPa are needed for the latter, in both cases using 10 min and 25 °C. Therefore, LAB present in milk can survive, whereas pathogenic microorganisms are eliminated. Rod-shaped bacteria are more sensitive than cocci, and endospores are highly resistant to HHP treatments, particularly *Clostridium* spp., which usually requires pressures around 1000 MPa. This is a significant issue when aiming to prevent late blowing defects in ripened cheeses. Because yeast and mold vegetative forms are the most pressure sensitive [52], HHP can prevent their growth during storage [53].

Microorganisms are more sensitive to high pressure treatments in a buffer solution than in the cheese matrix. A starter with *Lactococcus lactis* in a buffer solution was subjected to HHP in the range of 100–400 MPa for 20 min to produce microbial lysis and release enzymes to accelerate cheese maturation. Simultaneously, 1-day-old cheese with the same untreated strains was submitted to HHP. In the latter case, the HHP conditions did not improve starter autolysis [54]. A HHP treatment of 200 MPa for 20 min was applied either to starters (*Streptococcus thermophilus*, *L. lactis*, and *Lactobacillus bulgaricus*) or to ripened sheep cheese at the beginning of ripening, and cheese characteristics were compared with those of untreated control cheeses. All cheese samples were stored for 90 days at 4 °C. Cheeses from HHP-treated starters presented the higher sensory scores, and no bitterness was detected during storage. Secondary proteolysis was higher in these cheeses than in the other cheese samples, whereas the HHP-treated cheeses showed the highest aminopeptidase activity [47].

When high pressure treatment is applied to milk, microbiological quality comparable to that of pasteurized milk can be achieved [55]. Thus, thermal milk pasteurization can potentially be replaced by HHP treatment for cheesemaking [56]. However, milk HHP treatments can modify the physico-chemical structure of proteins, causing the fragmentation of casein micelles and denaturation of whey proteins, mainly due to the generation of non-covalent disruptions [57][58][59].

HHP processing is applied to cheese rather than to starters or milk [60]. HPP treatment has been shown to be an effective tool in eliminating cheese-borne pathogens. Such is the case with *Escherichia coli* tested in model semi-hard cheeses [61] or *Listeria* spp. in Iborea cheese [62]. However, the optimal processing parameters and the best time for HPP application may vary depending on cheese variety [51]. In this regard, cheese defects of microbial origin can be controlled by HPP treatments. Coliform growths responsible for the early blowing defect, particularly in cheese made from raw milk, can be limited using moderate HPP treatments (200–400 MPa) [45][50][63]. It was reported that pre-treatment of cheese at moderate pressure (300–500 MPa) induced the germination of *Clostridium tyrobutyricum* spores but a further high-pressure HPP treatment increased the microbial lethality [64]. In vacuum-packed 7-day ripened sheep cheese, the HHP treatment at 300 MPa avoided the late blowing defect, but these

pressurized cheeses showed a fracturable texture and low color, and the generation of certain volatile compounds was retarded [48].

Moschopoulou et al. [60] indicated that HHP treatment at 200 or 500 MPa applied to sheep cheese after 15 ripening days did not modify the cheese chemical composition. HHP treatments at 200 MPa were sufficient to inhibit coliform growth, and 500 MPa significantly delayed the growth of other microorganisms (total aerobic mesophiles, thermophilic starters, and non-starter bacteria (NSLAB). Arqués et al. [63] found a significant reduction of spoilage microorganism using HHP treatments (300–400 MPa for 10 min) applied to raw milk ewe's cheeses after 2 and 50 ripening days. HHP applied to 50 day matured cheeses did not affect their sensory properties, whereas the treatment applied at early ripening stages had a negative effect on cheese flavor. Similar results were obtained when pressures of 400 and 600 MPa were applied at three different ripening times (1, 30, or 50 ripening days) to raw goat's milk cheeses. Both HHP treatments reduced undesirable microorganisms in all cases. However, both HHP treatments applied at the first ripening day changed the texture profile, appearance, and flavor. In the case of a 600 MPa treatment applied to cheese at 30 and 50 ripening days, no sensory and proteolytic changes were observed, whereas spoilage microorganisms experienced a greater reduction. The content of short chain FFAs only decreased in cheeses treated at 600 MPa at the first ripening day. Medium and long FFA content did not vary with any of the HHP treatments [45][65]. Inácio et al. [56] reported a significant reduction in the microbial count of *Enterobacteriaceae*, *Listeria innocua*, molds and yeasts in raw milk ewe's cheeses treated with high pressure. HHP treatment (400–600 MPa) applied to cheese at 45 ripening days did not significantly modify its physico-chemical characteristics, although lipid oxidation was reduced in comparison with non-pressurized cheeses after 100 ripening days. Treatments of 400 MPa for 20 min in Cheddar cheese slurries inoculated with microorganisms produced a 3 log unit reduction in *Staphylococcus aureus*, and 6 and 7 log reductions in *E. coli* and *Penicillium roqueforti*, respectively, in addition to a reduction in the growth of molds and yeasts [53].

The application of HPP to cheese during ripening may lead to either the acceleration or the reduction of the ripening process. HHP treatments can influence cheese proteolysis by modifying the conformational structure of the proteins, activation or inactivation of proteinases, and inhibition or acceleration of the microbial growth and metabolism [66]. Studies showed that pressure intensity and the time of application are crucial to maintain the cheese's texture and flavor characteristics [54][60][65]. Changes during ripening can be due to an increase in primary or secondary proteolysis when using 200–400 MPa HHP treatments. The mechanism by which ripening is accelerated is still unclear and further research is needed. HHP treatments induce conformational changes in proteins, affecting the enzyme modulation sites [47]. In this regard, some authors have attributed the enhanced proteolysis to cell lysis and enzyme release. In addition, a higher pH value (0.1–0.2 units) in HPP-treated cheese is more favorable for starter peptidase activity and can improve proteolysis during cheese ripening [67][68]. Proteinases and peptidases responsible for peptides and free amino acid (FAA) release can be modified by HHP treatments and, consequently a reduction in ripening time can be expected in most cases [47][48][69].

Edam cheese proteolysis was examined with the aim to determine the possibilities of accelerating the cheese ripening process, or cheese preservation. Cheese samples were subjected to pressures of 200 and 400 MPa, after salting and after four, six and eight ripening weeks. Control samples were traditionally ripened Edam cheeses.



Pressures of 200 and 400 MPa had no significant effect on proteolysis, although HHP treatments improved cheese consistency [70]. By comparison, a 100-fold reduction in LAB growth together with a retarded growth of NSLAB was observed in a 180-day-old Cheddar cheese when 400 MPa HHP treatment was applied for 10 min on the first day post-processing. In this case, there was little effect on the primary proteolysis, because the activities of chymosin and plasmin were not affected by the treatment. The HHP-treated cheeses showed color alteration. After 90 ripening days, these cheeses presented higher scores in some sensory attributes (animal cooked fat flavor and butter odor), but the overall flavor intensity was lower in HHP-treated cheeses than in untreated cheeses [71]. Several references describe the effect of HHP on different types of cheese during ripening; Cheddar [54][72], Hispánico [73][74][75][76], Serena [77], ewe's milk [68], and Reggianito cheese [69]. Moreover, the effect of HHP (600 MPa) on partial or total inactivation of microorganisms and enzymes is effective in retarding proteolysis and lipolysis, and in reducing the formation of some undesirable volatile compounds [51].

The reduction in biogenic amine content induced by HPP is mainly due to the elimination of NSLAB with amino acid decarboxylation activity [51]. HPP treatments of 400 and 600 MPa applied for 5 min to ewe's milk cheeses at 21 or 35 ripening days were found to be useful to reduce the formation of biogenic amines in 60-day-old cold-stored cheeses. The 600 MPa level was more effective than that of 400 MPa. The decline in biogenic amines was attributed to reduced counts of enterococci and lactobacilli in HHP-treated cheeses, the decrease in decarboxylase activity, and the low concentration of FAAs [78]. From day 180 onwards, similar HHP effects on the biogenic amine content were found in raw cow's milk cheese, with similar HHP treatment applied at 14 or 21 ripening days. Lower short chain FFA concentrations in cheeses treated with 400 or 600 MPa were found in comparison to untreated cheeses after 140 ripening days; this may be due to a lower esterase activity in HHP-treated cheeses. By comparison, no significant differences were observed in flavor preference and intensity between HHP-treated and untreated cheeses, but bitterness was higher from day 60 onwards in 400 MPa-treated cheeses [50][79].

To achieve more regular cheese production throughout the year and reduce the storage time, particularly for ewe's and goat's cheeses, HHP has been applied to raw milk curd followed by frozen storage. The ewe's raw milk curd was treated at 400 or 500 MPa, and goat's raw milk curd at 400 MPa, for 10 min and kept frozen at  $-24^{\circ}\text{C}$  up to five months. Cheese manufacture was a mix of 20% (by weight) HHP-treated ewe's curd and 80% of freshly made curd from pasteurized cow's milk. In goat cheesemaking, the mixture of HHP-treated and freshly curd was 30:70. Control cheeses were made with the same curd mixtures but without HHP treatment. For both sheep and goat cheeses, at day 60, no differences were found between control and experimental cheeses in total viable microbial counts, Gram (–) bacteria and LAB growth, but staphylococci presented higher counts in 400 MPa-treated ewe's cheeses than in the other cheeses. Aminopeptidase activity showed the same levels in pressurized cheeses as in control cheeses for both sheep and goat cheeses. Proteolysis was higher in cheeses made with all pressurized curds, and a greater release of FAAs in ewe's cheeses treated at 500 MPa was observed. Esterase activity and total FFAs showed higher levels in treated cheeses at 400 MPa than in control cheeses. Long chain-FFAs were 11% lower in goat-pressurized cheese, whereas no significant differences were found for short- and medium-chain FFAs. In the same manner, no differences were observed in the sensory attributes between control and pressurized cheeses, with the exception of flavor quality scores, which were higher in goat-pressurized curd

cheeses. In addition, these authors indicated a potential benefit for the cheese industry by increasing the yield and reducing the ripening time of the pressurized ripened cheeses [80][81].

The results described above indicate that HHP treatments have been applied to starters, milk, curds, and hard and semi-hard cheeses at different ripening days. HHP treatment at moderate doses (200–400 MPa) can be a reliable technique to reduce or eliminate cheese pathogens and undesirable microorganisms that cause defects in cheeses. However, spore-forming bacteria, such as *Clostridium* spp., need higher pressures (over 1000 MPa) to be effective. Many studies on HHP treatments have focused on accelerating or delaying cheese ripening in order to diminish storage costs or produce cheeses with optimal sensory characteristics after long storage periods. Moderate pressures (100–400 MPa) tend to accelerate proteolysis and, in consequence, shorten ripening and storage time, so these treatments could be especially useful for hard cheeses. Higher pressures (>500 MPa) are usually more effective in delaying proteolysis and lipolysis, and may be useful in the case of semi-hard cheese production. Ripening modulation can benefit small ruminant seasonal cheeses, and may be used to overcome seasonal shortages or production surpluses. Furthermore, the optimal ripening time for applying HHP treatment is another factor that should be taken into account. In this regard, the pressurization applied during the first ripening days leads to significant biochemical and sensory changes in cheeses. When the pressurization treatment is applied at later ripening stages, cheese flavor is little affected. The color, flavor, and texture of pressurized cheeses are often the most affected sensory parameters, independently of the HHP treatment and application ripening time.

A limiting factor is the high equipment cost. Thus, a significant proportion of newly installed HPP equipment operates under a toll service regime [51][52][82]. From an environmental perspective, another unfavorable aspect is the use of plastic packaging materials necessary to apply the pressurization treatment. HHP has good potential to be applied to ripened cheeses in order to prolong their shelf-life, but further studies are necessary for each cheese variety to optimize HHP conditions, and to verify the effects of pressurization treatments on biochemical, textural, and sensory characteristics of cheeses.

## 4. Food Additives

The direct addition of additives to foods is one of the simplest and oldest preservation techniques used to extend their shelf-life. At present, cheese preservation is often undertaken via chemical or biological additives. These substances are added to cheese in order to avoid defects caused by microorganisms, and extend the cheese's shelf-life, improve its physical properties and chemical composition, and preserve its nutritional value [23][43].

## 5. Packaging

Packaging is an important step in the food manufacturing and commercialization process. The objective of packaging is not only to contain food, but also to protect and maintain the quality and safety during the food's shelf-life, at a limited business cost [83]. Cheese packaging is mainly directed to avoid certain degradation processes, such as oxidation or dehydration, protect against the growth of undesirable microorganisms and external

contamination, and reduce or allow the continuance of the metabolic activities of ripening strains [84]. In packaging techniques, material properties, such as water vapor and gas barrier, and the shape and size of the package, are crucial to ensure cheese quality and safety [85]. For optimal packaging selection, it must be considered that cheese is a complex dynamic biological matrix in which several microbial, physical, and biochemical changes occur during storage. The growing consumer demand for portioned cheese sold as blocks, slices, or grated has led to the design of specific packaging conditions that ensure the desired shelf-life of this food product [86].

## 6. Cheese Post-Processing Technologies

The degree of microbial contamination that can occur during handling, slicing, and packaging steps greatly influences the quality of the final food products. In addition, post-processing cross-contamination of cheese can lead to both safety risks and significant compound losses due to spoilage, so additional control methods are needed to inactivate microorganism growth on cheese surface after the packaging step [87][88].

Light-emitting diode (LED) technology has recently received increased attention as a novel preservation technology for bacterial inactivation. Bacterial cells are excited when exposed to light photosensitizers, such as endogenous porphyrin, resulting in the release of reactive oxygen species, which may damage cell membranes, enzymes, proteins, or deoxyribonucleic acid (DNA), leading to cell death [89][90]. It has been recently shown that 460–470 nm LED illumination was able to inactivate *L. monocytogenes* and *Pseudomonas fluorescens* growth on the surface of packaged sliced cheese, especially when combined with refrigeration temperatures [91].

Pulsed ultraviolet (UV) light is more advantageous than continuous UV light in terms of microbial inactivation efficiency [92], and pulsed UV light can significantly reduce the microbial growth on the cheese surface [93]. Several authors have reported that the application of UV light in combination with other preservation treatments (refrigeration, MAP, antimicrobial substances) has significant benefits for safety and the shelf-life of cheese [91][94][95]. Therefore, these new technologies can represent a good option for minimizing deterioration phenomena during cheese storage.

The addition of antimicrobial substances (2.5% nisin and 50 mg/L natamycin solutions) to the cheese surface may synergistically increase the antimicrobial effectiveness of pulsed UV light (9.22 J/cm<sup>2</sup>), when antimicrobial substances are added after the light treatment [87]. Similarly, the combination of pulsed UV light (1.2–6 KJ/m<sup>2</sup>) and antimicrobial (0.001% sodium benzoate/30% citric acid) starch films were effective in reducing *L. innocua* growth on the surface of Cheddar cheese. However, significant changes in physico-chemical properties of the treated cheeses were observed after seven days of refrigerated storage [96]. These results highlight the opportunity to use pulsed UV light as a final preservative treatment in cheese pre-packaged with clear materials, and may become a very attractive solution to mitigate surface cheese contamination in manufacturing, distribution, and retail environments [93]. It has been demonstrated that pulsed UV light (44 J/cm<sup>2</sup>) has the potential for post-processing decontamination of the surface of semi-hard cheese [97]. Although the current trends are based on minimizing the impact on cheese quality parameters by combining treatments applied at low intensity, the combination of

preservative treatments may not always result in synergistic effects, and interactions between treatments need to be studied before being applied at the commercial level [87].

Food irradiation has the ability to disrupt the microorganism DNA, thereby prolonging shelf-life and enhancing food safety, without a detrimental effect on the food sensory and nutritional quality when applied at an appropriate dose [98]. Ionizing irradiation at less than 3 kGy has proven to be an effective technology to control *L. monocytogenes* growth in cheese [99]. However, there are some discrepancies regarding the occurrence of off-flavors and depreciation of sensory quality in cheese. Probably for this reason, only some studies have been found in the scientific literature on cheese irradiation. Nevertheless, it appears that lower radiation doses do not affect the composition of different cheese types [98][100]. In Cheddar cheese, off-flavors were detected immediately after E-beam treatment, although off-flavors progressively disappeared during storage when radiation doses were lower than 2 kGy [101]. X-ray radiation at a high dose of 0.8 kGy was suitable to reduce microbial contamination of packaged sliced Cheddar cheese without affecting product quality; thus, X-ray radiation may be applied as a new post-processing antimicrobial technology for cheese preservation [88]. Ras cheese treated with  $\gamma$ -irradiation (5–15 kGy) showed higher degradation of biogenic amines without any detrimental changes in cheese chemical composition after six storage months compared to non-irradiated cheese samples. The results of the irradiation treatment resulted in adequate cheese suitability and wholesomeness, together with consumer acceptability of the sensory attributes [102].

In summary, cheese irradiation is found to be safe, with a potential application in the preservation and shelf-life extension in the case of certain cheeses. However, full acceptance of this preservation technology, and its incorporation into the food dairy industry, is slow and often controversial. Further studies are needed for the successful adaptation at the industrial level [98].

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