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Review

Sustainable Approaches in Whey Cheese Production: A Review

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Abstract: Whey cheeses have been produced from the very early steps of cheesemaking practices as a sustainable way to utilize whey, which is the main by-product of cheesemaking. Traditional whey cheeses, manufactured with similar processes, are Ricotta, Ricotta salata or Ricottone, and Ricotta fresca in Italy; Anthotyros, Myzithra, Manouri, Xynomyzithra, and Urda in Greece; Urda in Serbia and Romania as well as in other countries such as Israel; Lor in Turkey; Anari in Cyprus; Skuta in Croatia and Serbia; Gjetost and Brunost in Norway; Mesost and Messmör in Sweden; Mysuostur in Iceland; Myseost in Denmark; Requeijão in Portugal; and Requesón in Spain and Mexico. The production of whey cheese is based on the denaturation of whey proteins by heating to 88–92 °C. The specific processing conditions and aspects of the microbiology of whey cheeses are discussed. The special characteristics of whey cheeses, which are high pH and high moisture content, make them susceptible to microbial growth. Due to the limited shelf life of these products, extended research has been carried out to extend the shelf life of whey cheese. The sustainable preservation approaches, such as modified atmosphere packaging, addition of herbs and/or plant extracts, and bio-preservation methods are reviewed. Moreover, novel whey cheeses focused on functional properties have developed during the last 10 years.

Keywords: whey cheese; Ricotta; Anthotyros; Manouri; Urda; Lor; Requeijão; sustainable preservation; bio-preservation; functional whey cheeses

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1. Introduction

The nutritional properties of whey were very early recognized, and from the fifth century BC, the Greek physician Hippocrates prescribed cheese whey for gastrointestinal disorders and to enhance the immune system [1,2]. It is not known when exactly whey was first utilized to produce whey cheese. From the seventh century BC, rennet-coagulated Caprino and Pecorino were produced in Tuscany; Columella, in his treatise on agriculture, *De Re Rustica* (50 AD), describes the production of Ricotta cheese [3–6]. Whey cheese was very common in Roman times for breakfast and *Caseum sanctuarie* was the cheese made with the milk provided on the celebration of St. J. Baptist [5,6]. Anari cheese is produced from the whey released during the production of Halloumi and its manufacture dates back to Halloumi making [7]. Norwegian whey cheeses have their origins back to the Vikings, about a thousand years ago [8].

Whey cheeses are, nowadays, produced globally, under various names: Ricotta, Ricotta salata or Ricottone, and Ricotta fresca in Italy; Anthotyros, Myzithra, Manouri, Xynomyzithra, and Urda in Greece; Urda in Serbia and Romania as well as in other countries such as Israel; Lor in Turkey; Anari in Cyprus; Skuta in Croatia and Serbia; Gjetost and Brunost in Norway; Mesost and Messmör in Sweden; Mysuostur in Iceland; Myseost in Denmark; Requeijão in Portugal; and Requesón in Spain and Mexico [5,7–9].

Among the different whey cheeses, Ricotta di Bufala Campana and Ricotta Romana in Italy, Brocciu corse in France, Manouri and Xinomyzithra Kritis in Greece, and Requeijão da Beira Baixa and Requeijão Serra da Estrela in Portugal, all having proved connection with regional history and culture, have been designated as protected designation of origin (PDO) [10].

The manufacture of whey cheeses has traditionally been developed as a sustainable way to utilize the main by-product of rennet and acid-coagulated cheesemaking practices. Traditional cheese production has positive, social, and economic impacts in terms of maintaining local employment and retaining farmers and their families in rural areas [11]. According to the Codex Alimentarius [12], 'whey cheeses are solid, semi-solid, or soft products which are principally obtained through either of the following processes: (1) the concentration of whey and the molding of the concentrated product or (2) the coagulation of whey by heat with or without the addition of acid'.

The aim of the current review is to present the sustainable aspects of utilization of whey to produce traditional and novel whey cheeses and to give an overview of the properties of these products.

2. Whey Types and Composition

Whey has a greenish-yellow color, and it is the part of milk which remains after the removal of the coagulum formed during cheese manufacture; the coagulum can be formed either by using acids or rennet and other plant-based coagulants [13,14]. Whey is considered as a pollutant by-product of the cheesemaking process and high amounts are produced; it is estimated that the whey produced annually is 145–200 million tons [15,16], causing serious environmental problems when disposed as a waste material with a BOD of 40,000 mg/kg [17]. Thus, environmental issues have led to the study of the properties of whey, its composition, nutritional value, and technological uses. It should be noted that whey maintains a high nutrient value, i.e., mainly residual proteins, and consequently, several studies are focusing on the whey valorization [18].

The whey produced by rennet- or plant-coagulated cheeses is called cheese whey or sweet whey (pH > 6.0); the whey with an acidic pH (<5.8), obtained from acid-coagulated cheese is called acid whey [19–22], and acid whey is also produced from Greek or other strained yoghurts [23]. The whey released from the production of whey cheese, such as Ricotta fresca, is called second whey cheese [24,25], which is an acidic whey and has lower fat and protein contents in comparison to the primary whey [25–27]; second, whey is also released from the production of non-acidified whey cheeses and has higher pH. The composition of whey from different sources is presented in Table 1.

Table 1. Chemical composition (g/100 g) and pH of whey from different milk and cheese sources.

	pH	Fat	Lactose	Protein	Total Solids	Reference
Buffalo whey	na	0.50	4.90	0.73	6.50	[28]
Cow whey	5.7–6.3	0.20	5.00	0.80	6.60	[29,30]
Cow whey (from Feta-type)	6.3	0.25	4.72	0.82	6.32	[31]
Cow whey (from Graviera)	6.3	0.60	4.90	0.90	6.90	[31]
Cow whey (from Kefalotyri)	6.4	0.40	4.85	0.80	6.55	[31]
Cow whey (from Cottage)	4.6–4.5	0.04	4.90	0.75	5.50	[31]
Goat whey	na	na	4.10	0.40	5.10	[32,33]
Sheep whey (from Feta)	na	0.39	5.33	1.61	7.87	[31]
Sheep whey (from Graviera)	6.3	1.26	5.27	1.52	8.74	[31]
Sheep whey (from Kefalotyri)	6.2	0.70	4.99	1.41	7.48	[31]
Sheep whey	na	1.50	3.70	1.80	9.50	[32,34]

na: not available.

Great variation in the chemical composition of whey is reported, and this differs according to the type of cheese produced, which is the main cheesemaking product, and the kind of milk from which the main cheese is made [35–38]. Additionally, seasonal differences due to lactation or other reasons or feed differences also cause the observed variations [39]. Due to the composition of sheep milk, sheep whey consists of significantly higher levels of total solids, lipids, and proteins than cow whey [14,40]. Whey proteins have a higher nutritive value than casein and are considered as one of the richest proteins available. Whey contains essential amino acids, e.g., branched-chain amino acids (isoleucine, leucine, and valine) and sulfur amino acids (cysteine and methionine), and antioxidant properties have been reported [41]. Sheep whey contains more β-lactoglobulin, about the same proportion of α-lactalbumin, and lower proportions of serum albumin and immunoglobulins compared to cow whey [42]. Goat whey contains twice as much α-lactalbumin and lower levels of β-lactoglobulin, serum albumin, and immunoglobulins compared to cow whey [43,44]. Nudda et al. [45] observed seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep whey used to make Ricotta cheese; conjugated linoleic acid and vaccenic acid decreased as lactation progressed, probably due to variation in pasture availability and fatty acid composition of grass.

The nutritional value of whey has been well documented [32,46–56]. Lorieau et al. [57,58] observed that whey cheese showed different digestion and absorption behavior in comparison to the rennet-coagulated cheese. Lorieau et al. [58] observed a higher amino acid bioavailability in whey cheese, and cheese based on whey proteins has been suggested for muscle synthesis in elderly people. Whey proteins are valued for their multiple health benefits, i.e., they enhance immunity, inhibit HIV, and have anti-cancer effects against colon cancer [51,55,59,60].

3. Sustainable Valorization of Whey

Based on the principles of circular economy, whey is nowadays used in the manufacturing of various products, such as whey cheeses, whey powder, edible films, beverages, as well as feed supplement and biogas production. Whey has been proved to be a valuable product due to its components and their functional properties, and food-related applications containing whey components, mainly proteins, have been developed [21,22,61,62]. The valorization of whey has been reviewed by Smithers [2], Papademas and Kotsaki [63], Prazeres et al. [64], Panghal et al. [65], Wang et al. [66], Lappa et al. [67], Asunis et al. [68], Rocha-Mendoza et al. [69], Tsermoula et al. [70], Barba [16], Arshad et al. [71], Osorio-González et al. [72], León-López et al. [73], Buchanan et al. [74], Gergihon et al. [75], and Rocha and Guerra [76]. Rama et al. reviewed the potential applications of whey, as cryoprotectants for freeze-drying and as encapsulating agents for the spray-drying used to produce lactic acid bacteria (LAB) cultures [77]. Valta et al. [78] investigated the technologies of treatment of cheese whey applied by Greek dairies and suggested potential whey valorization opportunities.

Membrane processing, such as ultrafiltration, microfiltration, and reverse osmosis technologies, has been used in the cheese industry to fractionate whey components and to enable whey proteins and other valuable constituents to be retained in the cheese [79–83]. New or improved products derived from whey have gained commercial success due to the enhanced functional properties, which have enabled their incorporation into a number of food and drinks [63,79]. Whey cheeses such as Ricotta cheese, have been produced using ultrafiltrated whey, with reduced cost and improved cheesemaking efficiency [26].

Cheese whey exploitation by the Brazilian dairy industry was studied using a questionnaire survey [84]; most of the dairy industries produced and processed sweet cheese whey, using it in whey dairy beverages, Ricotta, and different types of whey concentrates. Kotoupas et al. [85] compared five alternative scenarios for the treatment of cheese whey wastewater resulting from a cheese factory and concluded that the production of Manouri cheese was the most profitable one. Masotti et al. [86] reviewed the effect of different technologies used for the fortification of cheese with whey proteins: heat treatment of cheese milk, membrane separation technologies, and the direct addition of milk- or whey-based derivatives and more recent technologies such as high hydrostatic pressure, ultra-high-pressure homogenization, transglutaminase treatment of cheese milk, and direct addition of buttermilk.

4. The Manufacture of Traditional Whey cheeses

Whey cheeses belong to a special category of cheeses that are produced by heating the whey at high temperatures to form a coagulum following the denaturation of whey proteins. Whey cheeses are globally produced by traditional methods either as artisanal or standardized industrial processes as a sustainable method of utilizing the whey according to the principles of circular economy [14,32]. There are many whey cheeses that were originally produced from sheep or goat whey as by-products. Nowadays, since their consumption has increased, dairies have started to produce whey cheese from cows' whey or add cows' milk. Most of them are heat- and/or acid-coagulated cheeses, and their technology varies in respect of the raw material (type of whey) used, the quantity and type of the milk and/or cream added, and the cheesemaking recipe followed [87,88]. The most important whey cheeses and their composition and processing characteristics are shown in Table 2.

Table 2. Composition (%) and specific processing conditions of whey cheeses.

Cheese	Country	Source of Whey	Specific Processing Conditions	pH	Fat	Protein	Salt	Moisture	Reference
Anari (fresh)	Cyprus	S, G	Fresh Anari has a short shelf life, that is 2–3 days once packaging is opened	na	21.7	11.0	na	65.4	[7]
Anari (dry)	Cyprus	S, G	Salted fresh Anari cheese is dried until it becomes hard and easy to grate	na	34.9	21.7	na	33.8	[7]
Anthotyros	Greece	S, G	Sheep or goat cream and/or milk is added	6.4	16.6	6.9	na	68.4	[31]
Anthotyros	Greece	S, G	Sheep or goat cream and/or milk is added	6.3–6.4	16.5–16.6	9.6–9.7	0.5–0.6	65.0–66.5	[89]
Arishi (from sweet whey)	Lebanon	C	Whey is coagulated by heating at 85 °C for 5–10 min	5.8	4.9	14.4	na	70.2	[90]
Arishi (from acid whey)	Lebanon	C	Citric acid is added	4.4	7.0	8.1	na	77.6	[90]
Bračka skuta	Croatia	S	The whey is heated until proteins are coagulated and then is further boiled at 95–97 °C	6.5	27.9	10.9	na	58.7	[91]
Gjestost	Norway	G	Caramelized lactose in concentrated whey is combined with whey proteins and fat	na	34	9.7	na	13.4	[7,92–95]
Lor	Turkey	S, G, C	The raw cheese is strained in a hemp or other straining cloth and press is applied	na	5.3–15.3	9.7–13.5	0.9	64.3–72.4	[96]
Manouri PDO	Greece	S, G	Salt is added at 1% and milk and/or cream up to a proportion of 25%	5.9	36.7	10.9	0.8	48.1	[7,31]
Mesost	Sweden	G, C	The whey is boiled to evaporate water	na	17.0	na	na	20.0	[7]
Messmör	Sweden	G, C	The whey is boiled to evaporate water; the concentrated mass is homogenized	na	2.0	na	na	30.0–35.0	[7]
Mysost	Norway	C, G	It is produced either as a hard or soft-spread cheese	na	24.7	11.5	na	83.8	[14]
Myzithra	Greece	S, G	It is produced either from sweet or acid whey; in case of sweet whey, partial acidification is required during heating	6.0	15.9	13.1	0.8	66.4	[31]
Myzithra dry	Greece	S, G	The cheese is dried in the air	4.7	20.8	25.4	8.7	38.6	[31]
Myzithra Kalathaki	Greece	S, G	Similar to Myzithra	6.8	15.0–20.0	na	1.0–1.5	75.0	[97]
Primost	Norway	G	Addition of cream	na	35	10.9	na	13.8	[7]

Table 2. *Cont.*

Cheese	Country	Source of Whey	Specific Processing Conditions	pH	Fat	Protein	Salt	Moisture	Reference
Requeijão	Portugal	S, G	90% S whey and 10% G milk; at 95 °C for at least 15 min under stirring; this cheese is usually eaten fresh	na	29.5	8.5	na	59.0	[98]
Requesón	Mexico, Spain	C, S, G	10% milk is added to the whey	6.1	4.1–13.0	5.1–7.0	na	na	[14,99]
Ricotta	Italy	S	Sweet whey is used, mixed with 5–10% milk, salt is added at 0.1% and citric acid at 0.11 kg/L and heating is carried out up to 80–85 °C	na	26.0	4.1	na	56.8	[14,100]
Ricotta	Italy	B	See above (Ricotta)	6.6–6.9	22.5	5.63	na	65.5	[101]
Ricotta	Serbia	C	See above (Ricotta)	5.7–5.9	6.4	16.9	2.2	69.8	[102]
Ricotta	US	C	Addition of 20% milk	na	11.6	16.3	na	66.5	[103]
Ricotta	US	C	Addition of 5% milk	na	3.3	7.0	na	81.6	[104]
Ricotta (buffalo)	Italy	B	See above (Ricotta)	na	18.0	10.0	na	65.0	[100]
Ricotta (cow)	Italy	C	See above (Ricotta)	na	10.0	9.0	na	76.0	[100]
Ricotta (sheep)	Italy	S	See above (Ricotta)	na	18.0	8.0	na	70.0	[100]
Ricotta di Bufala Campana DOP	Italy	B	See above (Ricotta)	6.2–6.7	na	na	na	na	[105]
Ricotta forte	Italy	na	Freshly produced Ricotta is put into a small tank and thoroughly mixed; then, the tank is covered and kept in a cool place for at least 6 months at room temperature; natural fermentation takes place; at the end of the ripening period, salt is added (20–40 g/kg)	4.7	21.4	12.2	na	64.1	[106]
Ricotta Romana	Italy	S	See above (Ricotta)	na	15.5	8.9	na	71.1	[107,108]
Ricotta salata (0 days)	Italy	S	See above (Ricotta)	6.54	19.5	14.8	3.42	57.9	[109]
Ricotta salata (60 days)	Italy	S	See above (Ricotta)	6.04	21.2	15.2	3.1	56.3	[109]
Ricotta salata (90 days)	Italy	S	See above (Ricotta)	5.68	20.1	15.2	3.4	57.2	[109]
Skuta	Serbia	S, G	Milk and/or cream can be added to the whey, before or after coagulation	na	11.7	na	na	30.0	[102,110]

Table 2. *Cont.*

Cheese	Country	Source of Whey	Specific Processing Conditions	pH	Fat	Protein	Salt	Moisture	Reference
Skuta Dalmatian	Croatia	na	Acidification of Skuta is achieved by the addition of sour whey in an amount up to 10%, vinegar or citric acid	na	35.4	na	na	26.4	[110]
Urda	Serbia	C	Sweet whey is heated to 80–85 °C; citric acid is added	5.77–5.97	5.63	10.7	1.9	79.6	[102]
Urda dry	Greece	S, G	Fresh Urda is ripened on wooden shelves in ventilated room at 19 °C and 70–75% RH for 25 days	5.04–5.46	43.5–45.7	18.4–21.9	3.4–3.6	27.5–30.8	[11]
Urda fresh	Greece	S, G	Artisanal Urda cheese is naturally fermented by spontaneous fermentation and undergoes ripening	6.39–6.41	28.8–29.7	12.6–14.6	0.5–0.6	54.1–57.0	[11]
Xynomyzithra Kritis PDO	Greece	S, G	Myzithra is first manufactured and, after draining, press is applied for 7 days; salt is added, and the cheese is ripened for 60 days at 5–10 °C	4.8	na	na	1.86	38.6	[111]

B: buffalo, C: cow, G: goat, S: sheep, na: not available.

In general, to produce whey cheese, the whey is heated at a temperature of 88–92 °C while stirred. The rate of heating is important to be such as to attain the selected temperature in 40–45 min. Another critical step is the rate of stirring. The first small particles of denaturated whey proteins appear at temperatures around 80 °C. This temperature could be lower according to the type of whey, the acidity and the quantity and proportion of milk and/or cream added [14,112]. The final temperature is lower when whey cheeses are to be consumed fresh, whereas higher temperature heating is applied for dried whey cheeses. The curd obtained is left for 15–20 min on the surface at the selected temperature to lose part of its moisture and then it is scooped and molded for drainage; whey cheese is mainly consumed fresh [14,112].

In the cases in which only the whey is used for the production of whey cheese, the particles of denatured proteins floating on the surface are small and the yield is low. Usually, a certain amount of milk and/or cream is added during heating; the quantity of milk is 30–50 mL/L; however, for higher quality cheeses, milk percentage could be 10% or higher, and cream may also be added [112]. Whey cheese, in most cases, is consumed fresh, that is, without fermentation and ripening; only a few whey cheeses, e.g., Ricotta forte and Xynomyzithra, undergo the ripening process [111,113]. Coprecipitation of caseins may be improved by acidification; the yield is improved when addition of acid is coupled with the addition of calcium chloride [112].

Salt may also be added to the whey, as well as an acidification agent; the latter is usually needed in the case of cow whey to help denaturation and final precipitation of the whey proteins [114]. Acidification is applied in the form of an aqueous solution of citric acid at the rate of 6 mL/L and this quantity decreases the pH to about 5.2 [112,114].

Kaminarides et al. [115] studied the effect of the addition of milk and cream on the characteristics of Greek whey cheeses and reported that the addition of milk and cream led to higher yields; cheese produced with 79% whey, 15% milk, and 6% cream had the most preferred sensory characteristics. More recently, Kaminarides et al. [27] studied the effect of whey fat content on the yield, physicochemical, textural, and sensory properties of cheeses made from sheep whey; cheese with 5% added cream of 50% fat was the most preferred in sensory evaluation. Similarly, Ricotta can be produced by using whey resulting from cow, buffalo, sheep, and goat cheese manufacturing, as well as mixtures of them (Table 2). Biancolillo et al. [116] investigated the fatty acid profiles of 240 samples of Ricotta cheese made from sheep, goat, cow, or buffalo milk. Low-salt whey cheeses can be manufactured with a combination of NaCl and KCl salts to lower the sodium content in Requesón while maintaining the accepted sensory characteristics [117].

As shown in Table 2, there is a great variability in the composition of whey cheeses. The variability is mainly reflecting the differences in composition of the whey (Table 1), differences in the cheese-making technology, the kind of cheese milk used for the main cheese, the kind of milk and/or cream added, or the use of sweet or acid whey; these factors lead to a large variability in the characteristics of whey cheeses. Sensory properties of Ricotta cheese, such as softness, granularity, and greasiness, as well as the fatty acid profile, were significantly different among goat breeds [118].

5. Aspects of the Microbiology of Whey Cheese

Whey cheeses are undergoing a high temperature heat treatment at 88–92 °C for the denaturation of whey proteins, and this treatment has a severe impact on the microbiota of the whey or of the milk and/or cream added to the whey. The indigenous microbiota is inactivated and no starter cultures are applied after heating [7–9].

Escherichia coli O157:H7 was reported to grow in Myzithra, Anthotyros, and Manouri whey cheeses at 12 °C and survive at 2 °C storage [119]. The growth of *L. monocytogenes* in the same whey cheeses was reported to be very rapid; maximum populations were significantly lower in Myzithra cheese at 5 and 12 °C than maximum populations in Anthotyros and Manouri cheese at the same temperatures independent of the inoculated strain. In addition, *Aeromonas hydrophila* was recovered in 4 (10.2%) and 3 (8.3%) of the Anthotyros and Manouri cheese samples analyzed, respectively, but not countable populations were noted [120]. *A. hydrophila* grew in Myzithra and Anthotyros, but no growth was observed in Manouri [121].

Anthotyros showed high numbers of coliforms (10^6–10^7), micrococci and staphylococci (10^4–10^5), yeasts (10^5–10^6), and enterococci (10^5–10^6) [122]. Lioliou et al. [123] studied the microbiology of Manouri cheese throughout storage at 4 °C for 20 days and concluded that the counts, of all the microbial groups studied, increased throughout storage. Suzzi et al. [124] studied the microbiological properties of Manteca cheese and the technological significance of the different naturally occurring yeast species.

Theodoridis et al. [125] reported 4% and 2% of Manouri and Anthotyros cheese samples, respectively, positive for *Listeria* spp. Additionally, 2% of samples of Anthotyros were reported positive for *L. monocytogenes*. Later, Angelidis et al. [126] in a survey of *L. monocytogenes* in soft cheeses for sale in retail outlets found no positive soft cheeses, including Anthotyros, Manouri, fresh Myzithra, dry Myzithra and Ricotta, and only 1 sample out of the 137 cheese samples examined contained *Listeria innocua* at levels of 4.5×10^2 cfu/g. Different physicochemical and microbiological characteristics of cheeses may affect *L. monocytogenes* potential to grow and/or survive during storage [127]. A study conducted on Sardinian dairies revealed that the prevalence of *L. monocytogenes* on Ricotta salata rind was approximately 18% [128]. Requesón showed a 6.7% prevalence of *L. monocytogenes* [129,130], whereas, 30 samples of Ricotta examined were free of *L. monocytogenes* [131]. Villarruel-Lopez et al. [99] studied the presence of aerobic mesophilic bacteria, coliform bacteria, yeasts, molds, and certain pathogenic bacteria and staphylococcal enterotoxins in Mexican commercial Requesón cheeses; although they reported samples at high risk of contamination, neither *L. monocytogenes* nor staphylococcal enterotoxins were detected. Scatassa et al. [132] reported that during a 15 year (2002–2016) investigation of the microbiological profile of Sicilian Ricotta, made from sheep whey, Ricotta represented a safe cheese from a hygienic point of view, and during the entire period of investigation, the pathogenic bacteria (*L. monocytogenes*, *Salmonella* spp. and *Brucella* spp.) were never detected, while the levels of undesired microorganisms such as *Enterobacteriaceae*, *E.coli*, and coagulase-positive staphylococci were found in reduced numbers.

Whey cheeses have reported to be able to support the growth of *L. monocytogenes* and low initial levels of contamination can lead to concentration potentially threatening to human health [133–137]. Evidence that *L. monocytogenes* can contaminate whey cheeses and that these cheeses support its growth, even during refrigerated storage, has been documented [133–138].

Adame-Gómez et al. [139] reported 9 positive samples of Requeson cheese out of 16 examined with *Bacillus cereus* contamination at a 5.52 log cfu/g, whereas Sattin et al. [140] identified a *Bacillus* strain that caused pink discoloration in the same cheese. Cosentino et al. [141] reported 78% positive samples for *Bacillus* spp. in Ricotta cheese, with counts rarely exceeding 10 cfu/g, but also counts higher than 1000 cfu/g were found (16% of positive samples). The level of *B. cereus* vegetative cells in naturally contaminated Ricotta salata decreased during refrigerated storage, while the presence of spores was rare [142,143].

Post-process handling of the curd results in cross-contamination with a diverse native microbiota, mainly psychrotrophic Gram-negative bacteria, lactic acid bacteria (LAB), staphylococci, spore-forming bacilli, clostridia, yeasts, and molds [14,144–147]. Considering that whey cheeses have a high pH, high moisture content, and a low salt content (Table 2) these dairy products are very susceptible to microbial spoilage, especially under abuse temperatures. Additionally, whey cheeses constitute a major food safety concern due to their lower hurdle effect compared with ripened cheeses, showing a higher incidence and potential for survival/growth of pathogens [14,144–147]. Psychrotrophic food-borne pathogenic bacteria possessing elaborate cold-adaptation mechanisms can grow in milk and whey products during refrigerated storage [148]. The main route of contamination is the contact with surfaces of the processing plant environment and equipment [149]. Spanu et al. [135] studied the effect of a post-process heat treatment on the survival of *L.*

monocytogenes cells in artificially contaminated Ricotta salata and demonstrated that the reduction in *L. monocytogenes* count was entirely accountable to the hot water treatment.

6. Sustainable Preservation Approaches of Whey cheeses

6.1. Modified Atmosphere Packaging

The use of effective and sustainable methods for the preservation and safety of whey cheeses is necessary to control cross-contamination of fresh whey cheeses throughout their storage. The increased consumer demand for fresh, preservative-free foods has led to extended research on advances in cheese preservation focusing on 'clean labels' [150]. The use of modified atmosphere packaging (MAP) as a technique to improve product' safety and extend the shelf life satisfies the chemical preservative-free strategies for various foods [151]; MAP is widely used as a storage method, extending the shelf life and improving the appearance of several foods. The potential of MAP and active packaging for extending shelf life of dairy products, including cheese, has been demonstrated [152–154].

Several MAP conditions were used to assess the effect of MAP on the characteristics, that is, free fatty acids, lactose, lactic acid, moisture, pH, and texture of whey cheeses [24,155–164]. The MAP conditions and the best practice and/or effect on whey cheeses is presented in Table 3. Different mixtures have been applied in different whey cheeses; in most studies, a specific mixture of gases provided an extension in the shelf life.

Table 3. Modified atmosphere packaging (MAP) conditions and the best practice and/or effect on whey cheeses.

Cheese	MAP Conditions Used	Best Practice/Effect	Reference
Anthotyros	40% CO_2/55% N_2/5% O_2, 60% CO_2/40% N_2 and 50% CO_2/50% N_2	60% CO_2/40% N_2 and 50% CO_2/50% N_2 mixtures proved to be most effective for inhibiting total mesophilic microorganisms and *E. coli*	[158]
Anthotyros	30% CO_2/70% N_2 and 70% CO_2/30% N_2	The use of MAP conditions 70% CO_2/30% N_2 extended the shelf-life of fresh cheese for 20 days	[159]
Anthotyros	40% CO_2/60% N_2 and basil essential oil (0.4% *v/w*)	Extend the shelf life by approximately 10–12 days compared to aerobic packaging	[160]
Lor	40% CO_2/60% N_2, 60% CO_2/40% N_2 and 70% CO_2/30% N_2	60% and 70% CO_2 were the most effective mixture for inhibition of growth of micro-organisms	[161]
Lor	80% CO_2/20% N_2 and 60% CO_2/40% N_2	80% CO_2/20% N_2 was the most effective for inhibiting growth of micro-organisms	[162]
Myzithra Kalathaki	20% CO_2/80% N_2, 40% CO_2/60% N_2 and 60% CO_2/40% N_2	40% CO_2/60% N_2 was the most effective treatment for the inhibition of psychrotrophs in Myzithra cheese until days 40; control samples were sensorily unacceptable after 10–12 days of storage	[97]
Requeijao	100% CO_2, 100% N_2 and 50% CO_2/50% N_2	CO_2 alone ensured more consistent cheese composition until 15 days of storage and provided protection against lipolysis	[163]
Ricotta fresca	30% CO_2/70% N_2 and 100% N_2	No evidence that MAP conditions used in Sardinian dairies allowed to extend the shelf life to 21 days	[24]

Ramírez-Rivas et al. [164] studied the effect of packaging and salt content and type on antioxidant and angiotensin-converting-enzyme (ACE)-inhibitory activities in Requeson. Di Pierro et al. [165] studied the application of chitosan/whey protein film as active coating in combination with 40% CO_2/60% N_2 MAP conditions to extend the shelf life of Ricotta cheese; the treatment reduced the viable numbers of mesophilic and psychrotrophic bacteria and delayed the development of undesirable acidity, better maintained the texture, and did not modify the sensory characteristics.

6.2. Addition of Herbs and/or Plant Extracts

There are a number of studies in the literature on the addition of various herbs and/or plant extracts and their potential use in combination with different packaging materials to extend the shelf life of whey cheeses [20,164–170]. Black cumin, thyme, and rosemary are frequently added to cheeses because of their sensory and antioxidant properties [20,166]. Akpinar et al. [20] studied whey cheeses packaged in goat-skin bags used for the storage of traditional cheeses with a long shelf life (semi-hard and hard cheeses) and alternatively studied cheeses in artificial casings. In addition to different packaging materials, the potential usage of different plants (black cumin, thyme, and rosemary) in Lor cheese production was evaluated.

Christaki et al. [170] studied the effect of oregano essential oil and extracts on two Greek whey cheeses. The authors evaluated the feasibility of employing oregano essential oil and extracts in whey cheese to control fungal contamination that causes cheese spoilage and important economic losses and reported that the combination of oregano essential oil and extracts produced nanoemulsions with potent antioxidant and antifungal activity against *Penicillium expansum* in vitro.

6.3. Bio-Preservation

The use of natural antimicrobial compounds from a wide variety of natural sources, or protective LAB cultures, i.e., bio-preservation, has been studied extensively [144,171–190]. Bio-preservation is of high interest due to its ecological sustainability and consumer friendly nature [177]. Unlike artificially synthesized chemical preservatives that have toxic effects over long-term usage, bio-preservatives offer little or no harmful health effects [182–187]. Nisin, a bacteriocin produced by strains of *Lactococcus lactis* subsp. *lactis* has been used as a natural preservative to extend the self-life of certain dairy products [170,178]. Nisin inhibits Gram-positive bacteria, including *L. monocytogenes* and *B. cereus* and outgrows the spores of members of the genus *Bacillus* and *Clostridium* [171,178]. Wu et al. [188] concluded that the combined use of nisin with other antibacterial methods showed more advantageous activities against *L. monocytogenes* than single use.

Samelis et al. [179] investigated the use of nisin as a bio-preservative to control *L. monocytogenes* introduced post-processing on Anthotyros stored in refrigerated storage in vacuum packages for up to 45 days; they reported that this treatment suppressed *L. monocytogenes* growth below the inoculation level for 30 and 45 days [179]. Interestingly, nisin resulted in a switch in the natural spoilage flora of Anthotyros from Gram-positive to Gram-negative [179]. Aspri et al. [180] applied a bacteriocin-producing *Enterococcus faecium* isolated from donkey milk in the bio-control of *L. monocytogenes* in fresh whey cheese. Spanu et al. [181] investigated bio-preservatives to control the growth of spoilage microorganism, that is *Pseudomonas* spp., on the surface of MAP-packed Ricotta fresca during refrigerated storage and *Carnobacterium* spp. inoculated on the surface of the finished product; they reported promising results in controlling contamination of Ricotta fresca with certain spoilage microorganisms. In another study, *Carnobacterium* spp. significantly reduced the growth of *Pseudomonas* spp. at 1.28 log and 0.83 log after 14 and 21 days of refrigerated storage, respectively [182]. Fernández et al. [187] studied the effect of nisin alone and in combination with Microgard, that is bacteriocin-like inhibitory products, on the microbial flora of Ricotta cheese.

Bacteriocin production in situ from several LAB and its impact on food preservation have been examined; thermophilin T, a bacteriocin produced by *Streptococcus thermophilus* ACA-DC 0040, had an inhibitory activity against a large number of related LAB as well as *Clostridium* spp. [189]. Kaminarides et al. [190] evaluated the effect of thermophilin T, previously produced in fermented milk, on the microbiological and physicochemical characteristics of Myzithra cheese.

More recently, Sameli et al. [185] studied the effect of an enterocin A-B-P crude extract on the spoilage microbiota in fresh Anthotyros whey cheeses stored at refrigeration temperatures in vacuum for 40 days; *Pseudomonas* spp., *Aeromonas* spp., *Hafnia* spp., and *Serratia* spp. grew faster than LAB during early storage. Later, LAB outgrew the Gram-negative bacteria and prevailed by mid- to late storage in all cheese batches, causing an acidification effect [185]. Sameli and Samelis [144] assessed the growth and bio-control of inoculated *L. monocytogenes* in Anthotyros whey cheeses, without or with 5% of a crude enterocin A-B-P extract during storage at 4° C. From day 15 to the sell-by date (days 35–40), *L. monocytogenes* growth ceased, and progressively, the populations of the pathogen declined in most cheeses; this was due to an unmonitored, batch-dependent natural acidification by spoilage lactic acid bacteria, predominantly *Leuconostoc mesenteroides*, which reduced the cheese pH to 5.5 and finally to a value of 5.0 [144].

6.4. Novel Treatments for Extending the Shelf Life

During the last 10 years, a number of novel treatments have been applied to whey cheeses in order to extend the shelf life and to enhance their functional and sensory characteristics. Duarte et al. [191] proposed an alternative to refrigerated storage technology for Requeijão; they stored the cheese under 100 MPa hyperbaric storage at variable room temperature, and total aerobic mesophiles and *Enterobacteriaceae* growth was inhibited. For LAB, yeasts, and molds, hyperbaric storage showed an additional microbial inactivation effect. This technology is an environmentally friendlier technology, with a carbon footprint estimated to be about 26-fold lower compared to refrigerated storage [191]. The possibilities of using the continuous type of UV-C light on the surface of Lor cheese was studied, and it was found that the application of UV-C light (1.617, 4.018, and 36.832 kJ/m^2) to the cheese surface allowed delaying mold growth during storage; however, extreme doses could induce lipid and protein oxidation reactions, leading to quality deterioration [192].

Two novel food preservation technologies were applied to Ricotta, that is pulsed light [193] and plasma treatment [194]. The authors concluded that both methods could be used to extend the shelf life of Ricotta cheese. To extend the shelf life of buffalo Ricotta cheese, a process was assessed that included a second heat treatment followed by homogenization and hot packaging. In another study, Tripaldi et al. [101] reported that homogenized buffalo Ricotta cheese had a longer shelf life than traditional Ricotta cheese, although the process could be optimized to reduce the total bacterial load during storage.

7. Novel Whey Cheeses and Whey Products

A modified form of Myzithra cheese was produced by substituting the fresh cheese whey by dried whey protein concentrate 65% [195]. The modified Myzithra cheese had a higher lactose, potassium, and sodium content and lower total bacteria, protein, ash, calcium, hardness, and adhesiveness than control Myzithra cheese, while yeasts and molds were absent. Attempts to incorporate fresh whey protein concentrates in Queso fresco showed good results in terms of yield and acceptance, although excessive levels may cause detrimental changes in the texture and flavor of Cheeses and the process needs to be optimized [38]. The incorporation of whey cheese in milk used to make Queso fresco was suggested as an alternative technology for small and medium scale producers for revalorizing cheese whey, increasing yield, and lowering production costs, although physicochemical characteristics were affected [196].

An application for salted whey produced from Domiati, Ras, and Cheddar cheese making is to use it as an ingredient in processed cheese [197]. An added-value whey cheese through addition of pine nuts was developed by Semenium et al. [198]; different concentrations of pine nuts were added to whey cheese, and the whey cheese sample with 4% pine nuts was the most appreciated by the sensory panel [198]. A whey cheese analogue was made from 65% whey protein concentrate and vegetable fat with 15% olive oil, and the product was enriched with thyme leaves, film-packed, and then subjected to heat treatment; the product was characterized as a new whey cheese easy to produce, with improved health enhancing properties, acceptable textural characteristics, a rich aroma profile, and extended shelf life [199].

In addition, novel dairy products using whey cheese or whey components have been studied. Ice cream made from sheep's milk was manufactured with addition of whey protein concentrates [200]. Ricotta cheese powder, prepared from different blends of whey and milk, was used for the manufacture of dried cheese [201]. Marnotes et al. [202] manufactured frozen yoghurts using goat and sheep liquid whey concentrates produced by ultrafiltration; different concentrations of inulin were also added. Maragkoudakis et al. [203] studied the use of scotta, that is, the second whey remaining after the manufacture of Ricotta, as substrate for bacterial fermentation with the objective of obtaining a novel fermented drink and a selection of a starter consisting of *S. thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus,* and *L. acidophilus*, was chosen; the authors speculated that further studies are needed to optimize the organoleptic aspects of the final product. Moreover, an economic and feasible option for dairy products is the production of butter using cheese whey; whey cream is separated from the aqueous fraction of cheese whey and processed to obtain whey cream butter [204–206]. Costa et al. [206] reviewed the technology of production of butter from whey cream and the physico-chemical, sensory, and nutritional characteristics of the product.

8. Functional and Probiotic Products

Although Hippocrates (460–370 BC) said 'Let food be thy medicine and medicine be thy food', and the term 'functional food' was first mentioned in Japan [207], nowadays, there is an increasingly awareness that a good health is directly associated with food and nutrition habits, and functional foods are increasingly demanded by the consumers. Dairy products are leading the research and development of health-promoting and therapeutic products [23,208–211]. Madureira et al. [212–214] effectively applied probiotic cultures in whey cheese, and this type of cheese has proven to be a good vehicle to deliver probiotic bacteria into the gastrointestinal tract. Whey cheese and whey products have several functions to be classified as functional foods [215–217]. Whey-protein-derived peptides have several beneficial effects, e.g., ACE-inhibitory peptides in the treatment of hypertension and β-lactotensin peptide in cholesterol-lowering, anti-stress, and memory-enhancing activities [91,215–217].

Dimitrellou et al. [218] evaluated the feasibility of producing a novel whey cheese with extended shelf life using probiotic lactobacilli immobilized on casein. Pires et al. [219] evaluated the application of kefir culture and probiotics, i.e., *Bifidobacterium* spp., which has previously added and fermented the cream added to the whey for the manufacture of Requeijão cheese. In another study, the addition of *L. delbrueckii* ssp. *bulgaricus* NCFB 2772 was investigated to manufacture a functional acid whey cheese [220]. Nzekoue et al. [221] suggested Ricotta cheese as a cheese matrix for vitamin D fortification.

By considering the potential of whey cheese as a probiotic carrier, the combination of traditional Requeijão with second cheese whey protein, probiotic cultures, and ingredients improving the organoleptic characteristics of the final product was studied [222]; this approach enabled minimizing the environmental impact of the cheese whey and especially whey by-products, assuring a sustainable cheese supply chain. Rosa et al. [223] evaluated the effect of probiotic strains of *L. acidophilus*, *Bifidobacterium* Bb-12, and *Lacticaseibacillus casei-01* on the characteristics of fermented whey/milk beverages during refrigerated storage for 30 days [221]. Schlabitz et al. [224] investigated the shelf life of a symbiotic fermented dairy beverage using Ricotta cheese whey, prebiotics, and probiotics; since the aim of the study was to reduce the amount of whey incorporated into the dairy wastewater, the authors concluded that the whey drink proved to be a viable way to use whey, reducing thus the effluent generated at dairy industries. In addition, Mileriene et al. [225] suggested a valuable way to utilize acid whey and apple pomace with the fermentation from *L. lactis* LL16, with the potential to produce a sustainable cheese with functional properties.

9. Conclusions

Whey cheese production has a long history as a sustainable way to utilize the released whey after the milk curdling. Whey cheeses are produced and consumed worldwide due to their nutritional value and acceptable sensory characteristics. There is a great variation in the technology used, the kind of whey, and thus the variation in composition and characteristics common among different types. In any case, the exploitation of whey for the manufacture of whey cheeses has many practical and financial benefits.

The special cheesemaking processes and the composition have an impact on the type of microorganisms present in the final product. Because whey cheeses have a neutral pH and high moisture content, they may be exposed to contamination and should be consumed in a short period after production. Initially, after the coagulation of whey proteins, and due to the heating of the whey, the natural milk and whey microbiota is inactivated, starter cultures are not applicable and thereby whey cheeses present very low counts of microorganisms after manufacture. However, handling of the fresh curd post-heating results in cross-contamination with a large variety of spoilage, mainly yeasts, molds, coliforms, pseudomonads, and potentially pathogenic bacteria. Therefore, hygienic practices throughout the handling, packaging, and storage are of high importance.

The functional properties of the whey have forced researchers to investigate novel and more attractive products based on whey, some of them with health-promoting potential. Although the production of whey cheese is one sustainable way to utilize the valuable whey, the current review presents specific knowledge concerning whey cheeses to help further research for the development of novel cheeses and other dairy products for sustainable valorization of whey, a proven valuable by-product of the cheesemaking process in a sustainable way.

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