Review: Technology, Chemistry and Microbiology of Whey Cheeses

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In whey cheese manufacture, whey, plain or added with milk, is heated by direct fire, bubbling steam or alternatively in jacketed vats. In some cases, salts or organic acids are previously added. At 80–85 °C, the first particles of curd form; at 85–95 °C, the curd may be cooked for a few minutes to reduce moisture content and/or to obtain the desirable level of browning. After drainage at room temperature during molding for ca. 4 h, whey cheese is stored at ca. 4 °C. The typical mass yield is 6%, but addition of milk, calcium salts and preliminary concentration of protein (by condensation or ultrafiltration techniques) may increase yield considerably. Some types of whey cheeses are supposed to be consumed within a short time upon manufacture (e.g., Ricotta, Requeijão and Manouri), whereas others bear a longer shelf life (e.g., Gjetost, Mysost and Myzithra). Whey cheeses are significantly different from one another in terms of chemical composition, which is mainly due to variations in the source and type of whey, as well as to the processing practices followed. Moisture content and pH of whey cheeses are usually high and favor microorganism growth (molds, yeasts, lactic acid bacteria and *Enterobacteriaceae* account for the dominant microflora in these cheeses). Adequate packaging of whey cheeses should be provided, and legislation should be prepared to fix standard characteristics of each type of whey cheese, and hence protect typical products from adulteration and fakes. Marketing efforts should also be aimed at increasing whey cheese consumption, either directly or incorporated in desserts, snack dips and pasta-type dishes.

Key Words: whey cheeses, manufacture, microflora, composition

En la fabricación de quesos de suero, el suero, solo o con adición de leche, se calienta directamente con fuego, con burbujeo de vapor o alternativamente en recipientes con camisas de calor. En algunos caos se añaden sales o ácidos orgánicos previamente. Entre 80 y 85 °C se forman las primeras partículas del cuajo; entre 85 y 95 °C el cuajo debe cocerse durante unos minutos para reducir el contenido de agua y además producir un pardeamiento si se desea. Después del drenaje en el molde y a temperatura ambiente durante aproximadamente 4 horas, el queso se almacena a 4 °C. El rendimiento másico es aproximadamente del 6%, aunque la adición de leche, sales de calcio y una concentración previa de las proteínas, pueden aumentarlo considerablemente. Se supone que algunos de los quesos de suero deben consumirse poco después su elaboración (p.e., Ricotta, Requeijão and Manouri), mientras que otros tienen vidas útiles más largas (p.e., Gjetost, Mysost and Myzithra). Los quesos de suero son significativamente diferentes unos respecto a otros en cuanto a su composición química debido principalmente a las variaciones en el origen y el tipo de suero, así como a la forma de elaboración. El contenido de agua y el pH de los quesos de suero son generalmente altos, lo que favorece el crecimiento de microorganismos (hongos, levaduras, bacterias acidolácticas y *Enterobacteriaceae* componen la microflora de estos quesos). Estos quesos deberían envasarse adecuadamente y establecerse la legislación que fije las características estándar de cada tipo de queso y así proteger al producto de adulteraciones y fraudes. También debe promoverse su consumo, tanto directo como incorporado en pasteles, galletitas y platos de pasta.

Palabras Clave: quesos de suero, fabricación, microflora, composición

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INTRODUCTION

Whey is the aqueous portion of milk obtained following acid-, heat- or rennet-driven coagulation. It is an opaque liquid possessing a greenish-yellow color, with total solid content generally ranging in 6.0–6.5% (w/v) and a biological oxygen demand (BOD) of at least 30,000. In Mesopotamia, about 5,000 B.C., Kanana discovered that warm milk stored in a bag made of fresh stomach skin of sheep (or goat) produced curds and, concomitantly, whey (Malcata, 1991). Later, nomad shepherds started boiling whey in copper kettles and eventually obtained a nourishing solid food (Kosikowski, 1982b).

Whey cheeses are manufactured all over the world, usually according to traditional protocols and in small scale, via denaturation of whey proteins (which also have associated residual fat). These cheeses bear distinct names, depending on the country and region where they originate (Table 1).

Whey cheeses have been manufactured chiefly from ovine whey in the Mediterranean basin, owing not only to the economic importance of such ruminants in that area, but also to its high protein content; the lower protein contents of bovine and caprine wheys lead in fact to lower yields of the corresponding whey cheeses. Ricotta is the most important, and well-known, whey cheese in the world; it was originally a product of Italy, but in recent years, Ricotta has become rather popular in the USA. This cheese has been manufactured from whey obtained from buffalo's skim milk, whey obtained from Cheddar cheese making and *paneer* whey (produced by acid precipitation of buffalo's milk at high temperature) (Prajapati and Mathur, 1981).

The literature on whey cheeses is scarce and is virtually limited to Italian, Portuguese, Norwegian and Greek types. Therefore, only whey cheese from these countries will be presented and further discussed in this review.

MANUFACTURE

Thermal denaturation, sequential aggregation and eventual precipitation permit whey proteins be used in the manufacture of soft and semi-soft whey cheeses. In the manufacture of these dairy specialties, whey can be used as such, but a small percent of milk is often added; hence, coprecipitation of caseins usually occurs when whey cheeses are produced. Coprecipitation of casein may be improved by acidification, and the maximum yield is obtained when addition of acid is coupled with maintenance of low levels of calcium chloride (Hill et al., 1982).

Ricotta is generally sold as a fresh, unripened grainy cheese, which is white, soft and moist. It is fairly bland, or at most semi-sweet, when it is manufactured from fresh, sweet whey. Ricotta was originally produced where an important production of ovine milk existed, viz., Lazio, Sicilia and mainly Sardinia; however, such whey cheeses were later distributed throughout Italy, and even exported to other countries, especially the USA. In this country, the early Ricotta, called Ricotone, was usually produced via high temperature heating of acidified cheese whey. In the eastern USA, the highest consumer acceptance was achieved when Ricotta was manufactured with the addition of whole milk, or partly skimmed milk (Kosikowski, 1982a); this product is indeed softer and creamier, and possesses a delicate texture and a pleasant flavor that resembles caramel. Since the 1970s some patents pertaining to manufacture of Ricotta cheese have been granted (Schmidt et al., 1972; Carswell, 1973; Pontecorvo, 1973; Edwards, 1977; Savaresse, 1981; Prella, 1987; Skovhauge and Simonsen, 1988; Rota, 1990; Magnanini et al., 1995; Tomatis, 1996; Sassu, 1998). The industrial processes most commonly found for the manufacture of Ricotta start from sweet whey mixed with 5–10% (v/v) milk, that has been previously heated to ca. 40–50 °C. Salt is then added to ca. 0.1% (w/v), and heating is carried on up to 80–85 °C. At this stage, an acidification agent is added (usually an aqueous solution of citric acid at ca. 0.11 kg/L),

Table 1. Whey cheeses produced worldwide.

Country	Name of Whey Cheese	Reference
Norway	Mysost, Primost, Gjestost, Grubransdalsost	Jelen and Buchheim, 1976
Switzerland	Schottenziegr, Hudelziger, Mascarpone	Kandarakis, 1986
Portugal	Requeijão	Kandarakis, 1986
Spain	Requesón	Kandarakis, 1986
France	Serac, Brousse, Broccio, Greuil	Kandarakis, 1986
Germany	Zieger, Schottenzieger, Schabzieger	Kandarakis, 1986
Greece	Manouri, Myzithra, Anthotyros	Kandarakis, 1986; Anifantakis, 1991
Italy	Ricotta (gentile, pecorina or romana)	Kosikowski, 1982a,b
Malta	Cacio-ricotta	Kandarakis, 1986
Cyprus	Anari	Williams and Syson, 1984; Kandarakis, 1986
Romania	Ziger, Urda	Kandarakis, 1986
Macedonia	Urda	Dzilenski et al., 1975
Czechoslovakia	Urda, Zincica	Kandarakis, 1986
ex-Yugoslavia	Scuta, Puina	Stefanovic and Djordjevic, 1969; Kosikowski, 1982b
ex-USSR	Nadigi, Kaukaz	Kandarakis, 1986
Tunisia	Klila	Kandarakis, 1986
Northern Africa	Nicotta	Kandarakis, 1986
Lebanon	Kariche	Kandarakis, 1986
Israel	Urda	Kandarakis, 1986
Iraq	Lour	Kandarakis, 1986
Argentina	Ricotta	Kandarakis, 1986
Brazil	Requeijão do Norte, Ricotta fresca	Jassen-Escudero and Rodriguez-Amáya, 1981
USA	Ricotone, Ricotta	Kosikowski, 1982b; Kandarakis, 1986

and gentle stirring is provided. Heating and stirring are discontinued when the curd rises to the free surface of the hot whey (Mathur and Shahani, 1981), and the mixture is then allowed to rest for ca. 5 min. The curd is then scooped from the surface into perforated tins. The whey cheeses are allowed to drain in those tins for 4–6 h in a cool room, and then they are covered with parchment and ice. Usually, 1 kg of Ricotta can be obtained from 15–20 L of whey (Mills, 1986), which corresponds to a yield of ca. 6% (w/v) (Kosikowski, 1982a). Weatherup (1986) described a method designed for Ricotta, which consisted of mixing 80% (v/v) Cheddar whey with 20% (v/v) whole milk, neutralizing to pH 6.5 with sodium hydroxide, and precipitating at 87 °C after acidification to pH 5–6 with citric or acetic acid; these conditions lead to a yield of 12% (w/v). An empirical equation was developed by Modler and Emmons (1991), which, based on milk composition, was able to estimate the final yield of Ricotta. Meanwhile, the low yield of plain whey cheese urged a few technological changes. In general, addition of milk or addition of calcium salts increases overall protein recovery, as observed by Mathur and Shahani (1977, 1981) and Shahani (1981) (Table 2); addition of those cations at the 500 ppm level may increase recovery of proteins from 48.2% up to 65.5%, and up to 77.8% when 2000 ppm is employed in the case of Cheddar whey, provided that neutralization to pH 6.8 had already been done. The desired fat level may also be reached by directly creaming the Ricotta curd from whey/dried skim milk mixtures (Shahani, 1981). An alternative manufacturing process to increase yield is a preliminary concentration of whey. Instead of whey obtained directly from the cheese vat, Nilson and Streiff (1978) and Streiff et al. (1979) preferred condensed whey to produce Ricotta and thereby obtained higher yields, i.e., increases from 10.2% (w/v) to 47.2% (w/w) in total solids; however, the whey cheeses, characterized by a total solids content above 21% (w/v), were not organoleptically acceptable because they were coarse, unclean and exhibited a grainy and mealy texture; manufacture of Ricotta with ca. 21% (w/v) condensed whey was thus recommended. Furthermore, acidification with acetic acid produces higher yields and better final product characteristics than with citric and lactic acids, and indirect heating produces greater yields of curd (and a better curd texture) than direct heating (Weatherup, 1986; Mahran et al., 1999).

Additionally, Pereira et al. (1982) observed that the rate of heating of whey did not significantly affect the characteristics of the final Ricotta, except when it was above 3.5 °C/min; in this latter case, a small reduction in yield was recorded, as well as a higher retention of fat. Holding the temperature at 85 °C for 10 min led to a firmer final whey cheese.

Table 2. Recovery of distinct fractions from whey systems containing varying amounts of added milk (adapted from Mathur and Shahani, 1981).

Composition of System (%, w/w)		Recovery of Protein (%, w/w)		
Whey	Whole Milk	Caseins	Whey Proteins	Total Proteins
100.0	0.0	95.5	30.6	48.2
97.5	2.5	94.7	55.4	64.7
95.0	5.0	94.2	62.0	71.4
90.0	10.0	95.5	61.2	73.3
75.0	25.0	94.4	64.4	79.5

Increase in the worldwide manufacture of Ricotta cheese to meet a growing demand by the various populations has been promoting technological optimization of Ricotta manufacture via, namely, concentration of proteins by ultrafiltration of a mixture of whey and milk, or whole milk in which no curd particles (or fat, for that matter) were lost prior to heating (Maubois and Kosikowski, 1978, 1981; Maubois, 1980; Nielsen, 1984; Eriksen, 1985) and continuous production of Ricotta, which shows advantages over conventional protocols owing to reduced capital and operating costs. In continuous methods, a mixture of sweet whey and 10–20% (v/v) whole milk (with pH adjusted to 6.3) or plain sweet whey (with pH adjusted to 7) is pasteurized or subject to HTST or (preferably) UHT procedures, cooled to 85 °C and typically passed through a holding tube (30.5 m long) at a flow rate of ca. 4.5 L/min, with injection of citric acid at a rate of 125–150 mL/min. The hot, acidified coagulum of protein is transferred to a slow-moving conveyor belt made of a nylon mesh with 43% open area (to allow whey separation from the curd) and is hooped and drained for ca. 1 h (Modler, 1984, 1988; Modler and Jones, 1987). Later, Palmas et al. (1994) presented a novel technology for the production of Ricotta cheese, which included mechanical handling of the curd, more intensive heat treatment, addition of citric or lactic acids, automatic packaging into sealed containers and cold storage; a higher yield and a longer shelf life were thus attained, which were associated with better microbiological properties than the traditional process.

Requeijão (in Portugal) and Requesón (in Spain) are manufactured mostly from bovine whey milk by dairy industries, although traditional Iberian whey cheeses are still manufactured, at the farm level, by adding a small percent of caprine milk to ovine whey. In Portugal, only 2% (v/v) of the total whey generated is used to manufacture Requeijão, which accounts for ca. 630 ton of final product (Ferraz, 1998). Said small fraction arises essentially from incipient marketing efforts, coupled with the realization that such a dairy product possesses a rather short shelf life; hence, the size of the market remains excessively limited for commercial exploitation. Although in the distant past Requeijão was sold door by door in limited regions of the interior of Portugal, it is nowadays an ubiquitous food specialty and is accordingly sold wrapped in paper or plastic packages at virtually every supermarket. This whey cheese is small (ca. 150 g), white and has a semi-sweet flavor.

The traditional way of manufacturing Requeijão consists of heating the starting material, i.e., bovine whey or ovine whey, or mixtures thereof with ovine or caprine milk, at a temperature ranging from 90 to 100 °C for ca. 15 min, under smooth stirring conditions. Near 85 °C, when coagulation starts, a small amount of cold water is sometimes added to improve coagulum formation. The curd rises spontaneously to the surface and is then scooped into wooden or plastic molds, where it is allowed to drain and cool for a few hours. In their studies pertaining to Requeijão, Roseiro and Wilbey (1991) obtained 1 kg of cheese from 15 L of plain ovine whey taken from the manufacture of either Spenwood or Rook's Nest cheeses, produced on a farmhouse level. They observed that variation in the acidity of whey (which was associated with a pH range of 5.26–5.86) was not a significant factor in the manufacture of Requeijão; however, high acidity could produce unacceptable sensorial properties, particularly if nonstarter organisms would contaminate whey. The relatively high pH of Requeijão makes it susceptible to spoilage, so good hygienic handling, addition of salt and use of chilled storage are crucial steps. Recently, Pintado et al. (1996) tried to optimize manufacture of Requeijão, according to a factorial design, using heating time, heating temperature and fractional addition of ovine/caprine milk as manipulated technological variables. A true local maximum existed for moisture content at a temperature of ca. 93 °C, a heating time of ca. 30 min and an addition ratio of ca. 17% (v/v) ovine milk, which lay well within the range chosen for experimentation. The heating temperature significantly affected the contents of fat, protein and moisture, whereas the heating time significantly affected only fat content. The sensorial analyses showed that the experimental whey cheeses produced were preferred to the traditional whey cheese. The best technological choice for production of Requeijão was via heating whey at 90 °C for 48 min, with addition of 8.2% (v/v) caprine milk.

Mysost, a unique bovine whey cheese, is currently produced in Norway. Consumer acceptance of Mysost outside Scandinavia has not been well documented; however, this product can be occasionally found in speciality cheese stores in Germany, the USA and Canada. Mysost is typically produced either as a hard or a spread-type cheese, from fresh sweet bovine or caprine whey. By the turn of the century, manufacturers started adding cream, so the generic name of such bovine whey cheese was eventually changed to Brunost. When plain caprine whey milk is employed, the cheese is called Gjestot. The cheese has a dark brown, coarse texture. The lactose content in that whey cheese (ca. 40% w/v) provides a long-lasting source of energy, suited for traveling people in Northern Norway. Cream is often added to caprine whey milk to improve sensorial characteristics, and then the product may be called Primost; in this case, it has a light tan color, a smooth, creamy body and a texture where lactose crystals are barely discernible. Its flavor is that of slightly sweetened, cream caramel. Grubransdalsost is the most popular Norwegian condensed whey cheese that is obtained from a mixture of ca. 88% (v/v) caprine whey and 12% (v/v) bovine milk.

In general, enough fresh cream is added to give ca. 3% (w/v) fat to the fresh sweet whey obtained from coagulation of full-fat caprine milk and bovine milk (at a ratio of 88:12) or from Cheddar, Swiss or Mozzarella cheese manufacture. The mixture of whey and cream is pumped into a two-stage (or double effect), stainless steel evaporating pan, which concentrates the creamed whey to about 50% (w/v) total solids. The heavy viscous fluid is then directly poured into a round vacuum kettle and boiled until the concentration reaches 80% (w/v) total solids, under strong, sustained agitation. The vacuum is then released, the cover is opened, and the concentrated whey is heated to ca. 95 °C and stirred until the desired brown color and typical flavor intensity are attained (Jelen and Buchheim, 1976). This plastic mass is removed by tipping the kettle over to a proper angle and dipping it into a round wooden tub equipped with a strong rotary sweep metal agitator. The mass is worked for ca. 20 min at room temperature, so as to produce a fine-grain, butter-smooth texture; uniform, steady agitation at this stage prevents formation of large lactose crystals. While still warm, the plastic mass is conveyed to a butter printer equipped with an Archimedes screw and wire-cutting bars, where it is extruded and cut into ca. 0.25, 0.5, 2.5 or 4.5 kg blocks, which are in turn lightly waxed, packaged and duly traded. Mysost cheeses keep their properties for up to 6 months (or even more) at 5 °C (Kosikowski, 1982b).

Greek whey cheeses are produced with names that also bear a quality meaning (Manouri is the highest quality whey cheese, and Myzithra is the lowest). They are made from all kinds of whey, but particularly from that derived from ovine milk when making hard and soft cheeses. According to the quality of the cheese that is required, certain quantities of milk or cream are added to the whey. For example, Manouri is considered to be an exceptional quality whey cheese when its fat content is 30%, as such, or is 65% in its dry matter. Anthotyros, on the other hand, is a whey cheese of good quality with 19–25% fat content, while Myzithra is considered a common whey cheese with fat content up to 19%. These whey cheeses are consumed as table cheeses, or are seasoned with salt or honey. The whey is heated in cheese vats, with capacity up to ca. 1000 L, by using steam, which is either directed to the double wall of the cheese vat or infused directly in the bulk of whey. The rate of heating is such as to attain the temperature of 88–90 °C in 40–45 min. Whole milk is added to the whey at the temperature of 40–72 °C in one or more batches, while cream is added with one of the batches of whole milk, or in certain cases before the addition of whole milk. Usually, the salt is added at 72–75 °C in the proportion of 1.0–1.5% (w/w). The first particles of curd appear at 78–80 °C, depending on the kind of whey, its acidity and the proportion of whole milk added. Heating is continued up to 88–92 °C, according to the product that is to be made and its intended use. Manouri and Myzithra, that are to be consumed fresh, are usually heated at a lower temperature, whereas a higher temperature is selected when the product is to be dehydrated afterwards. At those temperatures, the curd is left for 15–30 min to remove a large fraction of its moisture (i.e., to be cooked). During the removal of curd from the vat, it is placed in special molds, which are different for each kind of cheese; Manouri curd, for instance, is introduced in cloth bags of shape and size such that, after draining, the cheese becomes a cylinder of 10 cm in diameter and 25 to 30 cm in length. The cheese is consumed soon after production. Very often, the whey is sufficiently salted so that the resultant cheese has a salt content of ca. 0.5% (w/w) (Kalogridou-Vassiliadou et al., 1994). Myzithra curd, on the other hand, is introduced in metallic molds shaped as cones with diameter of 12.5 cm in the base and 18 cm at the top, with holes and crevices in order to facilitate drainage of the serum, or in cheese cloths which, when hung and properly tied, give the cheese a spherical shape. Drainage is done within 3–5 h in the cheese plant itself (Kandarakis, 1986). A method that is used for keeping Myzithra for a rather long time is its partial dehydration, which can be achieved either in a dedicated curing room or in the open environment, in combination with salting. Dehydration reduces the moisture to below 40% (w/w), so the cheese becomes sufficiently hard to be used for grating. The pieces of hard Myzithra are coated with paraffin, smoked or introduced in plastic bags and preserved in cold storage (Kandarakis, 1986).

Zerfiridis and Manolkidis (1978) tried to produce a new type of whey cheese resembling Myzithra cheese but with extended shelf life. To the curd produced via protein denaturation, 2–2.5% (w/w) salt was added at 45 °C, as well as 1% (v/v) lactic acid bacteria starter. After pressing, the cheese was maintained at 22 °C, under controlled humidity, for 5 d; after this period, it was sprayed with potassium sorbate, duly packaged and maintained at 8 °C for 2 months, so as to permit a shelf life of 6 months at 5 °C. Later, Zerfiridis et al. (1985) tried to improve the yield of this whey cheese, as well as that of Manouri and Anthotyros, and observed that adjustment of initial pH to 5.8 would be in order; they also concluded that addition of Ca^{2+} (at 40–120 ppm) did not have an effect in the level of protein recovery, whereas addition of NaCl (at 0.75–3.0% w/v) would gradually decrease it.

CHEMICAL COMPOSITION

The whey is the aqueous part of milk that remains after the separation of the curd during cheese making, as mentioned before. Some major components of milk remain in whey: 10 L of milk generate ca. 1 kg of cheese and ca. 9 L of whey, which retain 6.3–12.4% (w/v) lipids, 21.4–25.1% (w/v) nitrogen compounds, 88.0–99.3% (w/v) lactose, 61.8–88.5% (w/v) salts and 49–50% (w/v) total solids of the original milk (Monzón and Olano, 1977). The composition of the main constituents of whey differs according to the type of cheese and the kind of milk that it results from (Table 3). Precipitation of caseins during cheese making may be achieved by acidification not above pH 5.1, as in the manufacture of acid cheese (hence producing acid whey), or by enzymes, as in the manufacture of renneted cheese (hence producing sweet whey). Sweet whey is generally richer in lactose, while acid whey exhibits a higher concentration of minerals (Table 4), likely owing to solubilization of colloidal calcium phosphate of casein micelles that occurs concomitantly with acidification. The composition of bovine whey from several breeds is well known (Schingoethe, 1976; Regester and Smithers, 1991); composition changes essentially vary as a function of the cheese-making technique coupled with the physiological state of the animal (which undergoes seasonal variations), in addition to the producing species and breed. Ovine and caprine wheys are less frequently referred to in the literature, but their compositions are rather different from that of bovine, mainly in what concerns protein, fat and lactose concentrations (Table 4). The composition of whey cheeses is dependent upon such factors as source of whey (bovine, caprine or ovine), composition of whey (i.e., breed, stage of lactation, feeding regime and type of cheese), ratio of whey to milk (if milk or cream is added to whey prior to manufacturing cheese) and technological practices followed (i.e., heat temperature, time, batch versus continuous processes and possible use of ultrafiltration). Therefore, one expects large differences to show up between the whey cheeses described before in terms of chemical composition.

The composition of Ricotta was first reported by Vodret (1962, 1970); further in-depth characterization of this cheese has been made afterwards, according to the geographical location where the cheese was manufactured (Ta-

Table 3. Chemical composition and pH of whey from different types of bovine cheeses (adapted from Kandarakis, 1986).

Type of Cheese	Percentage (w/w)[1] Moisture	Protein	Fat	Lactose	Ash	pH
Camembert	93.0	0.9	0.3	5.1	0.6	n.a.
Cheddar	93.7	0.8	0.5	4.9	0.5	5.7–6.3
Cottage	93.5	0.8	0.1	4.9	0.5	4.6
Emmental	93.1	0.9	1.0	5.5	0.5	n.a.
Feta	93.7	0.8	0.3	4.7	0.5	6.3
Graviera	93.1	0.9	0.6	4.9	0.5	6.3
Kephalotyri	93.5	0.8	0.4	4.9	0.5	6.4

[1]n.a.: not available

Table 4. Composition of liquid whey from different animal sources (adapted from Frévier and Bourdin, 1977).

Constituent	Cow Sweet	Cow Acid	Sheep Sweet	Goat Acid
Dry matter (g/L)	70.84	65.76	83.84	62.91
Protein (g/L)	9.24	7.80	18.71	9.35
Nitrogen (g/L)	1.45	1.22	2.93	1.47
Non-protein nitrogen (g/L)	0.37	0.54	0.80	0.67
Ammonium nitrogen (g/L)	0.04	0.14	0.13	0.18
Fat (g/L)	5.06	0.85	6.46	0.40
Lactose (g/L)	51.81	45.25	50.98	39.18
Ash (g/L)	5.25	7.56	5.65	8.36
Calcium (g/L)	0.47	1.25	0.49	1.35

Table 5. Composition of whey cheeses.

Cheese/Location	Raw Material/Manufacture Technique	Moisture (%, w/w)	Protein (%, w/w)	Fat (%, w/w)	Lactose (%, w/w)	Ash (%, w/w)	Referece
Ricotta/Sardegna, Italy	Sheep whey/traditional	62.9 ± 3.3	8.7 ± 1.3	24.5 ± 3.7	3.1 ± 0.6	0.6 ± 0.1	Vodret, 1970
Ricotta/Diano Valley, Italy	Sheep whey/traditional	56.8	4.1	26.0	n.a.	n.a.	Cavaliere, 1988
Ricotta/Catania, Italy	Sheep whey/traditional	70–80	6.1–7.0	10.2–27.4	4.1	1.0–1.5	Ziino et al., 1993
Ricotta/Sassari, Italy	Cow whey/traditional	76.0 ± 4.2	n.a.	9.7 ± 2.3	n.a.	1.0–0.3	Cossedu et al., 1997
Ricotta/USA	Cow: 95% whey and 5% milk/traditional	81.63	7.0	3.3	n.a.	n.a.	Weatherup, 1986
Ricotta/USA	Cow: 80% whey and 20% milk/continuous	66.5	16.3	11.6	n.a.	n.a.	Modler, 1988
Ricotta/USA	Cow: 80% whey and 20% milk/ultrafiltration	19.8	15.9	2.4	n.a.	n.a.	Modler and Jones, 1987
Ricotone/USA	Cow: 100% whey	82.5	11.3	n.a.	1.5	n.a.	Kosikowski, 1982b
Requeijão/Portugal	90% Ovine whey and 10% caprine milk/traditional	59.0 ± 0.60	8.5 ± 0.6	29.5 ± 0.4	3.5 ± 0.2	n.a.	Pintado and Malcata, 1999
Mysost/Norway	Bovine: 100% whey/traditional	16.2 ± 18.5	11.5 ± 12.2	24.7 ± 28.7	34.4 ± 36.7	4.0 ± 4.5	Jelen and Buchheim, 1976
Anthotyro/Greece	Commercial cheeses	66.18	n.a.	18.50	n.a.	1.07	Tzanetakis et al., 1977
Manouri/Greece	90% Ovine whey and 10% cream/traditional	50.61	n.a.	33.67	n.a.	1.36	Tzanetakis et al., 1977
Manouri/Greece	Commercial cheeses	48.07 ± 2.97	10.86 ± 1.89	36.67 ± 3.70	2.49 ± 0.47	0.83 ± 0.22	Veinoglou et al., 1984
Myzithra/Greece	Commercial cheeses	80.39	n.a.	1.38	n.a.	0.79	Tzanetakis et al., 1977

n.a.: not available.

ble 5). Small compositional differences were found in the contents of fat, lactose and ash in whey cheeses made with different organic acids; lactic acid cheeses exhibited higher protein content than those made with acetic and citric acids (Mahran et al., 1999). Considering the high fat content of this cheese, several studies have been carried out to characterize fat composition. Vodret and d'Amaddio (1973a,b) reported that the free fatty acid composition of Ricotta Pecorina cheeses is accounted for by a high percentage of volatile free fatty acids, of which a notable concentration is justified by polyunsatured fatty acids. This composition changes throughout the sheep's lactation period, namely owing to a dynamic feeding regime; the typical trend is a marked rise in the proportion of palmitic and shorter acids when the winter feed of the animals is replaced by the spring pasture. Ziino et al. (1993) studied Ricotta from several locations in Catania and found that saturated free fatty acids constitute ca. 72% of the global inventory of free fatty acids in whole ovine whey, with palmitic acid predominating (20–25%). Monounsaturated fatty acids represent ca. 24% of the total, with similar values for the three regions tested, with oleic acid predominating (at levels above 20%). The major polyunsaturated fatty acids, i.e., linoleic and linolenic, were present at higher levels [5.6% (w/w)] in Ricotta from Solicchiata than from other origins [ca. 3.9% (w/w), on average].

The chemical composition of Requeijão made from ovine whey mixed with ca. 10% (v/v) caprine milk, according to the classical protocols followed in Portugal (Table 5) was recently reported by Pintado et al. (1996); however, these values varied depending on the source of whey, whether or not milk was added and drainage time. The composition in free fatty acids (Table 6) indicated that myristic and palmitic acids are the most important saturated free fatty acids (ca. 15–18% each), whereas linoleic and linolenic were the most important unsaturated ones (Pintado and Malcata, 1999).

Soft and hard Mysost cheeses found in the market exhibit pH values spanning the range 5.1–5.7 (Jelen and Buchheim, 1976). It is notorious that lactose is the major component of these cheeses (Table 5), which makes their crystals play a prominent role in the final structure; without proper control, such fact may actually bring about sandiness, which is an unacceptable organoleptic feature. Ultrastructural studies indicated that all Mysost cheeses consisted of fine lactose crystals bound together by a seemingly homogeneous fat protein matrix. Gjestost was found (Bakkene and Steinsholt, 1975) to display a stronger flavor than Mysost; the intensity of said goaty flavor was highly correlated with free fatty acid content, and could eventually be detrimental to product quality. A higher percentage of short-chain acids were also detected in the former than in the latter cheese. Primost is richer in fat (viz. 30.2%, w/v) than Mysost, owing to initial addition of cream to the whey as mentioned before; it is also characterized by a moisture content of 13.8% (w/w), a protein content of 10.9% (w/v) and a lactose content of 36.6% (w/w) (Kosikowski, 1982b).

The fat and moisture contents of Greek traditional whey cheeses are variable, depending on the manufacturing procedure and the origin of whey (Zerfiridis, 1976; Kandarakis, 1981). Several Manouri cheeses were purchased from local markets at random and were analyzed for chemical composition by Veinoglou et al. (1984); these authors recommended addition of ovine milk and cream at the ratio of 25% and 5% (w/v), respectively, so as to achieve a final content of 3.8% (w/v) fat and 2.2% (w/v) protein, hence decreasing the moisture content to 47.4% (w/w) and increasing the fat content to 29.9% (w/w) and the protein content to 12.9% (w/w); in this way, the yield could be raised, while fully maintaining its pleasant organoleptic properties.

Table 6. Composition in free fatty acids of traditional Requeijão, after 24 h of storage at 4 °C (adapted from Pintado and Malcata, 1999).

Free Fatty Acids	Concentration mg/g_{cheese}	Concentration % Total
Butyric ($C_{4:0}$)	0.024 ± 0.001	4.9
Caproic ($C_{6:0}$)	0.026 ± 0.000	5.1
Caprylic ($C_{8:0}$)	0.033 ± 0.001	6.6
Capric ($C_{10:0}$)	0.042 ± 0.002	8.4
Lauric ($C_{12:0}$)	0.043 ± 0.004	8.7
Myristic ($C_{14:0}$)	0.056 ± 0.001	11.3
Palmitic ($C_{16:0}$)	0.065 ± 0.001	13.0
Stearic ($C_{18:0}$)	0.070 ± 0.001	14.0
Oleic ($C_{18:1}$)	0.070 ± 0.002	14.1
Linoleic ($C_{18:2}$)	0.066 ± 0.001	13.3
Linolenic ($C_{18:3}$)	0.000 ± 0.000	0.0
Total	0.498 ± 0.006	100.0

MICROFLORA

In general, the growth of microorganisms depends on such factors as availability of nutrients, water activity, pH, ionic strength, temperature and overhead atmosphere composition. Considering that fresh whey cheeses exhibit high pH, high moisture content and low salt content, these dairy products are very susceptible to microbial spoilage, especially by molds, yeasts and *Enterobacteriaceae.* Even in the cases where acidified whey is used in cheese making, following heating at 80°C for 10 min, a high susceptibility to microbial spoilage is observed, owing likely to contamination and sporulation after heat processing of whey has taken place.

The microflora in whey, to be later used to manufacture commercial Ricotta, is drastically reduced after heat treatment; however, airborne contamination occurs to some extent (Ottogalli et al., 1981). Within the first 24 h following production, all microbial groups tended to grow in viable numbers, but later only psycrothrophs (mainly pseudomonas, yeasts, molds and coliforms) were able to grow; the high moisture content of the product, associated with the high relative humidities prevailing in the manufacture rooms, could account for this realization.

Enterobacteriaceae are present in Ricotta (Cicala et al., 1981); from the fourty-four strains of this family (which were resistant to tetracycline and/or ampicillin) isolated from thirty cheeses, six strains were classified as *Enterobacter hafnia,* eight as *Enterobacter aerogenes,* six as *Enterobacter cloacae,* four as *Citrobacter freundii,* five as *Escherichia coli* and another fifteen as irregular coliforms. Later, Cossedu et al. (1997) reported the absence of coliforms and *E. coli* in thirty-two samples of fresh bovine Ricotta. The presence of *Bacillus* spp. in Ricotta ($10–10^3$ cfu/g) was also assayed for (Cosentino et al., 1997), and ca. 78% of the samples analyzed were contaminated with strains belonging to this genus (ca. 10^2 cfu/g), with *B. cereus* predominating; other species isolated included *B. coagulans, B. liechenformis, B. mycoides, B. pumilis, B. subtilis* and *B. stearothermophilus.* However, in all cases, the level of contamination did not reach the threshold that leads to significant toxin production. In order for Ricotta to be safely consumed, Ottogalli et al. (1981) suggested, in addition to obvious standard sanitation practices, rapid cooling and storage at low temperature. The shelf-life of commercial Ricotta cheese will increase ca. 27 days if storage temperature is decreased from 25 to 6 °C (Hough et al., 1999). However, fresh Ricotta stored at 4 °C is rather susceptible to contamination by *Listeria* spp. and, as observed by Genigeorgis et al. (1991), it actually constitutes one of the best substrates for this genus, provided that no extraneous ecological competition is brought about by starter addition and that pH is not allowed to fall below 5.5. Use of bacteriocins (viz., nisin) has proven beneficial in controlling the viability of such pathogens as *Listeria monocytogenes* in Ricotta: addition at the level of 2.5 mg/L may effectively inhibit growth for at least 8 weeks, whereas nisin-free cheeses become unsafe within 1–2 weeks of incubation (Davies et al., 1997). However, *Listeria* spp. were not detected in commercial cheeses (Lescar, 1989). The effect of salt on the viable numbers of microorganisms in Ricotta was also studied; total viable counts were below 10^9 cfu/g for fresh unsalted Ricotta (but above 10^9 cfu/g for salted, ripened Ricotta), *Enterococci* (salt-tolerant bacteria) were present in ca. 30% and 60% of the samples, respectively, and sulfide-reducing bacteria were present in ca. 70% and 60%, respectively (Fadda et al., 1989). Additionally, yeasts and molds (viz., *Penicilium casei* and *P. notatum*) were present in 80% of the salted Ricotta cheeses analyzed, and *E. coli* in 38%.

Viable bacteria were virtually absent on the day of manufacture of Requeijão, as expected in view of thermal processing (at ca. 95 °C); however, by 2 days of storage, these whey cheeses exhibited high viable counts of *Bacillus* (1.1×10^4 cfu/g), *Pseudomonas* (4.3×10^3 cfu/g), *Staphylococcus* (7.2×10^3 cfu/g) and lactic acid bacteria (especially lactococci) (1.4×10^4 cfu/g). Yeasts and molds (3.6×10^3 and 1.1×10^6 cfu/g) and *Enterobacteriaceae* (8.7×10^3 and 7.8×10^5 cfu/g) underwent increases in viable counts later during storage, after 6 and 10 days, respectively. Spore-forming clostridia (4.9×10^2 cfu/g) were detected at 10 days of storage.

The microflora of Anthotyros was also duly characterized, and it exhibited high viable numbers of coliforms (5.5×10^6 cfu/g), yeasts (3.0×10^5 cfu/g), *Micrococcaceae* (3.0×10^4 cfu/g), enterococci (6.0×10^5 cfu/g) and lactic acid bacteria (1.5×10^7 cfu/g). In qualitative terms, lactic acid bacteria included *Lactococcus lactis* ssp. *lactis, Enterococcus faecalis, E. faecium* and *Leuconostoc paramesenteroides* (Kalogridou-Vassiliadou et al., 1994). *Escherichia coli, Citrobacter* spp., *Klebsiella* spp. and *Enterobacter* spp. were detected in 84 and 85% of Anthotyros and Myzithra samples, respectively; the same strains, except *E. coli* (which could never be found), were also detected in 75% of Manouri samples (Tzanetakis et al., 1977). The generation time of *Listeria monocytogenes* in Greek whey cheeses (Papageorgiou et al., 1996) is affected negatively by the ripening temperature (viz., ca. 18 h at 5 °C, ca. 6 h at 12 °C and ca. 2 h at 22 °C); furthermore, the maximum viable counts of this genus ranged from ca. 10^6 to 10^8 cfu/g, although the time necessary to reach this level depended upon temperature (viz., ca. 24–30 days at 5 °C, 5–12 days at 12 °C, and 56–72 days at 22 °C). The maximum numbers of *Listeria monocytogenes* in Myzithra, attained at 5 °C and 12 °C, were significantly lower than in Anthotyros and Manouri. Use of a starter culture to prevent spoilage of Greek whey cheeses was originally mentioned by Ramazanov and Rakitsaya (1973), but no clear information has yet been provided with regard to the specific taxonomic characterization of said culture. Later, Zerfiridis and Manolkidis (1978) concluded that both salt and starter contribute effectively toward controlling growth of coliforms and yeasts, and that coliforms vanish by 2–3 months of storage (which coincides with the onset of yeasts). This effect is likely due to acid development in cheese, which has a microbiostatic effect that is potentiated by the presence of salt.

PRESERVATION

As mentioned before, contamination of fresh whey cheeses occurs mainly after curd formation; hence, packaging seems necessary and useful to constrain microbial contamination. However, little attention has been paid to this straightforward fact, so studies encompassing packaging of whey cheeses and consumer safety issues are scarce.

Kosikowski and Brown (1970) claimed that packaging in preformed, rigid plastic containers followed by gaseous evacuation and flooding with CO_2 or N_2 could control multiplication of spoilage microorganisms effectively, while still retaining the fresh flavor in Ricotta for ca. 1.5 months at 5 °C. Optimization of the packaging characteristics, in terms of overhead modified atmosphere packaging composition, was studied for Requeijão (Pintado and Malcata, 2000a,b); packaging under plain CO_2 followed by refrigeration at 4 °C is the most effective set of processing conditions toward extending the shelf life of this cheese from 2 up to 15 days;

conversely, use of plain N_2 (even at subroom temperatures) can inhibit microbial growth of those groups only to a much more limited extent. No significant lipolysis takes place in Requeijão cheeses at 4 °C, irrespective of overhead atmosphere composition; in terms of overall visual appearance, all packaged cheeses were preferred to their unpackaged counterpart, but only whey cheeses stored at 4 °C exhibited significant differences relative to those stored at higher temperatures in terms of acidic smell.

The effect of vacuum packaging of Requeijão at 4 °C indicates that this type of packaging inhibits growth of yeasts and staphylococci, which may be explained by the fact that these microorganisms are aerobic. Surprisingly (because this bacterium is anaerobic), the growth of spore-forming clostridia in this whey cheese packaged under vacuum was also inhibited. This fact can probably be explained by the intolerance to acid of this bacterium; whey cheeses packaged under vacuum underwent relevant acidification, probably derived from lactose metabolism by lactic acid bacteria, which are also anaerobic microorganisms. Additionally, unpackaged Requeijão displayed significant loss of moisture and concomitant increase in rigidity, and developed high levels of free fatty acids by several days of refrigerated storage, whereas vacuum inhibited lipolysis despite the high microbial counts observed; this is obviously an advantage toward good sensory quality because excessively rancid and soapy flavors would be otherwise achieved (Pintado and Malcata, 2000c).

DOWNSTREAM APPLICATIONS

About one-half of the total whey produced worldwide is disposed of as waste effluent, dumped onto the land or into water systems (Zall, 1984), thus resulting in loss of an important source of food energy, as well as creating a major economic burden; fortunately, the other half is processed into various forms of foods and animal feed.

The market of whey cheeses is rather limited, owing to still incipient marketing efforts, coupled with the fact that fresh whey cheeses possess a short shelf life. However, if new and alternative uses of whey cheeses were devised and duly investigated, whey cheese production would increase considerably.

In Italy, Ricotta cheese has been used to prepare a typical Piedmontese cheese product (Brus) via grating (or cutting up) of this cheese onto pasta (Delforno, 1981). Ricotta has been also used to prepare cheese spreads, processed cheese foods, chip dips, cheesecakes, quiches and sour cream (Modler and Jones, 1987; Modler and Emmons, 1989a,b). The main use of Ricotta in the USA, is in production of desserts and pasta-type dishes, e.g., lasagna, ravioli and manicotti (Mathur and Shahani, 1981). Snack dip base formulations (e.g., blue cheese, chili and taco), containing 65–82% (w/w) Ricotta, were also successfully prepared (True and Patel, 1973). In India, Ricotta can replace between 25% and 50% of khoa solids (a heat-concentrated milk product) in gulabjaman (Indian sweet) manufacture, and between 70% and 100% in chhana solids (an acid-coagulated milk) in manufacture of sandesh (Indian sweet). Sandesh possessing an acceptable quality could be prepared from 70% (w/w) Ricotta and 30% (w/w) chhana, whereas at least 50% (w/w) khoa was necessary to produce satisfactory gulabjman (Prajapati and Mathur, 1981). Cheese spreads from fat-cultured cream, blended with Ricotta as protein base and further standardized with cultured buttermilk were successfully prepared (Kalab and Modler, 1985; Modler et al., 1985), and were actually saltier and creamier than their commercial counterparts. Scanning and transmission electron microscopy of this Ricotta cheese spread showed that individual, minute particles of coagulated whey proteins (99 ± 46 nm in diameter) were interconnected via a thin bridging material, whereas casein contributed by milk appeared in the form of small clusters. The observation of ultrastructure of spread cheeses prepared with whey protein precipitates also indicated that fat globules were dispersed throughout the matrix, but did not apparently interact with the protein network (Hill and Smith, 1992). Finally, Kalab et al. (1991) claimed that the use of Ricotta as an ingredient in processed cheese blends was economically advantageous. The primary limitation of use of Ricotta in processed cheeses derives from its meltability: Ricotta-based processed cheese will not flow upon heating, so its addition should be limited to no more than ca. 30% (w/w).

In Portugal, Requeijão is often incorporated into dessert formulations, viz., puddings and cakes (Santiago, 1993), but no other applications are known. In Greece, Myzithra is the only type of Greek whey cheese that is used for the preparation of certain foods and cheese pies; dry Myzithra is, in addition, used in food seasoning after grating (Kandarakis, 1986).

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