Bioactive components of ovine and caprine cheese whey

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Abstract

Cheese whey, also known as sweet whey, is a by-product of cheese-making that contains many valuable constituents. Among them, whey proteins stand out for their high nutritional value in terms of biological value and composition in essential amino acids. In recent years, the increasing demand for caprine and ovine cheeses has produced important amounts of whey from these species, boosting research on the biological activities of its constituent proteins. Different bioactivities have been associated to these proteins among them antihypertensive, antimicrobial, opioid, antioxidant and immunomodulant activity being the most studied. Although biological activities are present in the intact proteins, in many cases whey proteins act as precursors of bioactive peptides that are released from the hydrolysis of these proteins with different enzymes. This review presents an overview of the different biological activities described for caprine and ovine cheese whey proteins as well as for other whey components such as lactose, oligosaccharides or minerals.

Keywords: Cheese whey**,** sheep and goat whey proteins, bioactivity, bioactive peptides, health

1. Introduction

Goat and sheep milk have traditionally been a vital part of the cultural heritage and national economy of Mediterranean countries, particularly France, Italy, Spain and Greece. Rearing goats and sheep is gaining importance worldwide, and the production of derived dairy products have experienced an important increase, making them of particular economic value in these countries (Pandya and Ghodke, 2007; Raynal-Ljutovac et al., 2008). Excellent cheeses are made with goat and sheep milk. Some of the best known are Roquefort, Peccorino Romano, Feta, Manchego, Bryndza and the French goat milk cheeses. Specific texture and taste of sheep and goat milk, as well as their nutritional and functional properties, have made them a valuable and healthier alternative to cow milk. Even, many authors have called them "functional foods" because of their modulating activity of physiological functions and their reducing properties of chronic diseases risk (Correia and Cruz, 2006).

The average protein content in sheep milk (5.8%, w/w) is higher than in goat (4.6%, w/w) or cow milk (3.3%, w/w). Whey is the soluble fraction of milk, rich in proteins, minerals and lactose that are separated from casein during the manufacture of cheese or casein. Rennet-driven coagulation releases the sweet whey or cheese whey, while that obtained from the mineral or lactic acid coagulated casein is called acid whey. Whey from ovine and caprine species have a unique protein composition that depends on factors, such as the type of whey (acid or sweet), the time of the year, the type of feed, the stage of lactation and the quality of processing. Distribution of individual proteins in sheep and goat milk whey and its comparison with cow milk is shown in Table 1. The major whey proteins are β-lactoglobulin (β-Lg) and α-lactalbumin (α-La). Immunoglobulins (Igs), serum albumin and proteose–peptones are present in smaller concentrations. Another soluble protein found in small amounts is lactoferrin (LF) and, in the case of rennet whey, caseino-macropeptide (CMP) is also present. Other components including lactose, oligosaccharides or minerals are also important in the whey.

Traditionally, whey has been viewed as a by-product of dairy industry with little value for the consumers. However, in last decades, there is a growing interest focused on the nutritional properties of whey proteins and whey-derived products and its role upon human health. Milk whey proteins exhibit a range of biological activities that influence digestion, metabolic responses to absorbed nutrients, growth and development of specific organs, and resistance to disease. Moreover, hydrolysis of these proteins can release fragments, termed bioactive peptides, capable to exert specific biological activities, such as antihypertensive, antimicrobial, opioid, antioxidant, immunomodulant, or mineral binding (Yalcin, 2006; Hernández-Ledesma et al., 2008; Madureira et al., 2010). Unlike casein-derived bioactive peptides that can be released by either technological process during food production (e.g. cheese) or by enzymatic hydrolysis, whey protein-derived bioactive peptides are mainly produced by the second step using different enzymes or by chemical synthesis (Morris and Fitzgerald, 2008). Compared to bovine proteins, only few reports have been focused on peptides released from ovine and caprine milk whey proteins. Since primary structures of these proteins show a great homology with those observed for bovine proteins (see primary structure of β-Lg as an example, Figure 1), it is predictable that ovine and caprine whey proteins could also exert as source of numerous bioactive peptides. This review will be focused on the most recent research advances pertaining to the biological properties of ovine and caprine whey proteins and derived-peptides. Moreover, bioactivity of other components of cheese whey, such as lactose, oligosaccharides and minerals will be also included in this review.

2. Whey proteins

Nowadays, whey proteins are used in a variety of products appealing to nutrition-conscious consumers and for athletes. They can be consumed as ready-to-drink and powdered beverages, sport meals, nutrition bars, high-protein cookies and in tablet form. One of the reasons to explain the high consumption of this kind of products is that whey protein has been reported to support net muscle mass gains with resistance exercise (Phillips et al., 2009). In addition, whey protein seems to have potential as a functional food component to contribute to the regulation of body weight by providing satiety signals that affect both short-term and long-term food intake regulation (Luhovyy et al., 2007). Other physiological activities of the individual whey protein, some of them shared with peptides derived from their hydrolysis, are described in this review.

2.1. β-lactoglobulin

β-Lg is the major whey protein found in cow, sheep, goat and other ruminants' milk. Concentration of ovine and caprine β-Lg varies from 2.7 to 5.0 g/L and from 1.8 to 2.8 g/L, respectively, whereas concentration of this protein from bovine origin varied varies between 2.3 g/L and 4.9 g/L (Table 1). β-Lg is a small, soluble and globular protein, containing 162 amino acids in a single peptide chain with a molecular weight of 18.3 kDa. The primary sequence reveals two intra-chain disulphide bridges (Cys_{66}-Cys_{160} and Cys_{106}-Cys-$_{119}$) and a free thiol group at Cys_{121} (Creamer et al., 1983). Reported activities for β-Lg are summarized in Table 2. The homology of β-Lg tertiary structure with the plasma retinol-binding protein as well as its high stability to proteolytic action of digestive enzymes has suggested the role of this protein as a resistant carrier of retinol (a provitamin A). Moreover, fatty acid binding sites characterised on β-Lg allow this protein to participate in the digestion of milk lipids during the neonatal period. In addition, it has been shown that β-Lg enhances intestinal uptake of retinol, triglycerides, and long-chain fatty acids in pre-ruminant calves (Kushibiki et al., 2001), and it has been speculated that this protein may play a role in the absorption and subsequent metabolism of fatty acids. Other possible functions have been described for this whey protein, such as its role in developing passive immunity with IgG (Sutton and Alston-Mills, 2006). Wong and co-workers (1998) demonstrated that this whey protein stimulates normal murine spleen cells proliferation and Igs production. Moreover, β-Lg is a rich source of Cys, an essential amino acid that appears to stimulate glutathione synthesis, an anticarcinogenic tripeptide produced by the liver for protection against intestinal tumors (Mcintosh et al., 1995). The high nutritional and functional value of β-Lg is widely recognized and has made this protein an ingredient of choice in the formulation of modern foods and beverages (Chatterton et al., 2006).

2.2. α-lactalbumin

α-La is a metalloprotein that contains one atom of Ca per molecule and is physiologically important because of its requirements in lactose synthesis. While the concentration of bovine α-La varies between 0.8 g/L and 1.2 g/L, in goat milk is present at concentrations around 0.6-1.1 g/L and in ovine milk ranges between 1.2 g/L and 2.6 g/L (Table 1). α-La is a small and globular protein of approximately 14 kDa that consists of a single polypeptide chain with eight cysteine residues which form four disulphide bridges (Cys_{25}-Cys_{139}, Cys_{47}-Cys-$_{130}$, Cys_{80}-Cys_{96} and Cys_{92}-Cys_{110}). Both sheep and goat α-La possesses high homology with its bovine counterpart.

Some important bioactivities have been reported for α-La (Table 2). The best known is the anti-tumoral activity observed for the complex between human α-La and oleic acid called HAMLET (human alpha-lactalbumin made lethal to tumor cells). This complex is able to kill tumour cells by a process resembling programmed cell death. HAMLET has broad antitumor activity *in vitro*, and its therapeutic effect has been confirmed *in vivo* in a human glioblastoma rat xenograft model, in patients with skin papillomas and in patients with bladder cancer (Hallgren et al., 2008). Likewise, a complex of bovine α-La and oleic acid (BAMLET) killed tumor cells via a mechanism involving lysosomal membrane permeabilization, showing potent cytotoxic activity against eight different cancer cell lines (Rammer et al., 2010)

2.3. Serum albumin

Serum albumin is a 582 amino acids-protein that appears in milk after its passive leakage from blood. It possesses 17 intermolecular disulphide bridges and one free thiol group. Because of its size and structure, serum albumin binds free fatty acids, participating in synthesis of lipids (Choi et al., 2002). Moreover, its antioxidant activity has been reported to be effective on protecting lipids against phenolic induced oxidation (Smith et al., 1992). Laursen and co-workers (1990) revealed serum albumin's inhibitory activity of breast cancer cells proliferation through modulation of autocrine growth regulatory factors (Table 2).

2.4. Lactoferrin

LF, also known as lactotransferrin, is a globular multifunctional protein that binds, transports and supplies the organism with iron. The iron binding properties seem to vary between LF from different species (Sreedhara et al., 2010). The levels of this protein in sheep and goat milk are slightly higher than in cow milk, with values of approximately 0.1 mg/mL and 0.125 mg/mL, respectively (Table 1) (El-Agamy and Nawar, 2000). Traditionally it has been recognized to possess antimicrobial and antifungal properties (Orsi, 2004; Olakanimi et al., 2002; Table 2). LF exerts its antimicrobial activity by two different mechanisms. A bacteriostatic effect through the binding of iron ions necessary for cell growth, and a bactericidal effect similar for Gram-negative and Gram-positive bacteria involving direct interaction with the bacterial cell membrane (Ling and Schryvers, 2006).

Recent research has revealed that bovine LF induced apoptosis of human stomach cancer cells (Xu et al., 2010). LF also displays antiviral activity against both DNA- and RNA-viruses preventing their entry in the host cell either by blocking cellular receptors or by direct binding to the virus particles (Van der Strate et al., 2001, Seganti et al., 2004). Some *in vivo* studies have shown that LF exerts a protective effect against colitis in rats via modulation of the immune system and correction of cytokine imbalance (Togawa et al., 2002). Finally, purified ovine LF and its pepsin hydrolyzate inhibit thrombin-induced platelet aggregation (Quian et al., 1995a).

2.5. Caseinomacropeptide

CMP is composed by the 64 C-terminal amino acids of κ-casein, and it is released by chymosin (or pepsin) cleavage of κ-casein during cheese manufacturing. From a nutritional point of view, the CMP possesses limitations due to the lack of several essential amino acids (arginine, cysteine, histidine, tryptophan and tyrosine). However, its unique amino acid composition makes it adequate for special diets, for instance in phenylketonuria patients' diets since no phenylalanine is present. Additionally, CMP is rich in branched-chain amino acids (valine and isoleucine) and low in methionine, which makes it a useful ingredient in diets for patients suffering from hepatic diseases (Abd El-Salam et al., 1996). The biological properties of the CMP have received much attention in recent years (see review Thomä-Worringer et al., 2006). CMP is able to interact with toxins, viruses and bacteria due to its carbohydrate fraction, preventing the binding of different pathogens to cells or the adhesion of bacteria implicated in cariogenic processes (Manso and López-Fandiño, 2004). A recent study has demonstrated the CMP's protecting effect against acidic erosion of teeth (Setarehnejad et al., 2010). CMP also exerts inmunomodulating and prebiotic activity (Mikkelsen et al., 2006).

2.6. Other proteins

Ovine and caprine milk whey contain minor proteins, including Igs, transferrin, ferritin, proteose peptone, lactoperoxidase, lysozyme, calmodulin (calcium binding protein), prolactin and folate-binding protein (Park et al., 2007). Igs constitute a complex group of globular proteins produced by B-lymphocytes, consisting of two light polypeptide chains (with a molecular weight of 25,000 kDa) and two heavy chains (with molecular weight in the range from 50,000 to 70,000 kDa). They protect the gut mucosa against pathogenic microorganisms, also providing protection against diseases in the ruminant neonate until its own immune system is developed. Recently, it has been demonstrated that oral administration of ovine serum Ig modulates aspects of immunity, such as phagocytosis, lymphocyte proliferation, cytokine production, intestinal and plasma Ig concentrations in growing rats (Balan et al., 2010). These authors had previously reported the effect of this ovine Ig fraction improving growth performance, organ weight and gut morphology in growing rats (Balan et al., 2009).

Lactoperoxidase is one of the most abundant enzymes in plain milk, representing 1% (w/w) of the total protein pool in whey. It is an important part of the natural host defence system in mammals, which provides protection against invading microorganisms and virus (de Wit & van Hooydonk, 1996; Shin et al., 2005; Table 2). This enzyme, combined with lactoferrin in a cell culture, has also been reported to have suppressive effects on mitogen-activated lymphocyte proliferation through inhibition of IFN-γ production (Wong et al., 1997; Mercier et al., 2004). Recent studies have demonstrated that lactoperoxidase acts regulating expression of genes involved in metabolism, immunity, apoptosis, and cell cycle of epithelial intestinal cells (Wakabayashi et al., 2007).

Protease-peptone is defined as a heat-stable and acid-soluble fraction of milk whey. Lactophorin is a minor phosphoglycoprotein corresponding to component-3 of protease peptone found in bovine, ovine and caprine milk (Lister et al., 1998). It has been demonstrated to have immunomodulatory properties, being effective on IgM production of hybridoma cells (Sugahara et al., 2005). The C-terminal region f(113-135) of lactophorin, called lactophoricin, has been reported to display growth inhibitory activity against both Gram-positive and Gram-negative bacteria (Campagna et al., 2004).

3. Bioactive peptides derived from whey proteins

3.1. Antihypertensive peptides

During last years, food scientists and technologists have focused their studies on bioactivities associated with whey protein-derived peptides. Among the bioactive peptides known so far, those with angiotensin converting enzyme (ACE)-inhibitory properties have received special attention due their potential beneficial effects in the treatment of hypertension. Most published reports on ACE-inhibitory and/or antihypertensive peptides are associated with peptides derived from bovine milk. However, in recent years, sheep and goat milk whey proteins have become an important source of ACE-inhibitory peptides (Table 3). In a study where a caprine β-Lg hydrolyzate was prepared using thermolysin as proteolytic enzyme, two potent ACE-inhibitory peptides, which sequences were LLF and LQKW, were identified (Hernández-Ledesma et al., 2002). Subsequently, the antihypertensive effect of these two peptides in spontaneously hypertensive rats (SHR) has been reported (Hernández-Ledesma et al., 2007). Chobert and coworkers (2005) investigated the ACE-inhibitory activity of ovine β-Lg hydrolysed with trypsin, and of yoghurts made from ovine milk using different starters. These authors identified in this hydrolyzate several peptides responsible for this activity (Table 3).

Although less explored than β-Lg, several ACE-inhibitory peptides from α-La have been identified by different authors. Mullaly et al. (1996) synthesized three peptides from α-La sequence, YG, YGLF and LF, and studied their ACE-inhibitory activity. Despite none of these peptides showed very strong inhibitory activity, the cardiovascular effects of the tetrapeptide YGLF, known as α-lactorphin, was later tested in SHR and in normotensive Wistar Kyoto rats (WKY). α-Lactorphin, a peptide also produced by enzymatic hydrolysis with pepsin and trypsin, dose-dependently lowered blood pressure without affecting heart rate in SHR and WKY with evidences for an involvement of opioid receptors in its depressor action (Nurminen et al., 2000). Further studies to shed light on the antihypertensive mechanism of this tetra-peptide showed that its beneficial effect was directed towards endothelial function, improving vascular relaxation in adult SHR *in vitro* (Sipola et al., 2002). This peptide, α-lactorphin, has been obtained after protein hydrolysis of caprine α-La (Bordenave, 2000).

Didelot et al., (2006) used cheese microflora to produce several hydrolyzates of acid caprine whey with ACE-inhibitory activity. The highest activity was obtained after whey fermentation by the microflora from 18-months ripened Comté cheese that was further characterized as a co-culture of *Candida parapsilosis* and *Lactobacillus paracasei.* Fractionation by RP-HPLC and amino acid analysis allowed the identification of the α-La fragment f(104-108) in the most active fraction (Table 3). A more recent study also used microorganisms isolated from raw milk cheeses to produce several hydrolyzates of acid caprine whey with ACE-inhibitory activity, although no peptides were identified (Hamme et al., 2009).

ACE-inhibitory peptides have also been identified in hydrolyzates derived from ovine and caprine CMP. Manso and López-Fandiño (2003), found that undigested bovine, caprine and ovine CMP exhibited moderate ACE-inhibitory activity, but it increased considerably after digestion under simulated gastrointestinal conditions. ACE-inhibitory peptides MAIPPK and MAIPPKK, corresponding to κ-CN f(106-111) and f(106-112) respectively, were identified from CMPs via proteolysis with trypsin. These peptides showed moderate activity, but their digestion under simulated gastrointestinal conditions allowed the release of the potent antihypertensive tri-peptide IPP (IC_{50} value of 5 μM).

3.2. Antimicrobial and immunomodulatory peptides

Both *in vitro* and *in vivo* studies have demonstrated that certain whey-protein-based ingredients and individual whey proteins, such as β-Lg, can act as precursors of peptides with antimicrobial activity, enhancing the organism's natural defences against invading pathogens. (Rutherfurd-Markwick and Gill, 2005; Gauthier et al., 2006; Saint-Sauveur et al., 2008, 2009). Recently, the antimicrobial and immunostimulatory activities of β-Lg, among other food proteins, hydrolysed with four gastrointestinal proteinases (trypsin, chymotrypsin, pepsin and pancreatin) have been examined by Biziulevicius and co-workers (2006). Proteolytic digestion of bovine β-Lg by trypsin allowed the release of four peptide fragments [f(15–20), f(25–40), f(78–83), f(92–100)] with bactericidal activity against Gram-positive bacteria (Pellegrini et al., 2001). The immunomodulating potential of peptide fractions isolated from β-Lg enzymatic hydrolyzates has also been demonstrated (Prioult et al., 2004). Recently, peptides corresponding to β-Lg fragments f(15-20), f(55-60), f(84-91), f(92-105), f(139-148), and f(142-148) have been reported to stimulate murine splenocytes proliferation through the modulation of cytokine secretion (Jacquot et al., 2010). However, few data are reported about the release of antibacterial and/or immunostimulating peptides from ovine and caprine β-Lg. Only a peptic hydrolyzate of ovine α-La and β-Lg has been demonstrated by El-Zahar and co-workers (2004) to inhibit the growth of *Escherichia coli* HB101, *Bacillus subtilis* Cip5262 and *Staphylococcus aureus* 9973 in a dose dependent manner, but responsible peptides were not identified.

Additionally, peptides released from LF by enzymatic procedures have been reported with more potent activity than the precursor protein (Tomita et al., 1991). The antibacterial domains of bovine LF f(17-41) and human LF f(1-47), called respectively bovine and human lactoferricin (LFcin), have been purified and identified (Bellamy et al., 1992). These peptides showed a potent antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria (Wakabayashi et al., 2003). Hydrolysis of caprine and ovine LF by pepsin resulted in antibacterial hydrolyzates, and a homologous peptide to LFcin, corresponding to fragment f(14-42), was identified in the caprine LF hydrolysate (Table 3). The region corresponding to the LFcin within the sequence of ovine LF was hydrolysed by the action of pepsin, and hence, the activity observed in the ovine LF hydrolysate could be caused by other LF fragments (Recio and Visser, 2000).

3.3. Opioid peptides

Opioid peptides are defined as peptides which have an affinity for an opioid receptor, exerting opiate-like effects inhibited by naloxone. β-Lg has been found to be precursor of several opioid peptides (Pihlanto-Leppala, 2001). Digestion of bovine β-Lg with pepsin and trypsin, or trypsin and chymotrypsin yielded YLLF, corresponding to fragment f(102–105) and called β-lactorphin. A contracting effect of this peptide on smooth muscle has been determined in coaxially stimulated guinea pig ileum *in vitro* (Antila et al., 1991). β-lactorphin also improved the impaired vascular function in mesenteric rings of adults SHR (Sipola et al., 2002). This beneficial action was directed towards endothelial function and also enhanced endothelium-independent relaxation. β-lactotensin (HIRL) is a peptide isolated by Yoshikawa and co-workers from a chymotrypsin digest of β-Lg (Yoshikawa et al., 1991). It has a variety of actions including antinociceptive, cholesterol-lowering, anti-stress and memory-enhancing activities (Yamauchi et al., 2003a, b, 2006; Ohinata et al., 2007). Recently, it has been found that this peptide also suppresses food intake in mice after its oral administration (Hou et al., 2009).

3.4. Other biological activities of whey protein-derived peptides

It is assumed that “oxidative stress” is implicated in the aetiology of age-associated chronic diseases, such as cardiovascular diseases, diabetes, cataracts, neurodegenerative disorders, certain types of cancer and aging (Ames et al., 1993). In last few years, the searching for whey-derived peptides with radical scavenging and lipid peroxidation inhibitory activities is receiving a special attention. Hernández-Ledesma and co-workers (2005) investigated the antioxidant activity of hydrolyzates of β-Lg by commercial proteases (pepsin, trypsin, chymotrypsin, thermolysin and corolase PP). These authors found that Corolase PP was the most appropriate enzyme to produce β-Lg hydrolyzates having high oxygen radical scavenging activity. Several peptides were identified in the 3 kDa-permeate from these hydrolyzates. The radical scavenging activity of one of these peptides, with the sequence WYSLAMAASDI, was slightly higher (2.62 μmol Trolox equivs/μmol peptide) than that shown by the synthetic antioxidant butylhydroxyanisole (BHA) (2.43 μmol Trolox equivs/μmol BHA).

Whey proteins have been reported to exhibit a greater hypocholesterolemic effect in comparison with casein or soybean proteins in rats (Nagaoka et al., 1991, 1992). A posterior study of these authors provided the first evidence that β-Lg tryptic hydrolyzate had hypocholesterolemic activity in rats (Nagaoka et al., 2001). By using Caco-2 cell screening, these authors identified four kinds of novel peptide sequences which inhibited cholesterol absorption *in vitro*, i.e., IIAEK, GLDIQK, ALPMH, and VYVEELKPTPEGDLEILLQK, which corresponded, respectively, to fragments f(71–75), f(9–14), f(142–146), and f(41–60) of bovine β-Lg. These peptides are also present in caprine and ovine β-Lg, except for the longest sequence where aspartic acid (D) is replaced by asparagine (N) in the caprine and ovine sequences.

Qian et al. (1995b) hydrolysed ovine CMP with trypsin and identified three peptides with the sequences KDQDK f(112-116), TAQVTSTEV f(163-171) and QVTSTEV f(165-171) that completely inhibited thrombin-induced human platelet aggregation. Furthermore, bovine, ovine and caprine CMPs and their hydrolyzates with trypsin were found to be inhibitors of human platelet aggregation (Manso et al., 2002). In this work, the hydrolyzate obtained from ovine CMP showed the strongest effect, but the peptides responsible of this activity were not identified.

4. Other bioactive components in cheese whey

4.1. Lactose and oligosaccharides

Lactose is the major carbohydrate in milk with a content of 4.1 g/100 mL in goat milk and 4.9 g/100 mL in sheep milk (Park et al., 2007). This disaccharide is a valuable nutrient because favours the intestinal absorption of calcium, magnesium and phosphorous, and the utilization of vitamin C. On the other hand, milk oligosaccharides possess prebiotic and anti-infective properties. The amount of oligosaccharides in ovine milk is in the range of 20 mg/mL to 30 mg/mL while in caprine milk is in the range of 250 mg/mL-300 mg/mL. Many of these oligosaccharides contain sialic acid, a general name for N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). Sialic acid present in milk seems to promote the development of the infant's brain among other positive effects (Park, 2009).

4.2. Minerals

Many major and trace minerals play an important role in the physiology and metabolism of the human body. Sheep has around 0.9 % total minerals or ash, compared to 0.7 % in cow milk. The most abundant elements are Ca, P, K, Na, and Mg; Zn, Fe, Cu, and Mn are the trace elements. The levels of Ca, P, Mg, Zn, Fe, and Cu are higher in sheep than in cow milk; the opposite appears to be the case of K and Na. Goat milk contains about 134 mg of Ca and 121 mg of P/100g. Overall, goat milk has more Ca, P, K, Mg and Cl, and less Na and S contents than cow milk (Park et al., 2007).

Na, K, and Cl are almost entirely soluble and fully available in the whey. Ca, Mg, and P are associated in different proportions to the colloidal suspension of casein micelles and, therefore, are partly retained in the curd during cheese-making.

4.3. Vitamins

Vitamins are physiological, biochemical, and metabolical bioactive compounds occurring in milk. Most of the fat-soluble vitamins are incorporated to the curd during cheese-making while water-soluble vitamins go mainly to the whey. In the literature is described than sheep milk is richer than cow milk for most of the vitamins (Park et al., 2007). For instance, goat and sheep milk have higher amount of vitamin A than cow milk. However, goat milk has a significant drawback in folic acid and vitamin B_{12} compared to cow milk (Park, 2009).

Final considerations

This review shows the remarkable potential of goat and sheep cheese whey as source and precursor of bioactive compounds. The traditional view of cheese whey as a by-product of dairy industry with little value has disappeared, and now the whey is seen as a potential source of bioactive components that can be used in the formulation of multiple functional foods. Although less explored than bovine whey, the ovine and caprine counterparts are gaining importance due to the worldwide increase in the production of dairy products based on the milk of these animals. Among the different components of cheese whey, proteins are especially important in terms of biological activity. The advances in processing technology, including ultrafiltration, microfiltration, reverse osmosis or ion exchange among others, have resulted in the presence of multitude of whey products in the market. Many of these products, whey powders, reduced lactose whey, whey protein concentrates' etc.., proclaim their properties to growth or maintenance muscle mass, among other effects. However, as happens with other claimed activities for whey proteins including their effect on satiation and satiety, human studies documenting these benefits are limited. Recently, the European Food Safety

Authority (EFSA) delivered a scientific opinion on different health claims related to whey protein under Regulation (EC) No 1924/2006. Health claims were rejected based on the absence of human studies or because the existing ones were inappropriately done (European Food Safety Authority, 2010). Apart from the biological activities of the intact proteins, the recent interest has mainly focused on bioactive peptides encrypted within the whey proteins that are released by different means, such as enzymatic hydrolysis and/or fermentation processes. Among the plethora of bioactivities described for these peptides, stand out some activities like antihypertensive, opioid, antioxidant, antimicrobial and hypocholesterolemic. Unlike intact whey proteins more research on bioavailability, toxicity as well as animal studies have been carried out for bioactive peptides. However, additional studies in both whey protein-based products and those containing whey bioactive peptides are required in order to gain the official acceptance from EFSA. These studies should be especially focused on well-designed clinical trials to confirm the potential health effects of these products.

Conflict of interest statement

None of the authors (Blanca Hernández-Ledesma, Mercedes Ramos and José Ángel Gómez-Ruiz) has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled “Bioactive components of ovine and caprine cheese whey”.

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Bovine -Lg B

LIVTQTMKGL$_{10}$DIQKVAGTWY$_{20}$SLAMAASDIS$_{30}$LLDAQSAPLR$_{40}$VYVEELKPTP$_{50}$EGDLEILLQK$_{60}$WENGECAQKK$_{70}$IIAEKTKIPA$_{80}$VFKIDALNEN$_{90}$KVLVLDTDYK$_{100}$KYLLFCMENS$_{110}$AEPEQSLACQ$_{120}$CLVRTPEVDD$_{130}$EALEKFDKAL$_{140}$KALPMHIRLS$_{150}$FNPTQLEEQC$_{160}$HI

Ovine -Lg B

IIVTQTMKGL$_{10}$DIQKVAGTWH$_{20}$SLAMAASDIS$_{30}$LLDAQSAPLR$_{40}$VYVEELKPTP$_{50}$EGNLEILLQK$_{60}$WENGECAQKK$_{70}$IIAEKTKIPA$_{80}$VFKIDALNEN$_{90}$KVLVLDTDYK$_{100}$KYLLFCMENS$_{110}$AEPEQSLACQ$_{120}$CLVRTPEVDN$_{130}$EALEKFDKAL$_{140}$KALPMHIRLA$_{150}$FNPTQLEGQC$_{160}$HV

Caprine -Lg

IIVTQTMKGL$_{10}$DIQKVAGTWY$_{20}$SLAMAASDIS$_{30}$LLDAQSAPLR$_{40}$VYVEELKPTP$_{50}$EGNLEILLQK$_{60}$WENGECAQKK$_{70}$IIAEKTKIPA$_{80}$VFKIDALNEN$_{90}$KVLVLDTDYK$_{100}$KYLLFCMENS$_{110}$AEPEQSLACQ$_{120}$CLVRTPEVDK$_{130}$EALEKFDKAL$_{140}$KALPMHIRLA$_{150}$FNPTQLEGQC$_{160}$HV

Figure legends

Figure 1. Primary structure of β-Lg from cow, sheep and goat origin. Marked letters indicate differences between sequences.

Table 1. Concentration of the main whey proteins in cow, sheep and goat milk (Storry et al., 1983; Hahn et al., 1998; Fox et al., 2000).

	Concentration (g/L)		
	Bovine	Ovine	Caprine
Total whey proteins	5.0-9.0	8.8-10.4	3.7-7.0
β-lactoglobulin	3.2-4.0	2.7-5.0	1.8-2.8
α-lactalbumin	1.2-1.5	1.2-2.6	0.6-1.1
Serum albumin	0.3-0.6	0.55-0.6	0.26-0.3
Lactoferrin	0.05-0.2	0.10	0.12

Table 2. Biological functions of whey proteins

Protein	Biological function	Reference
β-lactoglobulin	Carrier of retinol, fatty acids and triglycerides	Pérez et al., 1992
	Transfer of passive immunity	Sutton and Alston-Mills, 2006
	Immunomodulatory activity	Wong et al., 1998
	Anti-cancerigen activity	Mcintosh et al., 1995
α-lactalbumin	Lactose synthesis	Markus et al., 2002
	Treatment of chronic stress-induced diseases	Ganjam et al., 1997
	Anti-cancerigen activity	Hallgren et al., 2008

Serum albumin	Synthesis of lipids	Choi et al., 2002
	Antioxidant activity	Smith et al., 1992
	Anti-cancerigen activity	Laursen et al., 1990
Lactoferrin	Antimicrobial activity	Orsi, 2004
	Antifungal activity	Olakanimi et al., 2002
	Anti-proliferative activity	Xu et al., 2010
	Antiviral activity	Van der Strate et al., 2001; Seganti et al., 2004
	Immunomodulatory activity	Togawa et al., 2002
	Anti-thrombotic activity	Quian et al., 1995a
Caseinomacropeptide	Anti-cariogenic activity	Oh et al., 2000 ; Kawasaki et al., 1992; 1993
	Immunomodulatory activity	Otani et al., 1995a, b; 1996
	Prebiotic activity	Azuma et al., 1984 ; Idota et al., 1994
	Anti-thrombotic activity	Manso et al., 2002
Immunoglobulins	Immunomodulatory activity	Balan et al., 2010
	Growth and development	Balan et al., 2009
Lactoperoxidase	Antimicrobial activity	deWit & van Hooydonk, 1996
	Antiviral activity	Shin et al., 2005
	Immunomodulatory activity	Wakabayashi et al., 2007

Table 3. Sequence of bioactive peptides derived from ovine and caprine milk whey proteins

Peptide fragment	Sequence	Biological activity	Produced by	References
β-Lg f(58-61)	LQKW	ACE-inhibitory (3.5 μM) Antihypertensive	Hydrolysis with thermolysin	Hernández-Ledesma et al. (2002) Hernández-Ledesma et al. (2007)
β-Lg f(103-105)	LLF	ACE-inhibitory (82.4 μM) Antihypertensive	Hydrolysis with thermolysin	Hernández-Ledesma et al. (2002) Hernández-Ledesma et al. (2007)
β-Lg f(142-148)	ALPMHIR	ACE-inhibitory	Tryptic hydrolysis	Chobert et al. (2005)
β-Lg f(1-8)	IIVTQTMK	ACE-inhibitory	Tryptic hydrolysis	Chobert et al. (2005)
CMP f(112-116)	KDQDK	Antithrombotic	Tryptic hydrolysis	Qian et al. (1995b)

CMP f(163-171)	TAQVTSTEV	Antithrombotic	Tryptic hydrolysis	Qian et al. (1995b)
CMP f(165-171)	QVTSTEV	Antithrombotic	Tryptic hydrolysis	Qian et al. (1995b)
CMP f(106-111)	MAIPPK	ACE-inhibitory	Tryptic hydrolysis	Manso et al. (2003)
CMP f(106-112)	MAIPPKK	ACE-inhibitory	Tryptic hydrolysis	Manso et al. (2003)
Lactoferrin f(14-42)	PEWSKCYQW-QRRMRKLGAP-SITCVRRTSA	Antibacterial	Hydrolysis with pepsin	Recio and Visser, (2000)
α-La (104-108)	WLAHK	ACE-inhibitory	Cheese microflora	Didelot et al., (2006)
α-La (104-108)	YGLF	ACE-inhibitory (733 μM)[a] Antihypertensive [d]	Hydrolysis with pepsin	Bordenave, 2000

[o] Ovine milk proteins,

[g] Goat milk proteins,

[a] Studies carried out in the bovine peptide