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4 **Bioactive components of ovine and caprine cheese whey**
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32 **Abstract**

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

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

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57 1. Introduction

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

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

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

101

102 **2. Whey proteins**

103 Nowadays, whey proteins are used in a variety of products appealing to nutrition-
104 conscious consumers and for athletes. They can be consumed as ready-to-drink and powdered
105 beverages, sport meals, nutrition bars, high-protein cookies and in tablet form. One of the reasons to
106 explain the high consumption of this kind of products is that whey protein has been reported to
107 support net muscle mass gains with resistance exercise (Phillips et al., 2009). In addition, whey

108 protein seems to have potential as a functional food component to contribute to the regulation of
109 body weight by providing satiety signals that affect both short-term and long-term food intake
110 regulation (Luhovyy et al., 2007). Other physiological activities of the individual whey protein,
111 some of them shared with peptides derived from their hydrolysis, are described in this review.

112

113 **2.1. β -lactoglobulin**

114 β -Lg is the major whey protein found in cow, sheep, goat and other ruminants' milk.
115 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,
116 respectively, whereas concentration of this protein from bovine origin varied varies between 2.3 g/L
117 and 4.9 g/L (Table 1). β -Lg is a small, soluble and globular protein, containing 162 amino acids in a
118 single peptide chain with a molecular weight of 18.3 kDa. The primary sequence reveals two intra-
119 chain disulphide bridges (Cys₆₆-Cys₁₆₀ and Cys₁₀₆-Cys₁₁₉) and a free thiol group at Cys₁₂₁
120 (Creamer et al., 1983). Reported activities for β -Lg are summarized in Table 2. The homology of β -
121 Lg tertiary structure with the plasma retinol-binding protein as well as its high stability to
122 proteolytic action of digestive enzymes has suggested the role of this protein as a resistant carrier of
123 retinol (a provitamin A). Moreover, fatty acid binding sites characterised on β -Lg allow this protein
124 to participate in the digestion of milk lipids during the neonatal period. In addition, it has been
125 shown that β -Lg enhances intestinal uptake of retinol, triglycerides, and long-chain fatty acids in
126 pre-ruminant calves (Kushibiki et al., 2001), and it has been speculated that this protein may play a
127 role in the absorption and subsequent metabolism of fatty acids. Other possible functions have been
128 described for this whey protein, such as its role in developing passive immunity with IgG (Sutton
129 and Alston-Mills, 2006). Wong and co-workers (1998) demonstrated that this whey protein
130 stimulates normal murine spleen cells proliferation and Igs production. Moreover, β -Lg is a rich
131 source of Cys, an essential amino acid that appears to stimulate glutathione synthesis, an
132 anticarcinogenic tripeptide produced by the liver for protection against intestinal tumors (Mcintosh
133 et al., 1995). The high nutritional and functional value of β -Lg is widely recognized and has made

134 this protein an ingredient of choice in the formulation of modern foods and beverages (Chatterton et
135 al., 2006).

136 **2.2. α -lactalbumin**

137 α -La is a metalloprotein that contains one atom of Ca per molecule and is physiologically
138 important because of its requirements in lactose synthesis. While the concentration of bovine α -La
139 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
140 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
141 approximately 14 kDa that consists of a single polypeptide chain with eight cysteine residues which
142 form four disulphide bridges (Cys₂₅-Cys₁₃₉, Cys₄₇-Cys₁₃₀, Cys₈₀-Cys₉₆ and Cys₉₂-Cys₁₁₀). Both
143 sheep and goat α -La possesses high homology with its bovine counterpart.

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

153

154 **2.3. Serum albumin**

155 Serum albumin is a 582 amino acids-protein that appears in milk after its passive leakage
156 from blood. It possesses 17 intermolecular disulphide bridges and one free thiol group. Because of
157 its size and structure, serum albumin binds free fatty acids, participating in synthesis of lipids (Choi
158 et al., 2002). Moreover, its antioxidant activity has been reported to be effective on protecting lipids
159 against phenolic induced oxidation (Smith et al., 1992). Laursen and co-workers (1990) revealed

160 serum albumin's inhibitory activity of breast cancer cells proliferation through modulation of
161 autocrine growth regulatory factors (Table 2).

162 **2.4. Lactoferrin**

163 LF, also known as lactotransferrin, is a globular multifunctional protein that binds,
164 transports and supplies the organism with iron. The iron binding properties seem to vary between
165 LF from different species (Sreedhara et al., 2010). The levels of this protein in sheep and goat milk
166 are slightly higher than in cow milk, with values of approximately 0.1 mg/mL and 0.125 mg/mL,
167 respectively (Table 1) (El-Agamy and Nawar, 2000). Traditionally it has been recognized to possess
168 antimicrobial and antifungal properties (Orsi, 2004; Olakanimi et al., 2002; Table 2). LF exerts its
169 antimicrobial activity by two different mechanisms. A bacteriostatic effect through the binding of
170 iron ions necessary for cell growth, and a bactericidal effect similar for Gram-negative and Gram-
171 positive bacteria involving direct interaction with the bacterial cell membrane (Ling and Schryvers,
172 2006).

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

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181 **2.5. Caseinomacropeptide**

182 CMP is composed by the 64 C-terminal amino acids of κ -casein, and it is released by
183 chymosin (or pepsin) cleavage of κ -casein during cheese manufacturing. From a nutritional point of
184 view, the CMP possesses limitations due to the lack of several essential amino acids (arginine,
185 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 immunomodulating 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).

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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,

238 the antihypertensive effect of these two peptides in spontaneously hypertensive rats (SHR) has been
239 reported (Hernández-Ledesma et al., 2007). Chobert and coworkers (2005) investigated the ACE-
240 inhibitory activity of ovine β -Lg hydrolysed with trypsin, and of yoghurts made from ovine milk
241 using different starters. These authors identified in this hydrolyzate several peptides responsible for
242 this activity (Table 3).

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

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

263 ACE-inhibitory peptides have also been identified in hydrolyzates derived from ovine and
264 caprine CMP. Manso and López-Fandiño (2003), found that undigested bovine, caprine and ovine
265 CMP exhibited moderate ACE-inhibitory activity, but it increased considerably after digestion
266 under simulated gastrointestinal conditions. ACE-inhibitory peptides MAIPPK and MAIPPKK,
267 corresponding to κ -CN f(106-111) and f(106-112) respectively, were identified from CMPs via
268 proteolysis with trypsin. These peptides showed moderate activity, but their digestion under
269 simulated gastrointestinal conditions allowed the release of the potent antihypertensive tri-peptide
270 IPP (IC₅₀ value of 5 μ M).

271

272 **3.2. Antimicrobial and immunomodulatory peptides**

273 Both *in vitro* and *in vivo* studies have demonstrated that certain whey-protein-based
274 ingredients and individual whey proteins, such as β -Lg, can act as precursors of peptides with
275 antimicrobial activity, enhancing the organism's natural defences against invading pathogens.
276 (Rutherford-Markwick and Gill, 2005; Gauthier et al., 2006; Saint-Sauveur et al., 2008, 2009).
277 Recently, the antimicrobial and immunostimulatory activities of β -Lg, among other food proteins,
278 hydrolysed with four gastrointestinal proteinases (trypsin, chymotrypsin, pepsin and pancreatin)
279 have been examined by Biziulevicius and co-workers (2006). Proteolytic digestion of bovine β -Lg
280 by trypsin allowed the release of four peptide fragments [f(15–20), f(25–40), f(78–83), f(92–100)]
281 with bactericidal activity against Gram-positive bacteria (Pellegrini et al., 2001). The
282 immunomodulating potential of peptide fractions isolated from β -Lg enzymatic hydrolyzates has
283 also been demonstrated (Prioult et al., 2004). Recently, peptides corresponding to β -Lg fragments
284 f(15-20), f(55-60), f(84-91), f(92-105), f(139-148), and f(142-148) have been reported to stimulate
285 murine splenocytes proliferation through the modulation of cytokine secretion (Jacquot et al.,
286 2010). However, few data are reported about the release of antibacterial and/or immunostimulating
287 peptides from ovine and caprine β -Lg. Only a peptic hydrolyzate of ovine α -La and β -Lg has been
288 demonstrated by El-Zahar and co-workers (2004) to inhibit the growth of *Escherichia coli* HB101,

289 *Bacillus subtilis* Cip5262 and *Staphylococcus aureus* 9973 in a dose dependent manner, but
290 responsible peptides were not identified.

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

301

302 **3.3. Opioid peptides**

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

314 al., 2007). Recently, it has been found that this peptide also suppresses food intake in mice after its
315 oral administration (Hou et al., 2009).

316

317 **3.4. Other biological activities of whey protein-derived peptides**

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

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

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

345

346 **4. Other bioactive components in cheese whey**

347 **4.1. Lactose and oligosaccharides**

348 Lactose is the major carbohydrate in milk with a content of 4.1 g/100 mL in goat milk and
349 4.9 g/100 mL in sheep milk (Park et al., 2007). This disaccharide is a valuable nutrient because
350 favours the intestinal absorption of calcium, magnesium and phosphorous, and the utilization of
351 vitamin C. On the other hand, milk oligosaccharides possess prebiotic and anti-infective properties.
352 The amount of oligosaccharides in ovine milk is in the range of 20 mg/mL to 30 mg/mL while in
353 caprine milk is in the range of 250 mg/mL-300 mg/mL. Many of these oligosaccharides contain
354 sialic acid, a general name for N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid
355 (Neu5Gc). Sialic acid present in milk seems to promote the development of the infant's brain
356 among other positive effects (Park, 2009).

357

358 **4.2. Minerals**

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

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

368

369 **4.3. Vitamins**

370 Vitamins are physiological, biochemical, and metabolical bioactive compounds occurring in
371 milk. Most of the fat-soluble vitamins are incorporated to the curd during cheese-making while
372 water-soluble vitamins go mainly to the whey. In the literature is described than sheep milk is
373 richer than cow milk for most of the vitamins (Park et al., 2007). For instance, goat and sheep milk
374 have higher amount of vitamin A than cow milk. However, goat milk has a significant drawback in
375 folic acid and vitamin B₁₂ compared to cow milk (Park, 2009).

376

377 **Final considerations**

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

391 Authority (EFSA) delivered a scientific opinion on different health claims related to whey protein
392 under Regulation (EC) No 1924/2006. Health claims were rejected based on the absence of human
393 studies or because the existing ones were inappropriately done (European Food Safety Authority,
394 2010). Apart from the biological activities of the intact proteins, the recent interest has mainly
395 focused on bioactive peptides encrypted within the whey proteins that are released by different
396 means, such as enzymatic hydrolysis and/or fermentation processes. Among the plethora of
397 bioactivities described for these peptides, stand out some activities like antihypertensive, opioid,
398 antioxidant, antimicrobial and hypocholesterolemic. Unlike intact whey proteins more research on
399 bioavailability, toxicity as well as animal studies have been carried out for bioactive peptides.
400 However, additional studies in both whey protein-based products and those containing whey
401 bioactive peptides are required in order to gain the official acceptance from EFSA. These studies
402 should be especially focused on well-designed clinical trials to confirm the potential health effects
403 of these products.

404

405 **Conflict of interest statement**

406 None of the authors (Blanca Hernández-Ledesma, Mercedes Ramos and José Ángel Gómez-Ruiz)
407 has a financial or personal relationship with other people or organizations that could inappropriately
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409

410

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687 **Figure legends**

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

690 **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).

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	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

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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 Antioxidant activity Anti-cancerigen activity	Choi et al., 2002 Smith et al., 1992 Laursen et al., 1990
Lactoferrin	Antimicrobial activity Antifungal activity Anti-proliferative activity Antiviral activity Immunomodulatory activity Anti-thrombotic activity	Orsi, 2004 Olakanimi et al., 2002 Xu et al., 2010 Van der Strate et al., 2001; Seganti et al., 2004 Togawa et al., 2002 Quian et al., 1995a
Caseinomacropeptide	Anti-cariogenic activity Immunomodulatory activity Prebiotic activity Anti-thrombotic activity	Oh et al., 2000 ; Kawasaki et al., 1992; 1993 Otani et al., 1995a, b; 1996 Azuma et al., 1984 ; Idota et al., 1994 Manso et al., 2002
Immunoglobulins	Immunomodulatory activity Growth and development	Balan et al., 2010 Balan et al., 2009
Lactoperoxidase	Antimicrobial activity Antiviral activity Immunomodulatory activity	deWit & van Hooydonk, 1996 Shin et al., 2005 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 μ M) ^a Antihypertensive ^d	Hydrolysis with pepsin	Bordenave, 2000

^o Ovine milk proteins,

^g Goat milk proteins,

^a Studies carried out in the bovine peptide