Health effects of cheese components with a focus on bioactive peptides

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Abstract

Cheese is a rich source of essential nutrients; in particular, proteins, fat, vitamins and minerals. In addition to their nutritional role, cheese components are also able to exert important health benefits, with calcium, fatty acids and bioactive peptides being the main players. This chapter presents an updated revision on the health effects of cheese components focusing on the most studied bioactive peptides activities. They are liberated during cheese ripening, where casein is hydrolyzed by proteases and peptidases from milk, rennet, starter culture, and secondary microbial flora. Some of the generated peptides can survive gastrointestinal digestion or serve as precursors from the final peptide form, responsible of a wide array of biological activities such as antihypertensive, antioxidant, antimicrobial or opioid.

In the chapter, the degree of evidence reached for the bioactive components described, as well as the bioavailability studies performed in each case are also discussed.

Keywords: cheese, bioactive peptides, cheese health effects, fat, proteins, vitamins, minerals, bioavailability

11.1. Introduction

Cheese is a nutritious, versatile dairy food with a long history in the human diet. The high content of fat and protein in cheese makes it an energy-rich and nutritious food recommended for all ages. Nowadays, a wide variety of cheese types are available to meet specific consumer requirements and allow convenience use. Europe (EU 27) is the biggest producer of cheese, with an average production of 8,998 kt during the period 2010-2012 . Average per capita consumption of cheese in EU 27 has been reported to be 17.2 kg during the period 2010-2012. From the integrant countries, France, Iceland, Luxembourg, Germany, and Finland were the biggest consumers with more than 23.5 kg/capita during 2012 (Bulletin of the International Dairy Federation, 2013).

Cheese is a rich source of essential nutrients; in particular, proteins, fat, vitamins and minerals. In addition to their nutritional role, cheese components have also revealed to potentially exert important health benefits (Walther et al., 2008). Calcium, present in large amounts in cheese, has been shown to have a positive effect on various disorders such as osteoporosis (Heaney, 2000) or dental caries (Kato *et al.*, 2002). Besides calcium, some fatty acids present in cheese such as conjugated linoleic acid (CLA) have demonstrated to display anti-carcinogenic and anti-atherogenic properties (Lee *et al.*, 2005; Battacharaya *et al.*, 2006). Bioactive peptides are also present in cheese. They are produced during cheese ripening, where casein is broken down by proteases and peptidases from milk, rennet, starter culture, and secondary microbial flora. Some of the generated peptides can survive gastrointestinal digestion or serve as precursors from the final peptide form, responsible of a wide array of biological activities (López-Expósito *et al.*, 2012).

This chapter presents an updated revision on the health effects of cheese components focusing on the most studied bioactive peptides activities. The degree of evidence reached, as well as the bioavailability studies performed in each case will be discussed.

11.2. Effects of cheese fat on health

The physical, organoleptic and nutritional properties that milk lipids impart to dairy products make them one of the most important components of milk. However, their high content of saturated fatty acids and cholesterol has been used by nutritionists as an argument to associate the consumption of these products with an increased incidence of obesity, metabolic disorders and cardiovascular diseases (CVD). Nevertheless, recent studies indicate that there are no demonstrated evidences to recommend the consumption of low-fat dairy foods in healthy people because of the biological activities demonstrated for several compounds present in milk fat (Fontecha *et al.*, 2011).

Conjugated linoleic acid (CLA) refers to a group of polyunsaturated fatty acids that exist as positional and stereo-isomers of conjugated dienoic octadecadienoate (C18:2). The fatty acid profile and the CLA content of cheese (Table 1) are influenced by environmental factors, farming practices and genetic and physiological aspects related to the animals (Collomb *et al.*, 2006). Moreover, factors involved in cheesemaking process, such as heat treatment of milk and/or curd, addition of starter cultures and ripening have an important effect on CLA levels in cheese. It has been shown that certain lactic acid bacteria used in milk fermentation possess the ability to generate CLA (Prandini *et al.*, 2007). Therefore, recent studies are focused on increasing the CLA content of cheese by supplementing the animal's diet with vegetable oils (rich in polyunsaturated fatty acids, Nudda *et al.*, 2014) and/or by adding dairy starters producers of CLA during cheese manufacture (de Lima Alves *et al.*, 2011). Rumenic acid, corresponding to the CLA isomer C18:2 *cis* 9, *trans* 11, is the most abundant isomer, accounting for around 80-90% of the total CLA (Parodi, 1999). A high number of *in vitro* studies and animal models have shown that it is responsible for the anti-carcinogenic and anti-atherogenic properties attributed to CLA (Lee *et al.*, 2005; Battacharaya *et al.*, 2006). Besides rumenic acid, other CLA isomers have a positive impact on human

health. As an example, the C18:2 *trans* 10, *cis* 12, has been reported to promote weight loss, decreasing glucose levels and insulin resistance in obese men with the metabolic syndrome (Riserus *et al.*, 2002), while isomer C18:2 *trans* 9, *trans* 11 acts inhibiting growing of human colon cancer cells (Coakley *et al.*, 2006).

Although a notable number of clinical studies have evaluated the biological properties of purified rumenic acid or CLA isomers mixtures on CVD risk factors (Wang *et al.*, 2012), relatively little information from dietary intervention studies with CLA-enriched cheeses is available. Sofi *et al.* (2010) observed beneficial effects of consumption by healthy subjects of a sheep cheese naturally rich in rumenic acid on several atherosclerotic biomarkers, in comparison with a commercially available cheese. Pintus *et al.* (2013) reported an improvement of the lipid profile and reduction of endocannabinoid synthesis in the plasma of hypercholesterolemic subjects after consumption of sheep cheese naturally enriched in α -linolenic, CLA and vaccenic acid. If these beneficial effects are confirmed by further studies, this will likely have important repercussion for human nutrition and food industry.

Short-chain fatty acids, such as butyric acid (C4), caproic (C6) and medium-chain fatty acids, such as caprylic (C8) and capric acids (C10), represent 10-12% of total saturated fatty acids. Apart from its antimicrobial properties (Huang *et al.*, 2011), the butyric acid, present in milk fat in concentrations ranged from to 2 to 5%, has been described as an anticarcinogenic agent inhibiting growth of a wide range of human cancer cell lines (Blank-Porat *et al.*, 2007). Furthermore, synergisms between butyric acid and other dietary compounds and common drugs in reducing cancer cell growth have also been shown (Parodi 2006). However, no human studies have been conducted to confirm these effects after consumption of cheese.

11.3. Minerals in cheese and their impact on health

The specific amount of minerals in cheese differs according to the manufacturing procedure of a cheese type. For example, the method of coagulation used and the acidity of the curd will have an effect on the salt concentration. Ripened cheeses constitute an abundant source of mineral compounds. Calcium, phosphorus, sodium and chloride will be in high amounts while elements such as zinc and potassium will be in at much lower concentration (Table 2). As shown in this Table, it is remarkable the higher daily value of calcium and phosphorus with regard to the rest of the minerals shown. Research has shown that an insufficient intake of calcium, in particular, raises the risk of obesity (Parikh and Yanovski 2003), hyperlipidemia and insulin-resistance syndrome (Teegarden 2003; Zemel 2004). On the contrary, a diet rich in dairy calcium intake enhanced weight reduction in type 2 diabetic patients (Shahar *et al.*, 2007).

A considerably body of research has been published on the cariostatic effect of cheese (Kashket and DePaola 2002). Assays on human subjects have suggested a caries-protective effect in childhood populations (Ohlund *et al.*, 2007). Similarly, Gedalia *et al.* (1991) showed that the consumption of hard cheese lead to significant rehardening of softened enamel surfaces by a cola type drink while a cheese with *Lactobacillus rhamnosus* as probiotic diminished caries-associated salivary microbial counts in young adults (Ahola *et al.*, 2002). Although more research is needed to elucidate the precise mechanisms for the cariostatic effects of cheese, there is enough evidence to support the consumption of cheese as an anticaries measure (European Food Safety Agency, 2008). The most plausible mechanism is related to the mineralization potential of the casein-calcium phosphate of cheese, to the stimulation of saliva flow induced by its texture, the buffering effects of cheese proteins on acid formation in dental plaque and the inhibition of cariogenic bacteria (O'Brien and O'Connor 2004).

One particular characteristic of cheese's calcium is its high bioavailability. The suggested factors responsible for it are the prevention of the formation of insoluble calcium salts in the intestine by casein phosphopeptides (CPPs) (Kitts *et al.*, 1992) and the optimal calcium:phosphorus ratio (Cámara-Martos *et al.*, 2013). In ripened cheeses, calcium availability is determined by fat content, being lower in full-fat cheeses (13.85%) than in semi-fat cheeses (24.13%) (Ibeagha-Awemu *et al.*, 2009). On the other hand, recent studies suggest that the use of probiotic cultures in the production of cheese increase the availability of calcium, magnesium, and phosphorus. The stimulating effect of probiotic cultures on the availability of mineral compounds can be attributed to intensified enzymatic conversion, mainly proteolysis and lipolysis (Aljewicz *et al.*, 2014).

11.4. Biological effects of cheese vitamins

As with other nutrients, the vitamin content is highly variable between types of cheese depending on its fat content. Most of the fat-soluble vitamins in milk are retained in the cheese fat. Absorption of vitamin A and other fat soluble vitamins such as vitamins D, E and K, are increased in the body by the presence of fats in the diet, making cheese a good vehicle to deliver them. It has been reported that cheese can provide one third of the reference nutrient intake of vitamin A, a vitamin with an important role for the normal functioning of the immune system, in the gene expression regulation and sustaining low-light vision (Russell 2000).

With regard to the water-soluble vitamins, such as riboflavin, vitamin B12 (niacin) and folate, their content in cheese is lower than in milk. However, the loss of some B vitamins is compensated by the microbial synthesis during ripening. A portion of a semi hard cheese will provide the average male and female adult with 80% for vitamin B12. This vitamin and folic acid will help to reduce high levels of homocysteine in blood, an amino acid that has been

linked with CVD (Quinlivan *et al.*, 2002). It must be highlighted that ovine milk is richer than cow's milk in most vitamins, and therefore 90 g of sheep milk cheese could cover completely the daily requirements for riboflavin (Recio *et al.*, 2009).

11.5. Cheese bioactive proteins and peptides

The content of proteins present in cheese depends upon the variety, ranging from 4% (cream cheese) to 40% (Parmesan cheese). Proteins are one of the major responsible for the nutritional, physicochemical, functional and sensory aspects of cheese. In addition, cheese protein is almost 100% digestible, as the ripening phase of cheese manufacture involves a progressive breakdown of casein by enzymes present in milk and those derived from rennet or microorganisms, releasing both amino acids and peptides. Interestingly, lysine is one of the major amino acids contained in cheese which bioavailability is very high because of the absence of Maillard reactions (de la Fuente and Juárez 2001). The type and quantity of milk proteins-derived peptides is affected by factors influencing proteolysis, such as pH, type of enzymes, salt-to-moisture ratio, humidity, and storage time and temperature (Park and Jin 1998). In this sense, different varieties of cheeses are likely to exhibit peptides with variable bioactivities, such as antihypertensive, antioxidant, opioid, antimicrobial, anti-proliferative and mineral absorption modulatory effects, among others (López-Expósito *et al.*, 2012).

11.5.1. Antihypertensive peptides

Hypertension is a chronic condition affecting up to 30% of the adult population in the developed countries that can lead to more severe and deadly diseases such as stroke and coronary heart disease (Chen *et al.*, 2009). One of the many physiological pathways that control blood pressure in the human body is the renin-angiotensin-aldosterone system. A key component of this system is the Angiotensin I-converting enzyme (ACE) that catalyzes the

conversion of angiotensin I into the potent vasoconstrictor angiotensin II and, simultaneously, the degradation of vasodilators bradykinin and kallidin. These two reactions cause a contraction of the blood vessels and the release of aldosterone, increasing sodium concentration and blood pressure. Thus, inhibiting this enzyme can lead to an antihypertensive effect. Most of hypertensive patients are treated worldwide with ACE-inhibitory drugs such as Captopril or Enalapril (Nussberger 2007). However, it has been demonstrated that small reductions in average blood pressure may be obtained by nutritional measures such as reducing sodium intake and increasing daily consumption of food-derived ACE-inhibitory peptides (Sieber *et al.*, 2010). Because of its content of sequences released during milk fermentation and ripening, cheese has been extensively studied as potential source of ACE-inhibitory peptides. As it is shown in Table 3, the water and/or ethanol soluble extracts (WSE, ESE) of many cheese varieties have been obtained and evaluated for their ACE-inhibitory properties. The influence of addition of certain probiotic strains (Ong and Shah 2008; Wang *et al.*, 2011) or simulated gastrointestinal digestion (Parrot *et al.*, 2003) on the ACE-inhibitory activity of different kind of cheeses has been also studied. In some studies, different fractionation steps have been performed to purify novel bioactive fragments in which their ACE-inhibitory activities have been evaluated. As an example, this strategy has been applied to isolated peptides from Manchego cheese (Gómez-Ruiz *et al.*, 2004a, b) and other Spanish cheeses such as Cabrales, Mahon, Idiazabal, and Roncal (Gómez-Ruiz *et al.*, 2006). These authors applied tandem mass spectrometry (MS/MS) to identify and quantify sequences contained in those cheese varieties. Some of them, derived from α_{s1} - and β -casein showed moderate ACE-inhibitory activity, indicating their potential contribution on the activity reported for the cheese WSE (Gómez-Ruiz *et al.* 2006). MS/MS technique has been also found useful to confirm the presence, in cheese samples, of peptides previously identified in other milk products obtained by enzymatic hydrolysis or fermentation. Antihypertensive β -

casein peptides VPP and IPP have been found in different cheese varieties, such as Appenzeller, Brie, Caprine, Cheddar, Edam, Fontina, Gouda, Gorgonzola, and Gruyere, among others (Bütikofer *et al.*, 2007; Stuknytė *et al.*, 2015). Interestingly, some of these varieties contained similar concentrations of VPP and IPP than fermented milk products with blood-pressure-lowering capacity. Similarly, antihypertensive α_{s1} -casein derived peptides AYFYPEL and RYLGY, identified and characterized from casein hydrolyzate by Contreras *et al.* (2009, 2013), were found in several cheese varieties, such as Brie, Caprino, Cheddar, Gorgonzola, and Maasdam (Stuknytė *et al.* 2015). Although several cheeses extracts have been characterized by their potent ACE-inhibitory activity, and the presence of antihypertensive peptides has been detected in some of them, confirmation of the antihypertensive effect of cheese in animal models or humans is still needed.

11.5.2. Antioxidant peptides

Oxidative stress, caused by an increased production of reactive oxygen species (ROS) associated with a decreased antioxidant capability of the cell, is thought to contribute to the pathogenesis of a number of human diseases including CVD, metabolic, inflammatory, neurodegenerative diseases and cancer, in addition to accelerate the aging process (Stadtman 2004). Natural antioxidants provide additional benefits to the endogenous defense strategies in the battle against oxidative stress (Erdmann *et al.*, 2008), with food derived peptides being potent antioxidants without important side effects (Sarmadi and Ismail 2010). Among them, milk proteins have been one of the most frequently studied sources.

First studies reporting antioxidant activity of cheese-derived peptides were carried out with raw and sterilized ovine and caprine cheese like systems coagulated with enzymes from the plant *Cynara cardunculus* (Silva *et al.*, 2006), although sequences of those peptides were not identified. Gupta *et al.* (2009) assessed the antioxidant activity of peptides in WSEs

obtained from Cheddar cheese prepared with *Lactobacillus casei* ssp. *casei* 300, *Lactobacillus paracasei* ssp. *paracasei* 22, and without adjunct cultures by three different methods based on radical scavenging activity. In all cheese samples, changes in the antioxidant activity correlated to the rate of formation of soluble peptides (proteolysis). Later on, Pritchard *et al.* (2010), Silva *et al.* (2012), and Bottesini *et al.* (2013) evaluated the radical scavenging activity of WSEs obtained from Australian Cheddar, Brazilian artisanal “Coalho” and Parmigiano-Reggiano cheeses, respectively. Gupta *et al.* (2010) identified two peptides corresponding to bovine β -casein (VKEAMAPK) and α_{s1} -casein (HIQKEDVPSE) whose potent radical scavenging activity indicated their key contribution on the antioxidant activity of Cheddar cheeses. Meister Meira *et al.* (2012) also identified peptides released during manufacture of a Roquefort-type cheese with sequences sharing great homology with peptides characterized previously by their antioxidant activity. Recently, three peptides derived from α_{s1} - and β -casein have been identified in a Burgos type cheese (Timón *et al.*, 2014). One of them, corresponding to α_{s1} -casein (SDIPNPIGSENSEKTTPLLW) is a new peptide with potential antioxidant activity. These preliminary results suggest the potential of cheese as source of antioxidant peptides, although animal models and human trials confirming these effects are needed.

11.5.3. Effects of cheese-derived peptides on nervous system

Opioid peptides are defined as opioid μ -receptor ligands with agonistic and antagonistic activities. Opioid receptors are located in the nervous, endocrine and immune systems as well as in the gastrointestinal tract of mammals (Teschemacher 2003). The most studied milk-derived opioid receptor ligands are those termed β -casomorphins (BCMs), derived from β -casein. They are characterized by the presence of a tyrosine residue at the N-terminal and another aromatic amino acid at third or fourth position which is an important

structural motif that fits into the binding site of opioid receptors (Nagpal *et al.*, 2011). In addition to their effects on the central nervous system, BCMs have demonstrated to induce intestinal mucus secretion (Plaisancié *et al.*, 2013), increase plasma insulin level, decrease glucagon level, and to elevate the activity of superoxide dismutase and catalase (Yin *et al.*, 2010). Furthermore, they also have been observed to prolong gastrointestinal transit time and to exert an anti-diarrheal action in both animals and humans (Daniel *et al.*, 1990). Other beneficial activities attributed to BCMs can be found in Ul-Haq *et al.* (2014).

Only a few studies deal on the presence and levels of BCMs in cheeses. BCM7 [β -casein (60-66)] and BCM5 [β -casein (60-64)] have been identified in extracts from Brie and Gouda cheeses, respectively (Jarmolowska *et al.*, 1999; Kostyra *et al.* 2004). Also, the content of agonistic BCM7 and BCM5, and antagonistic casoxin-6 and casoxin-C (derived from bovine κ -casein), and lactoferroxin A (derived from lactoferrin) opioid peptides in three semi-hard, Edamski, Gouda and Kasztelan, and in two ripening mould cheeses, Brie and Rokpol, was evaluated by Sienkiewicz-Szlapka *et al.* (2009). It was found that the content of BCMs was higher in mould cheeses than in semi-hard cheeses which contained fairly high amount of casoxin-6. All the extracts, except that from Gouda, were found to affect the intestinal mobility in isolated rabbit ileum similarly to morphine, with this effect being reversed by naloxone. Similarly, Gorgonzola, Caprino, Brie, Taleggio, Gouda, Fontina, Cheddar and Grana Padano cheeses as well as their digests have been analyzed by De Noni and Cataneo (2010) for the presence of BCM7 and BCM5. BCM7 was detected in all the cheeses evaluated with the exception of Taleggio, Caprino and Grana Padano samples. However, BCM5 was not found in any of them.

Peptides incorporating the BCM7 sequence, which could act as precursors during further digestion processes, have also been found in cheeses such as Parmigiano Reggiano (Addeo *et al.*, 1992), Cheddar and Jarlsberg (Stepaniak *et al.*, 1995), and Crescenza (Smacchi

and Gobetti 1998). BCM9 [β -casein (60-68)] and a number of peptides including the BCM7 sequence were identified in Cheddar cheese by Singh *et al.* (1995, 1997). BCM9 was also found in Gouda cheese by Saito *et al.* (2000). This finding was later on confirmed by Toelstede *et al.* (2008) who described the presence of BCM10 [β -casein (60-69)] in the WSE of a matured Gouda cheese. The caprine analogue of BCM9 was also detected in the WSEs of Caprino de Piemonte, an Italian goat cheese (Rizzello *et al.*, 2005).

Relatively little is known about the actual bioavailability of this kind of peptides. BCMs have been detected in the plasma of newborn calves after milk intake (Umbach *et al.*, 1985), and in plasma in 2- and 4- week old pups after ingesting a casein-based formula (Singh *et al.*, 1999). As for humans, immunoreactive material to human and bovine BCM7 and BCM5 has been detected in the plasma of infants fed with human and cow's milk (Kost *et al.*, 2009; Wasilewska *et al.*, 2011), although the method of detection employed by the authors raise many doubts regarding the cross-reactivity of the antibody with some other epitopes of antigen present in plasma. In any case, authors speculated that the intestinal mucosa of the newborn is more permeable to the relatively large peptides due to their immature tight junction through which peptides cross, thereby escaping hydrolysis by brush-border peptidases (Sienkiewicz-Szlapka *et al.*, 2009). It is necessary to continue exploring the role of BCMs in human health applying *in vivo* experiments with improved diagnostic techniques to verify their presence in the blood. Furthermore, more research needs to be done aimed to evaluate the absorption of these peptides in a complex food matrix such as cheese.

11.5.4. Antiproliferative peptides

There are several studies demonstrating the ability of bovine milk proteins such as lactoferrin and lactoferricin to suppress the growth of cancer cells both *in vitro* and *in vivo* by serving as apoptotic inducers in tumor development (Roy *et al.*, 2002; de Moreno de LeBlanc

et al., 2005; Mader *et al.*, 2005). However, regarding cheese, any peptides with antiproliferative properties have been yet characterized. To date, the antiproliferative effects of cheese extracts are being evaluated and all the described results are based on either cheese extracts or cheese whey. The potential role of 12 commercial cheese products on the cell growth and induction of DNA fragmentation in HL-60 human promyelocytic leukemia cells was investigated by Yasuda *et al.* (2010). Among them, extracts from Montagnard, Pont-l'Eveque, Brie, Camembert, Danablu and Blue cheeses revealed the highest activity. In addition, a positive correlation between the ripeness of various cheeses and their antiproliferative activity was found. It remains to be elucidated whether these active molecules from cheeses may be present at enough concentration to exert their activity, as well as their bioavailability and the specificity to cancer cells.

Mozzarella whey samples produced a 43% reduction in cell proliferation in Caco 2 cells (De Simone *et al.*, 2009). The absence of this effect in the peptide extract from original milk suggested that the release of specific bioactive compounds occurs specifically during the cheese's production process. Later on, it was identified a partially RP-HPLC purified peptide sub-fraction as the responsible of the activity observed (De Simone *et al.*, 2011). On the mentioned sub-fraction, a number of peptides mainly derived from κ -casein glyco-caseinmacropeptide, and β -casein were identified by MALDI-TOF/MS.

11.5.5. Antimicrobial peptides

The majority of antimicrobial peptides derived from food sources have been identified in milk, milk hydrolysates and fermented milks (López-Expósito and Recio 2006). However, there is almost no information considering the potential of cheese as a source of antimicrobial peptides. To date, all the publications dealing with the antimicrobial properties of cheese are based on WSEs, and very few of them identify the potential peptides responsible (Rizello *et*

al., 2005; Losito *et al.*, 2006, Lignitto *et al.*, 2012). Because cheese's WSE also contain organic acids and salts that could contribute to the inhibition of microbial growth, it is important to perform a purification step to remove them, prior to attributing to peptides the potential antimicrobial activity observed. Recently, Théolier *et al.* (2014) evaluated the antibacterial activity of WSEs from five commercial cheeses namely Mozzarella, Gouda, Swiss, and old and medium Cheddar. Before purification through a Sep-Pak cleanup column, WSEs from Mozzarella and Gouda cheeses inhibited the growth of *Listeria ivanovii* HPB28, *L. monocytogenes* Scott A3, *Escherichia coli* MC4100 and *E. coli* O157:H7 up to 4.66 log cycles. However, after desalting, the activity against *L. monocytogenes* Scott A3 and *E. coli* O157:H7 was almost negligible, highlighting the importance of carrying out a previous purification step. The same purified WSEs demonstrated to induce a delay in spore germination of several food-borne molds, reaching the lowest minimal inhibitory concentration values against *Penicillium camemberti* and *P. commune* strains, for Swiss, Gouda and Mozzarella extracts. Purified WSE of Asiago d'Alleva cheese displayed moderate antibacterial properties against *L. innocua* LRGIA01 growth (Lignitto *et al.*, 2012; Thi *et al.*, 2014). The bacterial growth inhibitory properties were affected by the ripening time with the highest activities observed after 6 months of maturation. Authors identified a big number of casein derived peptides present in the WSE, among which they found other potent antimicrobial fragments previously described such as isracidin [α_{s1} -casein (1-23)] (Hill *et al.*, 1974) and α_{s2} -casein (183-207) (López-Expósito *et al.*, 2006). Similarly, Losito *et al.* (2006) identified more than 30 peptides with a high level of homology with N-terminal, C-terminal or whole fragments of previously reported milk derived antimicrobial sequences from the WSEs of Pecorino Romano, Canestrato Pugliese, Crescenza, Caprino del Piemonte and Caciocavallo cheese varieties (Rizzello *et al.*, 2005). Recently, the antibacterial activity

against *L. monocytogenes* strain 162 of Emmental de Savoie purified WSE has also been reported (Thi *et al.*, 2014), although there was not identification of the responsible peptides.

To date, there are not any *in vivo* published data supporting the antimicrobial effects of cheese consumption. However, some data regarding the *in vivo* antimicrobial activity of one of the peptides identified in cheese extracts, isracidin, have been published (Lahov and Regelson, 1996). In mice, it exerts a protective effect against *L. monocytogenes*, *Streptococcus pyogenes* and *Staphylococcus aureus*. Also, in cows with mastitis, isracidin obtained a success rate of over 80% in the treatment of chronic streptococcal infection.

11.5.6. Modulatory peptides of mineral absorptions: Phosphopeptides

CPPs are bioactive peptides with various degrees of phosphorylation which are released *in vitro* and *in vivo* by enzymatic hydrolysis of the different casein fractions (Bouhallab and Bouglé 2004; Phelan *et al.*, 2009). As these peptides have a high net negative charge, they efficiently bind divalent cations with the formation of soluble complexes. This property makes these peptides perfect candidates as anticariogenic compounds (Cochrane and Reynolds 2009).

Apart from the metal binding properties, CPPs have other bioactive properties such as antioxidant (Kitts 2005), immunostimulatory (Kitts and Nakamura 2006), gastric secretion regulatory (Guilloteau *et al.*, 2009), antimicrobial (Arunachalam and Raja 2010), and stimulatory activity of growth and differentiation of osteoblastic cells (Tulipano *et al.*, 2010).

In the last years, several studies have isolated, characterized and identified CPPs from different types of cheese (Table 4). The presence of CPPs in these cheese types has been investigated by analyzing the total WSEs or using an enrichment step by precipitation. Although a number of enzymes are common to many cheese varieties, the peptide composition is unique from each cheese type and reflects a characteristic ripening process. In

cheeses some of the peptides appeared to be partly dephosphorylated. For instance, Addeo *et al.* (1992) found the α_{s1} -casein (41-75) partly dephosphorylated, containing only three of the seven native phosphate groups in Parmigiano-Reggiano cheese. Singh *et al.* (1997) observed a complete dephosphorylation of the peptide β -casein (8-23), which in intact β -casein contains four phosphate groups. These results suggest that phosphatase is active in these varieties of cheese, hydrolyzing the native CPPs to phosphate and partly dephosphorylated peptides.

Moreover, the presence of aminopeptidase activity has been deduced from the presence of a number of CPPs of different lengths with the loss of one or more residues from the N-terminus (Ferranti *et al.*, 1997). In Comté cheese, the original cleavage of β - and α_{s2} -casein is due to plasmin, an alkaline milk protease that has a trypsin-like specificity cleaving K₂₈-K₂₉ of β -casein and K₂₁-Q₂₂ of α_{s2} -casein. These two peptides are further hydrolyzed by the action of another endopeptidase and of an aminopeptidase, as well as of a lysyl carboxypeptidase (Roudot-Algaron *et al.*, 1994).

Therefore, CPPs could be regarded as transient intermediate components in cheese; they either accumulate or are degraded by cheese enzymes to shorter peptides and free amino acids, including phosphorylated serine. The most of CPPs come from the region (14-28) of β -casein (Sforza *et al.*, 2003). The accumulation of a specific peptide always depends on a delicate balance between producing and degrading enzymes and the availability of the parent substrate (Sforza *et al.*, 2012). In the case of Ragusano cheese the number of identified soluble peptides dramatically decreased after 4 and 7 months of ripening, from 123 to 47 and 25, respectively (Gagnaire *et al.*, 2011).

It has been reported that CPPs are released in the gut and accumulate in the lower part of the small intestine (ileum) where mineral absorption takes place (FitzGerald 1998; Zidane *et al.*, 2012). Therefore, several *in vivo* studies have investigated if CPPs could increase

mineral absorption, especially Ca^{2+} absorption. Although these studies provide considerable evidence for a potential effect of CPPs to improve mineral absorption, results are still controversial (Scholtz-Ahrens and Schrezenmeir 2000; Meisel and FitzGerald 2003; Mills *et al.*, 2011). This variability can be due to the diversity of the experimental approaches used to assess mineral bioavailability (Korhonen and Pihlanto 2006). Moreover, it seems that results may depend on the food matrix (Hansen *et al.*, 1997). On the other hand, the effect of CPPs may also be influenced by the physical status of the population (Teucher *et al.*, 2006), the levels of CPPs (Bennett *et al.*, 2000) and the ratio calcium:phosphorus present (Erba *et al.*, 2001, 2002).

11.6. Future prospects

Cheese constitutes an adequate source of micronutrients and bioactives, which have generated extensive investigation for the last six decades. Knowledge of minerals is relatively complete and precise. As for vitamins, data are lacking or there is discrepancy between them. However, there is no doubt about their important nutritional role and health benefits. As for lipid and protein components, beyond their nutritional role, the increased scientific knowledge on the biological activities has demonstrated that cheese might provide specific health benefits. Questions to be answered include the changes that may occur during processing, the role of the different components on the absorption and the interest in enriching cheese with micronutrients or bioactives. As an example, there are some reports on the presence of bioactive peptides in duodenal effluents from humans upon milk proteins ingestion (Boutrou *et al.*, 2013) and even the presence in plasma of the milk bioactive peptides IPP and VPP (Foltz *et al.*, 2007) or HLPLP (Sánchez-Rivera *et al.*, 2014) has been demonstrated *in vivo*. However, more research is needed to evaluate the absorption of these peptides in a complex

food matrix as cheese. Further intervention studies confirming the beneficial effects of cheeses containing bioactive peptides should be also conducted.

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Table 1. Fatty acid composition (g/100 g) and *cis*-9, *trans*-11 CLA content (mg/g fat) of different cheeses elaborated from cow, sheep and goat milk

Type of milk	Cheese type	SFA ^a content (%)	MUFA ^b content (%)	PUFA ^c content (%)	<i>Cis</i> -9, <i>trans</i> -11 CLA ^d (mg/g fat)	Reference
Cow	Alpine	65.23	31.19	3.57	4.79 ± 1.07	Prandini <i>et al.</i> (2007)
	Asiago	n.d.	n.d.	n.d.	5.91 ± 1.95	Cicognini <i>et al.</i> (2014) ^f
	Belpaese	n.d.	n.d.	n.d.	5.22 ± 1.97	Cicognini <i>et al.</i> (2014) ^f
	Caciotta	n.d.	n.d.	n.d.	5.98 ± 1.18	Cicognini <i>et al.</i> (2014) ^f
	Caciocavallao	n.d.	n.d.	n.d.	5.35 ± 2.77	Cicognini <i>et al.</i> (2014) ^f
	Camembert	58.41	25.70	3.20	9.50 ± 0.20	Domagała <i>et al.</i> (2010) ^g
	Caprino fresh	66.87	24.45	3.44	6.10 ± 2.65	Prandini <i>et al.</i> (2011)
	Cheddar	n.d.	n.d.	n.d.	5.86 ± 0.89	Cicognini <i>et al.</i> (2014) ^f
	Crescenza	n.d.	n.d.	n.d.	4.76 ± 0.79	Cicognini <i>et al.</i> (2014) ^f
	Dziurawiec	58.27	21.72	2.55	9.00 ± 0.90	Domagała <i>et al.</i> (2010) ^g
	Emmentaler	57.88	25.36	3.06	7.40 ± 0.10	Domagała <i>et al.</i> (2010) ^g
	Swiss Emmental	66.67	29.85	3.48	7.66 ± 1.42	Prandini <i>et al.</i> (2007)
	Fontina Valdostana	67.26	28.57	4.17	8.11 ± 1.53	Prandini <i>et al.</i> (2007)
	Gorgonzola	64.10	25.79	3.92	5.16 ± 0.29	Prandini <i>et al.</i> (2011)
	Gouda	56.38	24.89	3.45	12.00 ± 0.70	Domagała <i>et al.</i> (2010) ^g
	Gouda	n.d.	n.d.	n.d.	4.32 ± 1.43	Cicognini <i>et al.</i> (2014) ^f
	Grana/Parmigiano	68.52	27.90	3.58	3.85 ± 0.51	Prandini <i>et al.</i> (2007)
	Gruyere	n.d.	n.d.	n.d.	10.21 ± 2.47	Cicognini <i>et al.</i> (2014) ^f
	Mazdamer	56.05	26.51	3.20	9.80 ± 0.30	Domagała <i>et al.</i> (2010) ^g
	Montasio	n.d.	n.d.	n.d.	4.78 ± 1.23	Cicognini <i>et al.</i> (2014) ^f
	Mozzarella	67.04	29.20	3.75	0.24 ± 0.01 ^e	Caroprese <i>et al.</i> (2013) ^h
	Parmigiano	59.77	22.77	3.60	4.40 ± 0.20	Domagała <i>et al.</i> (2010) ^g
	Philadelphia	n.d.	n.d.	n.d.	5.41 ± 1.15	Cicognini <i>et al.</i> (2014) ^f
	Prato	n.d.	n.d.	n.d.	3.79 ± 0.50	Cortes Nunes and Guedes Torres (2010) ⁱ
	Provola	n.d.	n.d.	n.d.	6.96 ± 5.12	Cicognini <i>et al.</i> (2014) ^f
	Provolone	n.d.	n.d.	n.d.	4.92 ± 0.23	Cicognini <i>et al.</i> (2014) ^f
	Radamer	57.74	25.86	2.94	7.10 ± 0.50	Domagała <i>et al.</i> (2010) ^g
	Ricotta	n.d.	n.d.	n.d.	5.53 ± 1.08	Cicognini <i>et al.</i> (2014) ^f
	Robiola	n.d.	n.d.	n.d.	4.77 ± 1.26	Cicognini <i>et al.</i> (2014) ^f

	Salami	56.92	25.89	3.33	11.80 ± 0.70	Domagała <i>et al.</i> (2010) ^g
	Scamorza	n.d.	n.d.	n.d.	4.93 ± 0.31	Cicognini <i>et al.</i> (2014) ^f
	Stracchino	n.d.	n.d.	n.d.	5.01 ± 1.26	Cicognini <i>et al.</i> (2014) ^f
Sheep	Pecorino	67.69	26.83	5.48	7.77 ± 2.25	Prandini <i>et al.</i> (2007)
	Pecorino hard	65.94	23.80	4.93	10.90 ± 0.03	Prandini <i>et al.</i> (2011)
	Roquefort	70.10	21.11	3.92	8.81 ± 3.84	Prandini <i>et al.</i> (2011)
	Feta	70.20	21.00	4.70	9.24 ± 4.80	Zlatanos <i>et al.</i> (2002) ^j
	Feta	n.d.	n.d.	n.d.	8.50 ± 1.95	Cicognini <i>et al.</i> (2014) ^f
	Rokpol	55.47	27.55	3.71	13.10 ± 0.10	Domagała <i>et al.</i> (2010) ^g
Goat	Caprino fresh	66.92	23.89	4.08	6.80 ± 1.26	Prandini <i>et al.</i> (2011)
	Caprino hard	67.06	23.71	4.23	7.06 ± 3.42	Prandini <i>et al.</i> (2011)

^a Saturated fatty acids; ^b Monounsaturated fatty acids; ^c Polyunsaturated fatty acids; ^d Conjugated linoleic acid; ^e expressed as g of isomer/100 g fatty acids; n.d. not detected;

^f Cicognini, F. M., Rossi, F., Sigolo, S., Gallo, A., Prandini, A., 2014. Conjugated linoleic acid isomer (cis9,trans11 and trans10,cis12) content in cheeses from Italian large-scale retail trade. *International Dairy Journal* 34, 180-183.

^g Domagała, J., Sady, M., Grega, T., Pustkowiak, R., Florkiewicz, A., 2010. The influence of cheese type and fat extraction method on the content of conjugated linoleic acid. *Journal of Food Composition and Analysis* 23, 238-243.

^h Caroprese, M., Sevi, A., Marino, R., Santillo, A., Tateo, A., Albenzio, M., 2013. Composition and textural properties of Mozzarella cheese naturally-enriched in polyunsaturated fatty acids. *Journal of Dairy Research* 80, 276-282.

ⁱ Cortes Nunes, J., Guedes Torres, A., 2010. Fatty acid and CLA composition of Brazilian dairy products, and contribution to daily intake of CLA. *Journal of Food Composition and Analysis* 23, 782-789.

^j Zlatanos, S., Laskaridis, K., Feist, C., Sagredosa, A., 2002. CLA content and fatty acid composition of Greek Feta and hard cheeses. *Food Chemistry* 78, 471-477.

Table 2. Levels of minerals of different international cheeses, mg/serving portion of 30 g

	Calcium	Magnesium	Phosphorus	Sodium	Potassium	Iron	Reference
Camembert	116	6.0	104	253	56	0.10	Gaucheron 2011 ^b
% DV ^a	11	2	10	10	1	1	
Cheddar	216	8.4	154	186	29	0.20	Gaucheron 2011 ^b
% DV ^a	20	2	15	7	1	1	
Feta	147	5.7	101	335	19	0.19	Gaucheron 2011 ^b
% DV ^a	14	2	10	12	1	1	
Gouda	210	8.7	164	246	36	0.07	Gaucheron 2011 ^b
% DV ^a	20	2	16	9	1	1	
Gruyère	303	10.8	182	101	24	0.05	Gaucheron 2011 ^b
% DV ^a	29	3	19	4	1	1	
Idiazabal	295	14.3	188	166	33	0.19	Cámara-Martos <i>et al.</i> , 2013
% DV ^a	29	4	19	7	1	1	
Manchego	278	13.2	177	197	31	0.13	Cámara-Martos <i>et al.</i> , 2013
% DV ^a	27	4	18	8	1	1	
Parmigiano	413	15.3	242	558	32	0.29	Gaucheron 2011 ^b
% DV ^a	39	4	23	22	1	1	
Roquefort	199	9.0	118	543	27	0.17	Gaucheron 2011 ^b
% DV ^a	20	2	14	22	1	1	
Tetilla	243	9.5	154	162	31	0.08	Cámara-Martos <i>et al.</i> , 2013
% DV ^a	24	2	15	6	1	1	
Torta del Casar	158	9.9	117	161	34	0.16	Cámara-Martos <i>et al.</i> , 2013
% DV ^a	15	3	12	6	1	1	

^aPercentage of daily value, %DV, based on energy and nutrient recommendations for a general 2000-calorie diet

^bGaucheron, F., 2011. Milk and dairy products: A unique micronutrient combination. *Journal of the American College of Nutrition* 30, 400-409.

Table 3. Angiotensin I-converting enzyme inhibitory activity of different cheese varieties and peptides identified as potentially responsible for the observed effects

Type of cheese	Analyzed sample	ACE inhibitory activity		Identified peptides	Reference
		(%)	IC ₅₀ (mg/mL)		
Appenzeller					Bütikofer <i>et al.</i> , 2007
Classic, 3-month old	WSE ^a		17.6	VPP; IPP	
Surchoix, 4-month old	WSE ^a		8.0	VPP; IPP	
Extra, 6-7-month old	WSE ^a		16.8	VPP; IPP	
1/4 fat, 3-month old	WSE ^a		7.1	VPP; IPP	
1/4 fat, 7-10-month old	WSE ^a		4.2	VPP; IPP	
Brie	WSE ^a		25.9	VPP; IPP	Bütikofer <i>et al.</i> , 2007
	WSE ^a			VPP; IPP; RYLG; RYLGY; HLPLP; LHLPLP	Stuknytė <i>et al.</i> , 2015
Cabrales	WSE ^a	74.7		PP; MPI; MPL; EVVR; REL; DKIHPI; DKIHPIF; FPGPIH; PGPIH;	Gómez-Ruiz <i>et al.</i> , 2006
	Fraction < 1 kDa	76.1		GPIH; PVEP; PVEPF	
Caprino	WSE ^a			VPP; IPP; RYLG; RYLGY; AYFYPEL; LHLPLP	Stuknytė <i>et al.</i> , 2015
Cheddar	WSE ^a			VPP; IPP; RYLG; RYLGY; HLPLP; AYFYPEL; LHLPLP	Stuknytė <i>et al.</i> , 2015
+ <i>Lb. casei</i> LAFTI [®] L26 (36 weeks)	WSE ^a	~82	0.25	RPKHPIKHQ; RPKHPIK; RPKHPI; DKIHPIF; FVAPFPEVF; KKYKVPQLE; YQEPVLGPVRGPFPIIV	Ong <i>et al.</i> , 2007 ^d
+ <i>Lb. acidophilus</i> LAFTI [®] L10 (24 weeks)	WSE ^a		0.13	ARHPPHP; RPKHPIKHQ; RPKHPIK; RPKHPI; FVAPFPEVF; YQEPVLGPVRGPFPIIV	Ong and Shah 2008
Crescenza	FPLC fraction	37		LVYFPFGPIHNSLPQ	Smacchi and Gobetti 1998
Edam	WSE ^a		13.3	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Emmental	WSE ^a			VPP; IPP	Stuknytė <i>et al.</i> , 2015
Classic, 4-month old	WSE ^a		10.5	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Organic, 4-month old	WSE ^a		7.5	VPP; IPP	
Reserved, 8-month old	WSE ^a		9.9	VPP; IPP	
Cave-aged, 12-month old	WSE ^a		7.1	VPP; IPP	
Feta	WSE ^a		14.3	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Fontina	WSE ^a			VPP; IPP; HLPLP	Stuknytė <i>et al.</i> , 2015
Gamlost	WSE ^a	~77	~2.5	SLVYFPFGPIPNLSLPQNIPPLTQTPVVVPPFLQPEVM;	Qureshi <i>et al.</i> , 2013 ^e
		~70	~0.5	VYFPFGPIPNLSLPQNIPPLTQTPVVVPPFLQPE; HKEMPFPKYPVEPFT; SLTLTDVENLHLPLPLL; NLHLPLPLL;	

		HLPLPLLQ; WMHQPHQPLPPTVMFPPQSVL; FLLYQEPVLGPVRGPFPIIV		
Goat cheese	WSE ^a	72.8	QP; PP; RPK; EEL; EEI; DKIHPP; DKIHPPF; PLTQTP; VVVPP;	Gómez-Ruiz <i>et al.</i> , 2006
	Fraction < 1 kDa	59.4	GVPK; TDVEK; VRGP	
Gouda	WSE ^a		VPP; IPP	Stuknytè <i>et al.</i> , 2015
8-month old	WSE ^a	75.7 ^c	RPKHPIKHQ; RPKHPIKHQGLPQ; YPFPGPIP; MPFPKYPVQPF	Saito <i>et al.</i> , 2000
Old	WSE ^a	2.0	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Gorgonzola	WSE ^a	21.8	VPP; IPP	Bütikofer <i>et al.</i> , 2007
	WSE ^a		VPP; IPP; AYFYPE; HLPLP; AYFYPEL; LHLPLP	Stuknytè <i>et al.</i> , 2015
Grana Padano	WSE ^a		VPP; IPP; RYLG; HLPLP; LHLPLP	Stuknytè <i>et al.</i> , 2015
Graubünden-full fat	WSE ^a	9.2	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Graubünden-½ fat	WSE ^a	8.9	VPP; IPP	
Graubünden-¼ fat	WSE ^a	4.8	VPP; IPP	
Gruyere-5-month old	WSE ^a	14.9	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Gruyere-8-month old	WSE ^a	18.7	VPP; IPP	
Gruyere-10-month old	WSE ^a	14.2	VPP; IPP	
Gruyere-12-month old	WSE ^a	29.4	VPP; IPP	
Hobelkäse	WSE ^a	2.6	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Idiazabal	WSE ^a	87.5	QP; FP; NINE; PSE; ERYL; PQL; EIVPK; AWY; DKIHPP; DKIHPPF	Gómez-Ruiz <i>et al.</i> , 2006
	Fraction < 1 kDa	68.5	QNALIVRYTR; KALPMHIRLAF; EGPKLVAS;	
	WSE ^a and ESE ^b		PVGLVQPASATLYDY; KVGTKCCAKP; REKVLASS;	Sagardia <i>et al.</i> , 2013 ^f
			PKIDAMREKVLA; PRKEKLCTTS; YQEPVLGPVRG;	
			NAGPFTPTVNREQLSTS	
Küsnachter	WSE ^a	19.6	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Limburger	WSE ^a	6.4	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Maasdam	WSE ^a		VPP; IPP; HLPLP; AYFYPEL; LHLPLP	Stuknytè <i>et al.</i> , 2015
Mahon	WSE ^a	76.8	PK; PQ; RI; RL; PQEVL; EVLN; NENLL; ENLL; NLLRF	Gómez-Ruiz <i>et al.</i> , 2006
	Fraction < 1 kDa	56.6		
Manchego	WSE ^a	70.6	QP; PP; FP; PFP; HPIK; HQGL; DKIHPP; DKIHPPF; TGPIP; VRYL; VPSERYL; VPSERY; DVPSERYLG; KKYNVPQL;	Gómez-Ruiz <i>et al.</i> , 2004a,b, 2006
	Fraction < 1 kDa	65.3	KKYNVPQ; IPY; TQOKTNAIPY; VRGPPF; PVRGPF; LEIVPK;	
			RPKHPI; VPKVKE; REQEEL; DVPSERY; VRGPPF; LPQNILP	
			VPP; IPP	
	WSE ^b	29.5		Bütikofer <i>et al.</i> , 2007

Mexican + <i>Lb. casei</i>	WSE ^a	95.5	5.3	YQEPVLGPVRGPFPI; YQEPVLGPVRGPFPIIV; FVAPFPEVFGK; EVLNENLLRF	Torres-Llanez <i>et al.</i> , 2011 ^g
Mexican + <i>En. faecium</i>	WSE ^a	96.3	10.4	YQEPVLGPVRGPFPIIV; RPKHPIKHQGLPQEV; RPKHPIKHQGLPQEVLENNLLR; FVAPFPEVFGK; EVLNENLLRF	
Münster	WSE ^a		14.1	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Parmino	WSE ^a		28.6	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Provolone	WSE ^a			VPP; IPP; RYLG; LHLPLP	Stuknytė <i>et al.</i> , 2015
Provolone picante	WSE ^a			VPP; IPP; RYLG; LHLPLP	Stuknytė <i>et al.</i> , 2015
Raclette-raw milk	WSE ^a		8.8	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Raclette-pasteurized milk	WSE ^a		9.0	VPP; IPP	
Reblonchon	WSE ^a		17.0	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Roncal	WSE ^a	85.8		QP; PP; RPKHP; PKHP; HPIK; HQGL; DKIHPP; DKIHPPF; GPVR	Gómez-Ruiz <i>et al.</i> , 2006
	Fraction < 1 kDa	70.4			
Roquefort	WSE ^a		10.2	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Roquefort-type cheese	WSE ^a		0.34	KEMPFKYPVE; WMHQPPQPLPPTVMFPPQSVL; MHQPPQPLPPTVMFPPQSVL; HQPPQPLPPTVMFPPQSVL; YQEPVLGPVRGPFPI; QEPVLGPVRGPFPIV; QEPVLGPVRGPFPI; PVLGPVRGPFPI; LGPVRGPFPI; TDAPSFSDIPNPIGSENSGK; DIPNPIGSENSGKTTMPLW; IPNPIGSENSGKIT; NAGPFTPTVNR; YQGPIVLNPWDQVQR; YQGPIVLNPWDQVK; GPIVLNPWDQVQR; VLNPWDQVQR	Meister Meira <i>et al.</i> , 2012
St. Paulin	WSE ^a		23.5	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Sbrinz	WSE ^a		16.4	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Taleggio	WSE ^a			VPP; IPP; HLPLP	Stuknytė <i>et al.</i> , 2015
Tete de Moine	WSE ^a		7.1	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Tilsit-raw milk	WSE ^a		4.4	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Tilsit-pasteurized milk	WSE ^a		18.0	VPP; IPP	
Vacherin fribourgeois	WSE ^a		5.0	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Vacherin Mont d'Or	WSE ^a		21.4	VPP; IPP	
Wangener Geissmutschli	WSE ^a		7.9	VPP; IPP	Bütikofer <i>et al.</i> , 2007
Winzerkäse	WSE ^a		9.9	VPP; IPP	Bütikofer <i>et al.</i> , 2007

^a Water soluble extract; ^b Ethanol soluble extract; ^c Antihypertensive effect in spontaneously hypertensive rats (Dose of 6.1-7.5 mg/kg produces a decrease in Systolic blood pressure (SBP) of -24.7mmHg

^d Ong, L., Henriksson, A., Shah, N. P., 2007. Angiotensin converting enzyme-inhibitory activity in Cheddar cheeses made with the addition of probiotic *Lactobacillus casei* sp. Lait 87, 149-165.

^e Qureshi, T. M., Vegarudm G. E., Abrahamsen, R. K., Skeie, S., 2013. Angiotensin I-converting enzyme-inhibitory activity of the Norwegian autochthonous cheeses Gamalost and Norvegia after in *vitro* human gastrointestinal digestion. Journal of Dairy Science 96, 838-853.

^f Sagardia, I., Iloro, I., Elortza, F., Bald, C., 2013. Quantitative structure-activity relationship based screening of bioactive peptides identified in ripened cheese. International Dairy Journal 33, 184-190.

^g Torres-Llanez, M. J., González-Córdova, A. F., Hernández-Mendoza, A., Garcia, H. S., Vallejo-Cordoba, B., 2011. Angiotensin-converting enzyme inhibitory activity in Mexican Fresco cheese. Journal of Dairy Science 94, 3794-3800.

Table 4. Phosphopeptides derived from α_{s1} -, α_{ss} - and β -casein identified in different cheese types. Each peptide sequence is described by the one letter code, superscripts numbers are referred to the amino acid position into the protein sequence when the number of the amino acids is lower than 25.

Type of cheese	Analyzed sample	α_{s1} -casein	α_{s2} -casein	β -casein	Reference
Beaufort-6 month old	Enrichment by IMAC-Fe (III)	⁶⁷ SpSpEEIVPN ⁷⁴ ⁷⁵ SpVEQKHIQK ⁸³ ¹⁰³ KYKVPQLEIVPNSpAE ¹¹⁷ ¹⁰⁴ YKVPQLEIVPNSpAEERLH ¹²¹ ¹⁰⁶ VPQLEIVPNSpAEERLH ¹²¹ ¹⁰⁶ VPQLEIVPNSpAEERLHSMK ¹²⁴ ¹⁰⁹ LEIVPNSpAEERLH ¹²¹ ¹¹¹ IVPNSpAEERLH ¹²¹ ¹¹¹ IVPNSpAEERLHSMK ¹²⁴ ¹¹² VPNSpAEERLH ¹²¹ ¹¹⁴ NSpAEERL ¹²⁰	¹³⁷ KTVDMESpTEVFTK ¹⁴⁹ ¹³⁷ KTVDMESpTEVFTKK ¹⁵⁰ ¹³⁷ KTVDMESpTEVFTKKT ¹⁵¹ ¹³⁸ KTVDMESpTEVFTKK ¹⁵⁰ ¹⁴¹ MESpTEVFTKK ¹⁵⁰ ¹⁴³ SpTEVFTKK ¹⁵⁰	¹² IVESpL ¹⁶ ¹⁴ ESpLSpSpSpEESITRIN ²⁷ ¹⁵ SLSpSpSpEESITR ²⁵ ¹⁵ SLSpSpSpEESITRIN ²⁷ ¹⁶ LSpSpSpEESITR ²⁵ ¹⁶ LSSpSpEESITR ²⁵ ¹⁶ LSSpSpEESITRIN ²⁷ ¹⁷ SSpSpEESITRIN ²⁷ ¹⁷ SSpSEESITRIN ²⁷ ²⁹ KIEKFQSpEE ³⁷ ²⁹ KIEKFQSpEEQQ ³⁹ ²⁹ KIEKFQSpEEQQQTE ⁴² ²⁹ KIEKFQSpEEQQQTED ⁴³ ²⁹ KIEKFQSpEEQQQTEDELQ ⁴⁶ ²⁹ KIEKFQSpEEQQQTEDELQDK ⁴⁸ ²⁹ KIEKFQSpEEQQQTEDELQDKIHPF ⁵² ²⁹ KIEKFQSpEEQQQTEDELQDKIHPFAQT ⁵⁵ ³⁰ IEKFQSpEEQQQTEDELQDK ⁴⁸ ³⁰ IEKFQSpEEQQQTEDELQDKIHPF ⁵² ³⁰ IEKFQSpEEQQQTEDELQDKIHPFAQTQ ⁵⁶ ³² KFQSpEEQQQTED ⁴³ ³² KFQSpEEQQQTEDELQD ⁴⁷ ³² KFQSpEEQQQTEDELQDKIHPF ⁵² ³³ FQSpEEQQQTEDELQDKIHPF ⁵² ³³ FQSpEEQQQTEDELQDKIHPFAQTQ ⁵⁶	Dupas <i>et al.</i> , 2009 ^b
Cheddar (mature)	WSE ^a	¹¹⁵ SpAEERLH ¹²¹ ¹¹⁵ SpAEERLHSMK ¹²⁴	⁶¹ SpAEVATEEVK ⁷⁰	⁷ NVPGEIVESLSpSp ¹⁸ ⁷ NVPGEIVESLSpSpSp ¹⁹ ¹⁰ GEIVESp ¹⁵ ²⁹ KIEKFQSpEEQQ ³⁹ ²⁹ KIEKFQSpEEQQ....QTQ ⁵⁶ ³⁰ IEKFQSpE ³⁶	Singh <i>et al.</i> , 1997

Emmental- 2 month old	WSE ^a		¹ KNTMEHVSpSpSpEESIISpQETYK ²¹ ¹ KNTMEHVSpSpSpEESIISpQETYKQEK ²⁴ ⁷ VSpSpSpEESIISpQETYK ²¹ ¹⁶ SpQET ¹⁹ ³⁵ LCSTFCKEVVRNA....SpSpSpEESpAEVAT ⁶⁶	⁷ NVPGEIVESLSpSpSpEESITRINK ²⁸ ⁸ VPGEIVESLSpSpSpEESITRINK ²⁸ ¹¹ EIVESpLSpSpSpEESITRINK ²⁸ ¹¹ EIVESpLSpSpSpEESITRINKK ²⁹ ¹¹ EIVESpLSpSpSpEESITRINKKIEK ³² ¹² IVESpLSpSpSpEESITRINK ²⁸ ¹² IVESLSpSpSpEESITRINK ²⁸ ¹³ VESLSpSpSpEESITRINK ²⁸ ¹⁵ SpLSpSpSpEESITR ²⁵ ¹⁵ SpLSpSpSpEESITRINK ²⁸ ¹⁶ LSpSpSpEESITR ²⁵ ¹⁶ LSpSpSpEESITRINK ²⁸ ¹⁸ SpSpEESITR ²⁵	Gagnaire <i>et al.</i> , 2001 ^c
Grana Padano-14 month old	Enrichment by precipitation with barium nitrate	⁶¹ EAESpISpSpSpEEIVPN ⁷⁴ ⁶¹ EAESpISpSpSpEEIVPNSpVEQK ⁷⁹ ⁶² AESpISpSpSpEEIVPN ⁷⁴ ⁶² AESpISpSpSpEEIVPNSpVEQK ⁷⁹ ⁶³ ESpISpSpSpEEIVPN ⁷⁴ ⁶³ ESpISpSpSpEEIVPNSpVEQK ⁷⁹ ⁶⁴ SpISpSpSpEEIVPN ⁷⁴ ⁶⁴ SISpSpSpEEIVPN ⁷⁴ ⁶⁴ SpISpSpSpEEIVPNSpVEEK ⁷⁹ ⁶⁵ ISpSpSpEEIVPN ⁷⁴ ⁶⁵ ISSpSpEEIVPN ⁷⁴ ⁶⁵ ISpSpSpEEIVPNSpVEEK ⁷⁹ ⁶⁷ SpSpEEIVPN ⁷⁴ ⁶⁷ SSpEEIVPN ⁷⁴	⁷ VSpSpSpEESIISpQE ¹⁸ ⁷ VSSpSpEESIISpQE ¹⁸ ⁷ VSpSpSpEESIISpQET ¹⁹ ⁷ VSpSpSpEESIISpQETYK ²¹ ⁸ SpSpSpEESIISpQE ¹⁸	⁷ NVPGEIVESpLSpSpSpEESITRINK ²⁸ ¹² IVESpLSpSpSpEESITR ²⁵ ¹² IVESpLSpSpSpEESITRINK ²⁸ ¹³ VESpLSpSpSpEESIT ²⁴ ¹³ VESpLSpSpSpEESITR ²⁵ ¹³ VESpLSpSpSpEESITRIN ²⁷ ¹³ VESpLSpSpSpEESITRINK ²⁸ ¹⁴ ESpLSpSpSpEESIT ²⁴ ¹⁴ ESpLSpSpSpEESITR ²⁵ ¹⁴ ESpLSpSpSpEESITRIN ²⁷ ¹⁴ ESpLSpSpSpEESITRINK ²⁸ ¹⁵ SLSpSpSpEES ²² ¹⁵ SpLSpSpSpEESIT ²⁴ ¹⁵ SLSpSpSpEESIT ²⁴ ¹⁵ SpLSpSpSpEESITR ²⁵ ¹⁵ SpLSpSpSpEESITRIN ²⁷ ¹⁵ SLSpSpSpEESITRIN ²⁷ ¹⁵ SpLSpSpSpEESITRINK ²⁸ ¹⁵ SLSpSpSpEESITRINK ²⁸ ¹⁶ LSpSpSpEES ²² ¹⁶ LSpSpSpEESIT ²⁴ ¹⁶ LSpSpSpEESITR ²⁵ ¹⁶ LSpSpSpEESITRIN ²⁷ ¹⁶ LSpSpSpEESITRINK ²⁸	Ferranti <i>et al.</i> , 1997

Grana Padano-2-23 month old	WSE			¹⁴ ESLSpSpSpEESITRINK ²⁸ ¹⁶ LSpSpSpEES ²² ¹⁶ LSpSpSpEESITR ²⁵ ¹⁶ LSpSpSpEESITRINK ²⁸	Sforza <i>et al.</i> , 2003
Gruyère de Comté-12 month old	WSE ^a		⁵ EHVSpSpSpEESIIS?QETYK ²¹ ⁶ HVSpSpSpEESIISpQETYK ²¹⁻ ⁷ VSpSpSpEESIIS?QETYK ²¹	¹³ VESpLSpSpSpEESITRINK ²⁸ ¹³ VESLSpSpSpEESITRINK ²⁸ ¹⁴ ESpLSpSpSpEESITRIN ²⁷ ¹⁴ ESpLSpSpSpEESITRINK ²⁸ ¹⁴ ESLSpSpSpEESITRINK ²⁸ ¹⁵ SpLSpSpSpEESITRIN ²⁷ ¹⁵ SLSpSpSpEESITRIN ²⁷ ¹⁵ SpLSpSpSpEESITRINK ²⁸ ¹⁵ SLSpSpSpEESITRINK ²⁸ ¹⁶ LSpSpSpEESITRIN ²⁷	Roudot-Algaron <i>et al.</i> , 1994
Herrgard-10 month old	Enrichment by IMAC-Fe (III)	⁴⁰ LSKDIGSpESpTEDQAME ⁵⁵ ⁴² KDIGSpESpTE ⁵⁰ ⁴² KDIGSpESpTED ⁵¹ ⁴² KDIGSpESpTEDQAM ⁵⁴ ⁴³ DIGSpESpTE ⁵⁰ ⁴³ DIGSpESpTED ⁵¹ ⁴⁴ IGSpE ⁴⁷ ⁴⁴ IGSpESpTE ⁵⁰ ⁴⁴ IGSpESpTED ⁵¹ ⁴⁴ IGSpESpTEDQ ⁵² ⁴⁴ IGSpESpTEDQAM ⁵⁴ ⁶¹ EASpISpSpSpEEIVPNSpVQEK ⁷⁹ ⁶² AESpISpSpSpEEIVPNSpVQEK ⁷⁹ ⁶⁴ SpISpSpSpEEIVPN ⁷⁴ ⁷⁵ SpVEQK ⁷⁹ ⁷⁵ SpVEQKH ⁸⁰ ⁷⁵ SpVEQKHIQ ⁸² ⁷⁵ SpVEQKHIQKE ⁸⁴ ¹¹¹ IVPNSpAEER ¹¹⁹ ¹¹² VPNSpAE ¹¹⁷ ¹¹² VPNSpAEER ¹¹⁹ ¹¹⁵ SpAEER ¹¹⁹	⁵ EHVSpSpSpEESIISpQE ¹⁸ ⁵ EHVSpSpSpEESIISpQETY ²⁰ ⁵ EHVSpSpSpEESIISpQETYK ²¹ ⁶ HVSpSpSpEESIISpQE ¹⁸ ⁶ HVSpSpSpEESIISpQETYK ²¹ ¹⁴ IISpQE ¹⁸ ¹⁴ IISpQET ¹⁹ ¹⁴ IISpQETYK ²¹ ¹⁵ ISpQET ¹⁹ ¹⁵ ISpQETYK ²¹ ¹⁶ SpQETYK ²¹ ¹²⁸ LSpTSpEE ¹³³ ¹²⁹ SpTSEENSppKKTVD ¹⁴⁰ ¹⁴¹ MESpTEVF ¹⁴⁷ ¹⁴¹ MESpTEVFTK ¹⁴⁹	⁸ VPGEIVESpLSpSpSpEESITRINK ²⁸ ¹⁰ GEIVESpLSpSpSpEESITRINK ²⁸ ¹¹ EIVESpLSpSpSpEE ²¹ ¹¹ EIVESpLSpSpSpEESITRINK ²⁸ ¹² IVESpLSpSpSpEE ²¹ ¹² IVESpLSpSpSpEES ²² ¹² IVESpLSpSpSpEESITR ²⁵ ¹² IVESpLSpSpSpEESITRINK ²⁸ ¹³ VESpLSpSpSpEE ²¹ ¹³ VESpLSpSpSpEESITR ²⁵ ¹⁵ SpLSpSpSpEESITR ²⁵ ²⁹ KIEKFQSpEEQQQTED ⁴³ ²⁹ KIEKFQSpEEQQQTED ⁴⁶ ³⁰ IEKFQSpEEQQQ ⁴⁰ ³⁰ IEKFQSpE ³⁶ ³⁰ IEKFQSpEE ³⁷ ³⁰ IEKFQSpEEQQ ³⁹ ³⁰ IEKFQSpEEQQQTED ⁴³ ³⁰ IEKFQSpEEQQQTED ⁴⁶ ³² KFQSpEE ³⁷ ³² KFQSpEEQ ³⁸ ³² KFQSpEEQQQTED ⁴³	Lund and Ardö, 2004 ^d

		¹¹⁵ SpAEERL ¹²⁰ ¹¹⁵ SpAEERLH ¹²¹ ¹¹⁵ SpAEERLHS ¹²² ¹¹⁵ SpAEERLHSMK ¹²⁴		³³ FQSpE ³⁶ ³³ FQSpEE ³⁷ ³³ FQSpEEQQQ ⁴⁰	
Parmigiano-Reggiano-2 month old	WSE ^a		³ TMEHVS pSpSp PEESIISQE...PSKE ³³	⁸ VPGEIVES pL ¹⁶ ⁹ PGEIVES pL ¹⁶ ¹⁷ SpSpSp PEES ²² ²² SITRYNKKIEKFQSpEEQQQ....KIHPPAQ ⁵⁴	Addeo <i>et al.</i> , 1992
Parmigiano-Reggiano-6 month old	WSE ^a	⁴¹ SKNIG....SIS pSpSp PEEIVPNS ⁷⁵	⁴ MEHVS pSpSp PEESIISQE...PSKENLC ³⁶		Addeo <i>et al.</i> , 1992
Parmigiano-Reggiano-15 month old	WSE ^a		⁴ MEHVS pSpSp PEESIISQE...PSKENLC ³⁶		Addeo <i>et al.</i> , 1992
Parmigiano-Reggiano (Evolution 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 month old)	Enrichment by acid precipitation			¹¹ EIVES pLSpSpSp PEESITRINK ²⁸ ¹² IVES pLSpSpSp PEESITRINK ²⁸ ¹³ VES pLSpSpSp PEESITRINK ²⁸ ¹⁴ ES pLSpSpSp PEESITRINK ²⁸ ¹⁵ SpLSpSpSp PEESITRINK ²⁸ ¹⁵ SL pSpSp PEESITRINK ²⁸ ¹⁶ L pSpSp PEESITR ²⁵ ¹⁶ L pSpSp PEESITRINK ²⁸ ¹⁷ SpSpSp PEESITR ²⁵	Sforza <i>et al.</i> , 2012
Parmigiano-Reggiano-24 month old	Enrichment by IMAC-Fe (III)	⁶⁴ SIS pSpSp PEEIVPN ⁷⁴ ⁶⁵ IS pSpSp PEE ⁷⁰ ⁶⁵ IS pSpSp PEEIVPN ⁷⁴ ⁶⁷ SpSp PEEIVPN ⁷⁴ ¹¹⁵ SpAEERLH ¹²¹ ¹¹⁵ SpAEERLHSMK ¹²⁴	⁷ V pSpSp PEES ¹³	¹⁶ L pSpSp PEES ²²	Lund and Ardö 2004 ^d
Ragusano	WSE ^a	⁷⁵ Sp VEQKHQKE ⁸⁴ ¹⁰³ KYKVPQLEIVPNS pAE ¹¹⁷ ¹⁰⁴ YKVPQLEIVPNS pAE ¹¹⁷ ¹¹⁵ SpAEERLHSM ¹²³	¹ KNTMEHVS pSpSp PEESIIS pQE ¹⁸ ¹ KNTMEHVS pSpSp PEESIIS pQET YK ²¹ ¹⁴¹ MES pTEVFTKK ¹⁵⁰ ¹⁴³ SpTEVFTKK ¹⁵⁰	⁷ NVPGEIVES pLSpSpSp PEESITRINK ²⁸ ¹⁵ ISL pSpSp PEESITRINK ²⁸ ¹⁷ SpSpSp PEESITRINK ²⁸ ²⁸ KKIEKFQSpEEQQQTEDELQ ⁴⁶ ²⁸ KKIEKFQSpEEQQQTEDELQDKIHPP ⁵² ²⁹ KIEKFQSpEEQQ ³⁹	Gagnaire <i>et al.</i> , 2011

				²⁹ KIEKFQSpEEQQQ ⁴⁰ ²⁹ KIEKFQSpEEQQQTED ⁴³ ²⁹ KIEKFQSpEEQQQTEDEL ⁴⁵ ²⁹ KIEKFQSpEEQQQTEDELQ ⁴⁶ ²⁹ KIEKFQSpEEQQQTEDELQD ⁴⁷ ²⁹ KIEKFQSpEEQQQTEDELQDKIHP ⁵¹ ²⁹ KIEKFQSpEEQQQTEDELQDKIHPF ⁵² ²⁹ KIEKFQSpEEQQQTEDELQDKIHPFAQTQ ⁵⁶ ³⁰ IEKFQSpEEQQQTEDELQDKIHPF ⁵² ³² KFQSpEEQQ ³⁹ ³² KFQSpEEQQQ ⁴⁰ ³² KFQSpEEQQQTE ⁴² ³² KFQSpEEQQQTED ⁴³ ³² KFQSpEEQQQTEDELQ ⁴⁶ ³² KFQSpEEQQQTEDELQD ⁴⁷ ³² KFQSpEEQQQTEDELQDKIHPF ⁵² ³² KFQSpEEQQQTEDELQDKIHPFAQTQ ⁵⁶ ³³ FQSpEEQQQTED ⁴³	
Ragusano-4 month old	WSF ^a	⁴¹ SKDIGSpESpTEDQ ⁵² ⁴¹ SKDIGSpESpTEDQAM ⁵⁴ ⁴¹ SKDIGSpESpTEDQAMED ⁵⁶ ⁷⁵ SpVEQKHIQ ⁸² ⁷⁵ SpVEQKHIQKE ⁸⁴		¹ REL...IVESpLSpSpSpE...SpEEQQQTEDELQ ⁴⁶ ¹¹ EIVESpLSpSpSpEESITRINK ²⁸ ¹² IVESpLSpSpSpEESITRINK ²⁸ ¹⁵ ISpLSpSpSpEESITRINK ²⁸ ¹⁷ SpSpSpEESITRINK ²⁸ ²⁹ KIEKFQSpEEQQQTEDELQDKIHPF ⁵² ²⁹ KIEKFQSpEEQQQTEDELQDKIHPFAQTQ ⁵⁶ ³² KFQSpEEQQQ ⁴⁰ ³² KFQSpEEQQQTED ⁴³ ³² KFQSpEEQQQTEDELQDKIHPF ⁵²	Gagnaire <i>et al.</i> , 2011
Ragusano-7 month old	WSF ^a	⁴¹ SKDIGSpESpTEDQAM ⁵⁴ ⁴¹ SKDIGSpESpTEDQAMED ⁵⁶ ⁴² KDIGSpESpTEDQAMED ⁵⁶ ⁷⁵ SpVEQKHIQ ⁸²	¹²⁶ EQLSpTSEENSpKKT ¹³⁸	¹¹ EIVESpLSpSpSpEESITRINK ²⁸ ¹² IVESpLSpSpSpEESITRINK ²⁸ ¹⁷ SpSpSpEESITRINK ²⁸ ²⁹ KIEKFQSpEEQQQTEDELQDKIHPF ⁵²	Gagnaire <i>et al.</i> , 2011

^a Water soluble extract; Sp: Ser phosphorylated

^b Dupas, C., Adt, I., Cottaz, A., Boutrou, R., Molle, D., Jardin, J., Jouvet, T., Degraeve, P., 2009. A chromatographic procedure for semi-quantitative evaluation of caseinphosphopeptides in cheese. Dairy Science Technology 89, 519-529.

^c Gagnaire, V., Molle, D., Herrouin, M., Leonil, J. 2001. Peptides identified during Emmental cheese ripening: Origin and proteolytic systems involved. Journal of Agricultural and Food Chemistry 49, 4402-4413.

^d Lund, M., Ardö, Y., 2004. Purification and identification of water soluble phosphopeptides from cheese using Fe(III) affinity chromatography and mass spectrometry. *Journal of Agricultural and Food Chemistry* 52, 6616-6622.