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Detailed peptide profiling of “Scotta”: from a dairy waste to a source of potential health-promoting compounds

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Abstract

“Scotta” is a liquid waste deriving from Ricotta cheese production, which is wrongly considered only a dairy by-product. In this work, with the aim to elucidate the presence of valuable bioactive compounds in Buffalo’s Scotta, a peptide fraction under 3000 Da was isolated by ultra-filtration, purified by solid-phase extraction, and, subsequently, characterized in detail by liquid chromatography coupled to Orbitrap mass spectrometry. Analytical results revealed a complex profile, leading to the identification of 226 peptides, belonging to alpha, beta, and kappa caseins. A database-driven search approach was used to assess the biological effects of some of the identified peptides. A wide range of healthy properties was ascribed to the encrypted peptides, comprising antihypertensive, antimicrobial, immunomodulating, opioid, antioxidant, and antithrombotic. The peptidomic profile of Scotta was highlighted in depth for the first time, and the results revealed that this matrix should not be considered only a mere by-product, but a source of potential health-promoting peptides, which can be recovered and employed in nutraceuticals and functional foods.

Introduction

Food waste and by-products represent one of the main challenges for agro-food industries, which must face demanding economic costs for their treatment and disposal. Moreover, they can be a risky source of pollution, especially when illegal methods are employed. In this regard, dairy industry produces, yearly, tons of by-products, whose main component is whey, which corresponds to the liquid fraction remaining after milk clotting. Whey constitutes a huge problem for disposal due to its elevated content of organic matter and associated high biochemical oxygen demand (BOD). On the other hand, whey has been recognized as a source of functional and bioactive compounds such as lactose, minerals, and, especially, proteins (Brandelli et al. 2015). In Italy, a small percentage of whey is employed for the production of Ricotta cheese, obtained by heating of the cheese whey at 85–90 °C, thus leading to the precipitation and separation of whey proteins. The liquid fraction that remains after the cheese separation is called “Scotta,” which is the main by-product of Ricotta production process. Scotta is generally composed by lactose (4.8–5.0%), salts (1.0–1.13%), and proteins (0.15–0.22%) (Secchi et al. 2012). This by-product is considered, as well as whey, a significant source of pollution, possessing high values of BOD. While a weak interest in the recovery of Scotta has been focused on its conversion in bio-fuel and biotechnological substrate for fermented products (Sansonetti et al. 2009), almost nothing is known about the low-medium peptide fraction of this matrix so far. Since numerous bioactive peptides can be found in whey, a similar dairy waste, our study was focused on the identification of the peptides present in this by-product and the

investigation, through database-driven approach, of their potential bioactivity. With the aim to elucidate the peptide profile of Scotta, in particular, the one deriving from buffalo ricotta-cheese processing, we carried out an in-depth characterization through a liquid chromatography-high-resolution mass spectrometry (LC-HRMS) approach. A peptide fraction of 3000 Da was isolated and purified through ultrafiltration and solid-phase extraction (SPE); subsequently, the peptides were identified by LC-Orbitrap-based tandem mass spectrometry (MS/MS) with the help of online-driven database search for bioactivity assessment. A large number of potential bioactive peptides have been identified, still encrypted in the precursor oligopeptides, many with established health-promoting properties, including antihypertensive, antimicrobial, immunomodulatory, and antioxidant. These results could drive the dairy industry to its recovery, in order to re-evaluate this waste product as a possible source for nutraceuticals and personalized functional foods.

Material and methods

Chemicals

Ultra pure water (H_2O) was obtained by a Milli-Q system (Millipore, Milan, Italy). The following chemicals were all purchased from Sigma-Aldrich (Milan, Italy): acetonitrile (ACN) and formic acid (HCOOH) LC–MS grade. Centrifugal Filter Devices Amicon® Ultra-4 3K and 10K and Strata-X™ polymeric reversed phase SPE (500 mg/3 mL) cartridge were purchased from Millipore© and Phenomenex® (Castel Maggiore, Bologna, Italy), respectively, while filter paper Whatman® 540 from Sigma-Aldrich. Standard peptide mixture was purchased from Sigma-Aldrich.

Sampling and sample preparation

Buffalo's Scotta samples (500 mL each) were kindly donated by Di Lascio dairy factory (Capaccio, SA, Campania, Italy). The extraction was carried out in duplicate. Two samples were prepared by separating the residual fat fraction: 50 mL of Scotta was subjected to centrifugation at 4000×*g* for 30 min at 4 °C (Mikro 220R, Hettich, Germany) and further filtered by a filter paper.

Peptide fraction collection

Ultrafiltration was carried out by Millipore's Amicon® Ultra-4 centrifugal filter devices at different cutoffs. A preliminary filtration was carried out for all the samples using filters with 10,000 nominal molecular weight limit (NMWL), and subsequently, 4 mL of permeate was loaded on devices with 3000 NMWL. The devices were centrifuged for 40 min at 6000 rpm at 25 °C, using a centrifuge with fixed angle rotor (35°). In order to recover the peptides, the devices were washed with 4 mL of acidified water at pH 3.0 by formic acid. A peptide fraction, roughly 3.5 mL, with molecular weight ≤3000 Da was collected, filtered through a 0.45-µm pore cellulose membrane (Millipore©), and lyophilized for 24 h (LyoQuest-55, Telstar Technologies, Spain).

Solid-phase extraction

To remove salts and sugars, we employed a polymeric reversed phase cartridge, since this sorbent was more efficient in the purification of the peptide fraction instead of C18 cartridge (data not shown). The peptide fraction was solubilized in 0.1% (*v/v*) aqueous trifluoroacetic acid (TFA) and loaded on a Strata-X™ reversed phase polymeric SPE cartridge (Phenomenex®), previously equilibrated in 0.1% TFA, then eluted with 70/30/0.1 ACN/water/TFA (*v*/*v*/*v*), and finally re-lyophilized and stored at −20 °C. Lyophilized samples were solubilized in water and injected.

LC-HRMS analysis of peptide fraction

Peptide separation and identification were performed on an Accela 600 LC system coupled on-line to an LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany) through an electrospray source. For peptide separation, an Ascentis® Express Peptide ES C18 150 mm × 2.1 mm (*L* × ID), 2.7 μm 160 Å (Supelco, Bellefonte, PA, USA), was employed. Mobile phases were 0.1% HCOOH in H_2O *v*/*v* (A) and ACN plus 0.1% HCOOH *v*/*v* (B). LC gradient was the following: 0–22 min, 0–30% B, 22–27 min, 30–70% B, 27–28 min, 70–95% B, isocratic for 1 min, 29–34 min, returning to 0% B. Flow rate was set to 0.3 $mL.min^{-1}$. Column oven was set to 45 °C. Two microliters of sample was injected. For the MS part, spray voltage was set at +3.5 kV, sheath gas arbitrary units 30, auxiliary gas arbitrary units 10, and capillary temperature 250 °C. MS/MS spectra were collected in data-dependent mode, over the *m*/*z* range of 300–2000, at 30,000 resolution. All MS/MS spectra were collected using a normalized collision energy of 35% and an isolation window of 2 *m*/*z*, minimum signal threshold 150, and monoisotopic precursor enabled. Ion trap and Orbitrap maximum ion injection times were set to 50 and 100 ms, respectively. Automatic gain control was used to prevent over-filling of the ion traps and was set to 2×10^5 for full FTMS scan and 3×10^4 ions in MS/MS mode for the linear ion trap. Dynamic exclusion was enabled with a repeat count of 1 and a repeat duration of 30 s, list size 50, with exclusion duration of 30 s. All parameters were optimized by infusion of a standard peptide mixture at 20 $\mu L.min^{-1}$.

Peptide sequence identification

Raw MS/MS data files from Xcalibur software (Thermo Fisher Scientific) were converted in mzXML format, and a free trial of PEAKS 7.5 software (Bioinformatics Solutions Inc., Waterloo, Canada) was employed for sequence determination. Search was performed using a database search tool, by searching against Swiss-Prot/UniProt database (Release 2015_11) taxonomy mammals, with an improved algorithm that validates and assists the database search with de novo sequencing results. For database search, the following settings were chosen: digestion with no enzyme and peptide charges from +1 to +4, precursor mass search type: monoisotopic, fragmentation mode: CID (y and b ions), and precursor mass tolerance 15 ppm and fragment mass tolerance of 0.5 Da; oxidation (M) and phosphorylation (S, T, Y) were used as dynamic modifications; no static modification was selected. The LC-MS/MS injections were performed in triplicate. Identifications were accepted if the peptides were detected in at least two of the three replicates. To assess the bioactivity, all identified peptides were searched against the following free online databases: BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep), milkAMP (http://milkampdb.org/home.php), and EROP-Moscow (http://erop.inbi.ras.ru/).

Results and discussion

Sample isolation and peptide sequence determination

The growing interest in health-promoting molecules from food matrices pushes the research in the recovery of these bioactive compounds also from food processing materials and by-products. MS-based peptidomic approaches are the best strategies to characterize and monitor dairy bioactive peptides (Sánchez-Rivera et al. 2014). The interest in Scotta has been mainly restricted to the possible conversion of the matrix into bioethanol or for lactose production (Pisponen et al. 2013). Contrariwise, in this study, we focused on the isolation of peptides under 3000 Da, since many bioactive casein peptides are comprised in this range (Sánchez-Rivera et al. 2014). Peptide identification was performed through LC-MS/MS. The total ion chromatogram (TIC) relative to the separation of the isolated peptide fraction is depicted in Fig. 1. The complete list of peptides including

retention time, peptide sequence, precursor protein, position, and mass is reported in supplementary Table S1. As it can be appreciated from the TIC, a very complex profile was obtained. Despite this, the employment of a fused-core particle column provided a satisfactory and fast separation with an analysis time of 34 min, which led to the identification of 226 peptides, belonging to buffalo caseins (CN) α_{s1}, β, and k (UniProt Entries: O62823, Q9TSI0, P11840). No peptides belonging to α_{s2}-CN were detected, neither peptides deriving from whey proteins, since the thermal coagulation process leads to their incorporation into cheese. It is noteworthy that the only previous investigation on Scotta led to the identification of only 29 peptides (De Simone et al. 2009). This significant difference cannot be solely explained by a different processing method used by the factory but should be ascribed to a different analytical setup. All the previously identified peptides have been detected in this investigation. The suitability of the Orbitrap-MS analyzers for peptidomics has been, in fact, widely demonstrated (Capriotti et al. 2016).

The peptidomic profile of Scotta

Most peptides originated from β-CN (47%) followed by α_{s1} (40.5%) and k (12.5%). These data confirm a major degradation of β-CN, in particular at the C-terminal portion with $L^{192}Y^{193}$ and Y^{193}-Q^{194} residues together with the N-terminal portion with A^{1}-R^{2} residues, which are more susceptible to cleavage, probably by amino- and carboxypeptidases, as observed previously (Baum et al. 2013). Also, α_{s1}-CN was prone to degradation at the N-terminal portion, within the region comprising residues A^{1}-R^{2} and, in particular, F^{23}-F^{24} and F^{24}-V^{25}, which are known to be subject of cleavage by chymosin and cathepsin B, respectively. Kappa-CN was mainly represented by peptides deriving from k-CN macropeptide f(106–169), especially in the region containing residues N^{113}-Q^{114}. The sequence coverage was 83% for β-CN, 49% for α_{s1}-CN, and 41% for k-CN (supplementary Fig. S1). All the identified peptides were comprised in the range of 500–3000 Da (Fig. 2c).

Potential bioactive peptides in Scotta

As shown in Fig. 2a, b, a wide range of health-promoting properties can be attributed to Scotta peptides, many of them showing multifunctional activity. Table 1 shows the identified bioactive peptides reported in literature. It must be pointed out that many bioactive sequences are still encrypted in precursor oligopeptides, but they can be further released by the action of peptidases, as often occurs in gastrointestinal digestion process.

ACE inhibitory peptides

Numerous recognized anti-ACE peptides have been identified. Among them, the αs_1-CN peptide with sequence FVAPFPEVFG f(24–33) has been reported to exhibit strong ACE inhibitory activity (Robert et al. 2004), together with a large number of β-CN-derived peptides, for instance, NLHLPLPLLQ f(132–141), which contains the sequence LHLPLP that showed antihypertensive effect in rats (Miguel et al. 2006), and SLPQNIPPLTQTPV f(69–82) (Yamamoto et al. 1994). Neither of them was detected in the previous investigation on Scotta.

Antimicrobial peptides

Also, antimicrobial peptides (AMPs) were found in the sample. In this regard, particularly useful was the search against the milkAMP database (Théolier et al. 2014). Several fragments belong to α_{s1}-CN f(1–23), which shares some residues with the cow variant f(1–23) (isracidin) reported as antimicrobial (Lahov and Regelson 1996) and known to be subjected to chymosin cleavage. The shorter peptides, such as the caseicin B with sequence VLNENLLR f(15–22), showing activity against *Escherichia coli* at very low concentration (Hayes et al. 2006), could derive both from lactic

acid bacteria peptidases and from rennet enzyme action. The α_{s1} buffalo variant fragment 1–23 presents two amino acid substitutions (H^4-Q and E^{14}-G); therefore, the activity of these sequences should be confirmed. Among potential AMPs, numerous k-CN-derived fragments were found. In particular, the peptide AVRSPAQIL f(65–73) and the N-terminal portion f(105–122), deriving from peptide Kappacin f(106–169), were detected (López-Expósito et al. 2006), together with another peptide, namely QVTSTVV f(162–168), which is very similar to the C-terminal portion of Kappacin, which has been reported to possess antimicrobial activity. The antimicrobial activity of peptide QVTSTVV should be further investigated, since the buffalo variant possesses an amino acid variation (V^{167}-A). Moreover, an antithrombotic function was reported for the k-CN peptide MAIPPKKNQ f(105–113) (Jolles et al. 1986). Kappa-CN fragments f(65–73) and f(105–113) had not been detected before in Scotta.

Multifunctional peptides

Several peptides identified in the present investigation are characterized by a multifunctional activity. A clear example is casecidin 17, β-CN f(193–209), which exerts both anti-ACE (Yamamoto et al. 1994) and immunomodulating activities (Coste et al. 1992). In addition, also, the β-CN peptide LVYPFPGPIPK f(58–68) showed both anti-ACE and opioid activities, similarly to the α_{s1}-CN f(86–99) VPSERYLGYLEQLL, which includes α_{s1}-CN f(90–95) peptide with sequence RYLGYL, known as α-Casomorphin 6 (Loukas et al. 1983). The latter has not been previously reported in Scotta. Also present were antioxidant peptides, such as β-CN sequence f(98–106) and α_{s1}-CN f(80–90) (Gupta et al. 2010). Many other sequences have been identified in the sample, which are reported to provide other possible health benefits. To perform their activity, peptides must be absorbed in the intestinal tract and reach the systemic circulation. Nevertheless, if not absorbed, they can exert their functions on the local tissue or by binding to intestinal receptors. In this regard, several of the identified peptides have been detected in vivo, in the gastrointestinal tract, of both animal models and humans, after digestion of dairy matrices (Barbé et al. 2014; Boutrou et al. 2013). The activity of the identified peptides has been described by in vitro models in many of the reported papers; for this reason, in vivo and bioavailability studies are mandatory to understand in depth their mechanisms. Furthermore, many of the identified caseins peptides possess a high homology with the cow variant; thus, their activity may be very similar but should be further investigated. The isolation and purification of the different peptide fractions will be the following steps to elucidate and confirm the predicted biologic activity, together with the quantification of the most relevant peptides by multiple reaction monitoring (MRM) techniques. Although the protein content in Scotta is low, on average, 0.2%, high amounts of peptides can be easily recovered, since the production of this by-product can reach 1.0 Mt per year, and could be employed in nutraceuticals or personalized and enriched functional foods.

Conclusion

Similarly to whey, Scotta cannot be considered only a mere waste product of dairy industry. Our approach, based on the isolation of a peptide fraction ≤3000 Da, and subsequent LC-HRMS-based method have revealed a high complex profile. The peptidomic analysis highlighted a wide presence of valuable potential bioactive peptides, with recognized possible health benefits. These results could drive the dairy industry toward the recovery of Scotta, in order to turn this waste product into a possible source of bioactive peptides that could be potentially included in nutraceutical formulations and functional foods.

References

1. Barbé F, Le Feunteun S, Rémond D, Ménard O, Jardin J, Henry G, Laroche B, Dupont D (2014) Tracking the in vivo release of bioactive peptides in the gut during digestion: mass spectrometry peptidomic characterization of effluents collected in the gut of dairy matrix fed mini-pigs. Food Res Int 63:147–156
2. Baum F, Fedorova M, Ebner J, Hoffmann R, Pischetsrieder M (2013) Analysis of the endogenous peptide profile of milk: identification of 248 mainly casein-derived peptides. J Proteome Res 12:5447–5462
3. Boutrou R, Gaudichon C, Dupont D, Jardin J, Airinei G, Marsset-Baglieri A, Benamouzig R, Tome´ D, Leonil J (2013) Sequential release of milk protein-derived bioactive peptides in the jejunum in healthy humans. Am J Clin Nutr 97:1314–1323
4. Brandelli A, Daroit DJ, Folmer Corrêa AP (2015) Whey as a source of peptides with remarkable biological activities. Food Res Int 73:149–161
5. Capriotti AL, Cavaliere C, Piovesana S, Samperi R, Laganà A. (2016) Recent trends in the analysis of bioactive peptides in milk and dairy products. Anal Bioanal Chem 1–9 doi: 10.1007/s00216-016-9303-8
6. Coste M, Rochet V, Léonil J, Mollé D, Bouhallab S, Tomé D (1992) Identification of C-terminal peptides of bovine β-casein that enhance proliferation of rat lymphocytes. Immunol Lett 33:41–46
7. De Simone C, Picariello G, Mamone G, Stiuso P, Dicitore A, Vanacore D, Chianese L, Addeo F, Ferranti P (2009) Characterisation and cytomodulatory properties of peptides from Mozzarella di Bufala Campana cheese whey. J Pept Sci 15:251–258
8. Gupta A, Mann B, Kumar R, Sangwan RB (2010) Identification of antioxidant peptides in Cheddar cheese made with adjunct culture Lactobacillus casei ssp. Casei 300. Milchwissenschaft 65:396–399
9. Hayes M, Ross RP, Fitzgerald GF, Hill C, Stanton C (2006) Casein-derived antimicrobial peptides generated by *Lactobacillus acidophilus* DPC6026. Appl Environ Microbiol 72:2260–2264
10. Jolles P, Levy-Toledano S, Fiat AM, Soria C, Gillessen D, Thomaidis A, Dunn FW, Caen JP (1986) Analogy between fibrinogen and casein effect of an undecapeptide isolated from rc-casein on platelet function. Eur J Biochem 158:379–382
11. Lahov E, Regelson W (1996) Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides. Food Chem Toxicol 34:131–145
12. López-Expósito I, Minervini F, Amigo L, Recio I (2006) Identification of antibacterial peptides from bovine kappa-casein. J Food Prot 69:2992–2997
13. Loukas S, Varoucha D, Zioudrou C, Streaty RA, Klee WA (1983) Opioid activities and structures of alpha-casein-derived exorphins. Biochemistry 22:4567–4573
14. Miguel M, Recio I, Ramos M, Delgado MA, Aleixandre MA (2006) Antihypertensive effect of peptides obtained from *Enterococcus faecalis*-fermented milk in rats. J Dairy Sci 89:3352–3359
15. Pisponen A, Pajumägi S, Mootse H, Karus A, Poikalainen V (2013) The lactose from Ricotta cheese whey: the effect of pH and concentration on size and morphology of lactose crystals. Dairy Sci Technol 93:477–486
16. Robert MC, Razaname A, Mutter M, Juillerat MA (2004) Identification of angiotensin-I-converting enzyme inhibitory peptides derived from sodium caseinate hydrolysates produced by Lactobacillus helveticus NCC 2765. J Agric Food Chem 52:6923–6932
17. Sánchez-Rivera L, Martínez-Maqueda D, Cruz-Huerta E, Miralles B, Recio I (2014) Peptidomics for discovery, bioavailability and monitoring of dairy bioactive peptides. Food Res Int 63:170–181
18. Sansonetti S, Curcio S, Calabrò V, Iorio G (2009) Bio-ethanol production by fermentation of ricotta cheese whey as an effective alternative non-vegetable source. Biomass Bioenergy 33:1687–1692

19. Secchi N, Giunta D, Pretti L, Ruiz García M, Roggio T, Mannazzu I, Catzeddu P (2012) Bioconversion of ovine *scotta* into lactic acid with pure and mixed cultures of lactic acid bacteria. J Ind Microbiol Biotechnol 39:175–181
20. Théolier J, Fliss I, Jean J, Hammami R (2014) MilkAMP: a comprehensive database of antimicrobial peptides of dairy origin. Dairy Sci Technol 94:181–193
21. Yamamoto N, Akino A, Takano T (1994) Antihypertensive effect of the peptides derived from casein by an extracellular proteinase from Lactobacillus helveticus CP790. J Dairy Sci 77:917–922

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Ethics declarations

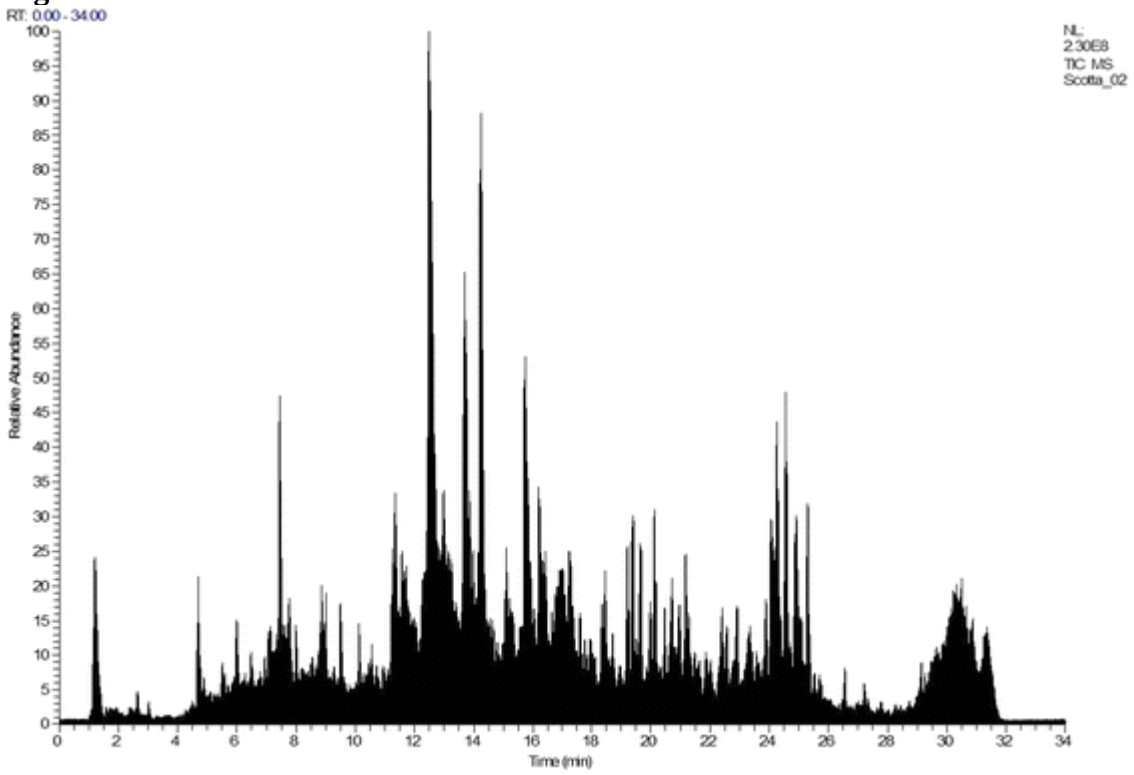
Conflict of interest

The authors declare that they have no conflict of interests.

Ethical approval

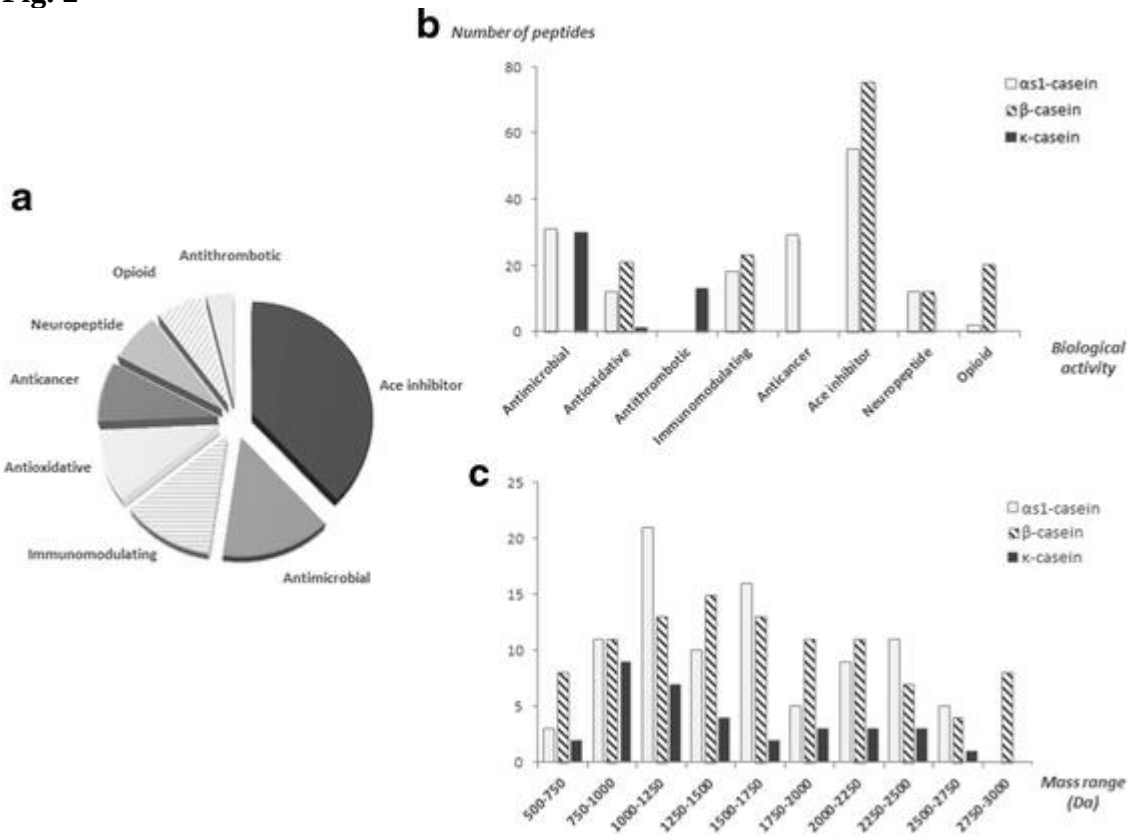
This article does not contain any studies with human participants or animals performed by any of the authors.

Fig. 1



Total ion chromatogram relative to the analysis of peptide fraction ≤3000 Da of Scotta. Column: Ascentis® Express Peptide ES C18 150 mm × 2.1 mm, 2.7 μm 160 Å. Detection: Orbitrap-MS

Fig. 2



Bioactive properties of identified peptides (**a**), relative contribution of each protein and corresponding peptides (**b**), and mass range order of the identified peptides (**c**)

Table 1 Bioactive peptides identified in Scotta

Activity	Position	Casein	Peptide Sequence	References
ACE inhibitor				
	24–33	α_{s1}	FVAPFPEVFG	Robert et al. (2004)
	23–34	α_{s1}	FFVAPFPEVFGK	Robert et al. (2004)
Antimicrobial				
	15–22	α_{s1}	VLNENLLR	Hayes et al. (2006)
ACE inhibitor, immunomodulating				
	193–209	β	YQEPVLGPVRGPFPIIV	Yamamoto et al. (1994); Coste et al. (1992)
Antioxidant				
	80–90	α_{s1}	HIQKEDVPSER	Gupta et al. (2010)

Electronic supplementary material

Detailed peptide profiling of “Scotta”: From a dairy waste to a source of potential health-promoting compounds

Figure S1: Sequence coverage for caseins σ, β, κ.

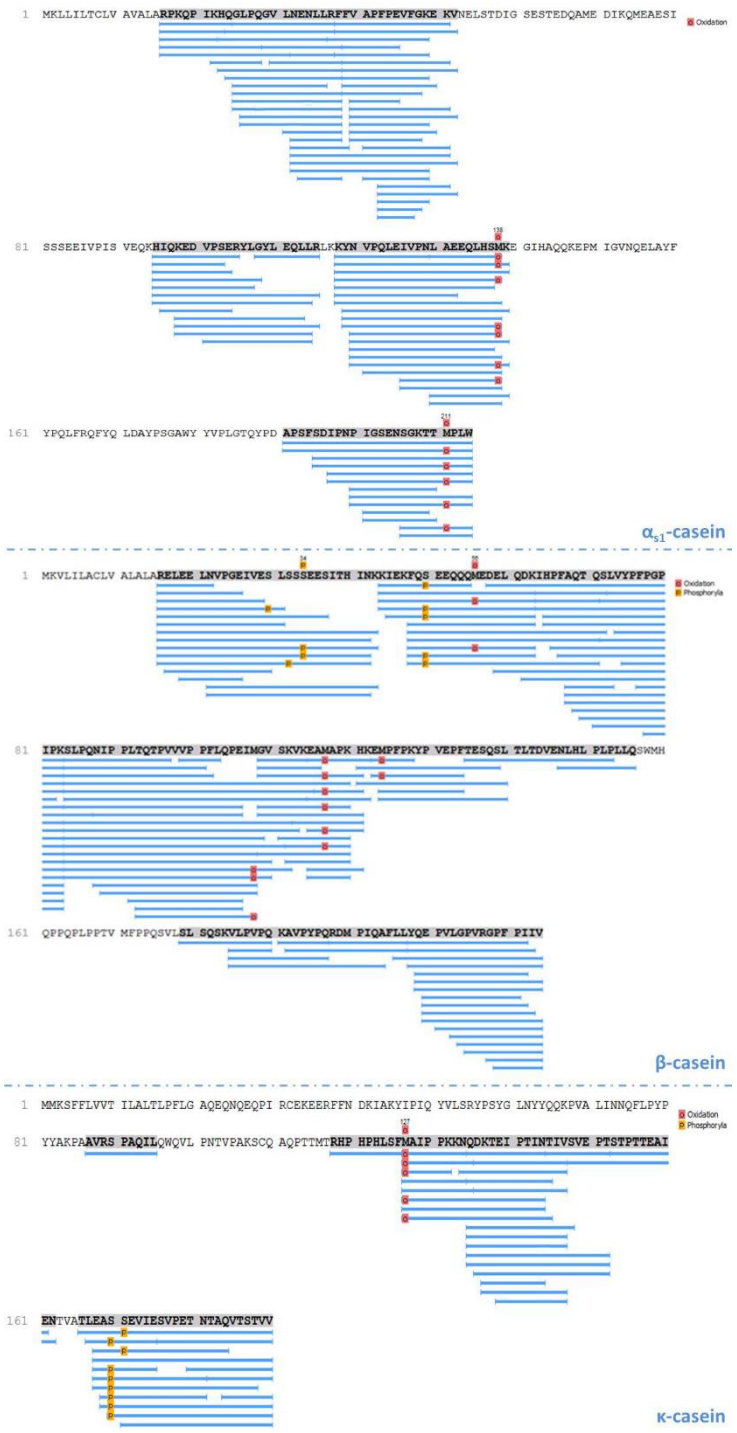


Table S1: Complete list of potential encrypted bioactive peptides identified by LC-MS/MS in Scotta.

n°	*t_r (min)*	*Mass*	*Error ppm*	*Casein*	*Amino acid*	*Peptide containing the sequence*	*Potential Bioactivity*	*References*
1.	5.06	865.5497	-1.3	α_{s1}	1-7	A.RPKQPIK.H	Antimicrobial, Immunomodulating	Hayes et al. 2006
2.	4.66	1002.6086	-2.4	α_{s1}	1-8	A.RPKQPIKH.Q	Antimicrobial, Immunomodulating	Hayes et al. 2006
3.	4.99	1130.6672	0.8	α_{s1}	1-9	A.RPKQPIKHQ.G	Antimicrobial, Immunomodulating	Hayes et al. 2006
4.	7.45	1525.8840	0.1	α_{s1}	1-13	A.RPKQPIKHQGLPQ.G	Antimicrobial, Immunomodulating	Hayes et al. 2006
5.	11.45	1795.0580	1.4	α_{s1}	1-16	A.RPKQPIKHQGLPQGVL.N	Antimicrobial, Immunomodulating	Hayes et al. 2006
6.	6.41	806.4399	-2.3	α_{s1}	7-13	I.KHQGLPQ.G	Antimicrobial, Immunomodulating	Hayes et al. 2006
7.	19.03	1530.8154	-0.1	α_{s1}	8-21	K.HQGLPQGVLNENLL.R	Antimicrobial, Immunomodulating	Hayes et al. 2006
8.	20.61	1833.9849	-0.8	α_{s1}	8-23	K.HQGLPQGVLNENLLRF.F	Antimicrobial, Immunomodulating	Hayes et al. 2007
9.	18.52	1549.8577	-1.1	α_{s1}	9-22	H.QGLPQGVLNENLLR.F	Antimicrobial, Immunomodulating	Hayes et al. 2007
10.	22.22	1696.9260	-2.1	α_{s1}	9-23	H.QGLPQGVLNENLLRF.F	Antimicrobial, Immunomodulating	Hayes et al. 2007
11.	20.74	1265.6979	-3.5	α_{s1}	10-21	Q.GLPQGVLNENLL.R	Antimicrobial, Immunomodulating	Hayes et al. 2007
12.	18.51	1421.7991	-1.4	α_{s1}	10-22	Q.GLPQGVLNENLLR.F	Antimicrobial, Immunomodulating	Hayes et al. 2007
13.	22.31	1568.8674	-2.5	α_{s1}	10-23	Q.GLPQGVLNENLLRF.F	Antimicrobial, Immunomodulating	Hayes et al. 2007
14.	21.87	1511.8459	-2.4	α_{s1}	11-23	G.LPQGVLNENLLRF.F	Antimicrobial, Immunomodulating	Hayes et al. 2007
15.	20.56	1173.6506	-2.8	α_{s1}	14-23	Q.GVLNENLLRF.F	Antimicrobial, Immunomodulating	Hayes et al. 2006
16.	19.22	1019.5764	-1.8	α_{s1}	15-22	G.VLNENLLR.F	Antimicrobial	Hayes et al. 2006
17.	18.32	1017.5607	-3.3	α_{s1}	16-23	V.LNENLLRF.F	Anticancer, Antimicrobial, Immunomodulating	Hayes et al. 2006
18.	9.65	757.4082	-1.2	α_{s1}	17-22	L.NENLLR.F	Anticancer, Antimicrobial, Immunomodulating	Hayes et al. 2006
19.	16.60	904.4766	-2.0	α_{s1}	17-23	L.NENLLRF.F	Anticancer, Antimicrobial, Immunomodulating	Hayes et al. 2006
20.	24.88	2123.1204	-2.4	α_{s1}	17-34	L.NENLLRFFVAPFPEVFGK.E	Anticancer	Robert et al. 2004
21.	24.30	2380.2578	-0.5	α_{s1}	17-36	L.NENLLRFFVAPFPEVFGKEK.V	Anticancer	Robert et al. 2004
22.	24.44	2479.3262	-1.9	α_{s1}	17-37	L.NENLLRFFVAPFPEVFGKEKV.N	Anticancer	Robert et al. 2004
23.	21.73	1539.8237	-3.8	α_{s1}	22-34	L.RFFVAPFPEVFGK.E	Anticancer	Robert et al. 2004
24.	23.63	1383.7227	-0.5	α_{s1}	23-34	R.FFVAPFPEVFGK.E	Ace inhibitor; Anticancer	Robert et al. 2004; Maruyama et al. 1982
25.	21.57	1640.8602	-1.2	α_{s1}	23-36	R.FFVAPFPEVFGKEK.V	Ace inhibitor; Anticancer	Robert et al. 2004
26.	22.26	1739.9286	-3.7	α_{s1}	23-37	R.FFVAPFPEVFGKEKV.N	Ace inhibitor; Anticancer	Robert et al. 2004
27.	17.89	805.4010	-2.1	α_{s1}	24-30	F.FVAPFPE.V	Ace inhibitor; Anticancer	Robert et al. 2004
28.	23.91	1108.5593	-1.8	α_{s1}	24-33	F.FVAPFPEVFG.K	Ace inhibitor; Anticancer	Robert et al. 2004
29.	21.18	1236.6542	-0.6	α_{s1}	24-34	F.FVAPFPEVFGK.E	Ace inhibitor; Anticancer	Robert et al. 2004
30.	21.21	1365.6968	-1.4	α_{s1}	24-35	F.FVAPFPEVFGKE.K	Ace inhibitor; Anticancer	Robert et al. 2004
31.	19.15	1493.7917	-3.3	α_{s1}	24-36	F.FVAPFPEVFGKEK.V	Ace inhibitor; Anticancer	Robert et al. 2004
32.	20.10	1592.8602	-0.8	α_{s1}	24-37	F.FVAPFPEVFGKEKV.N	Ace inhibitor; Anticancer	Robert et al. 2004
33.	13.59	658.3326	-2.1	α_{s1}	25-30	F.VAPFPE.V	Ace inhibitor; Anticancer	Robert et al. 2004
34.	21.06	961.4908	-1.3	α_{s1}	25-33	F.VAPFPEVFG.K	Ace inhibitor; Anticancer	Robert et al. 2004
35.	18.36	1089.5858	1.4	α_{s1}	25-34	F.VAPFPEVFGK.E	Ace inhibitor; Anticancer	Robert et al. 2004
36.	18.45	1218.6284	-2.8	α_{s1}	25-35	F.VAPFPEVFGKE.K	Ace inhibitor; Anticancer	Robert et al. 2004
37.	16.39	1346.7234	-1.5	α_{s1}	25-36	F.VAPFPEVFGKEK.V	Ace inhibitor; Anticancer	Robert et al. 2004
38.	17.61	1445.7917	-3.3	α_{s1}	25-37	F.VAPFPEVFGKEKV.N	Ace inhibitor; Anticancer	Robert et al. 2004
39.	17.13	990.5174	-2.4	α_{s1}	26-34	V.APFPEVFGK.E	Ace inhibitor; Anticancer	Robert et al. 2004
40.	15.22	1247.6549	-2.3	α_{s1}	26-36	V.APFPEVFGKEK.V	Ace inhibitor; Anticancer	Robert et al. 2004
41.	18.80	637.3112	-1.6	α_{s1}	28-32	P.FPEVF.G	Ace inhibitor; Anticancer	Robert et al. 2004
42.	17.98	694.3326	-2.6	α_{s1}	28-33	P.FPEVFG.K	Ace inhibitor; Anticancer	Robert et al. 2004
43.	15.38	822.4276	-2.4	α_{s1}	28-34	P.FPEVFGK.E	Ace inhibitor; Anticancer	Robert et al. 2004
44.	13.53	1079.5651	-2.4	α_{s1}	28-36	P.FPEVFGKEK.V	Ace inhibitor; Anticancer	Robert et al. 2004
45.	15.14	1178.6335	-3.0	α_{s1}	28-37	P.FPEVFGKEKV.N	Ace inhibitor; Anticancer	Robert et al. 2004

46.	7.28	1051.5298	-2.8	α_{s1}	80-88	K.HIQKEDVPS.E	Neuropeptide, Antioxidant	Gupta et al. 2010
47.	7.75	1180.5724	-0.6	α_{s1}	80-89	K.HIQKEDVPSE.R	Neuropeptide, Antioxidant	Gupta et al. 2010
48.	7.12	1336.6735	-1.6	α_{s1}	80-90	K.HIQKEDVPSER.Y	Neuropeptide, Antioxidant	Gupta et al. 2010
49.	11.61	1612.8209	-2.7	α_{s1}	80-92	K.HIQKEDVPSERYL.G	Neuropeptide, Antioxidant	Gupta et al. 2010
50.	11.09	1669.8423	-2.3	α_{s1}	80-93	K.HIQKEDVPSERYLG.Y	Neuropeptide, Antioxidant	Gupta et al. 2010
51.	22.93	2429.2590	-3.3	α_{s1}	80-99	K.HIQKEDVPSERYLGYLEQLL.R	Neuropeptide, Antioxidant	Gupta et al. 2010
52.	22.02	2585.3601	-3,3	α_{s1}	80-100	K.HIQKEDVPSERYLGYLEQLLR.L	Neuropeptide, Antioxidant	Gupta et al. 2010
53.	8.44	1043.5134	-0.7	α_{s1}	81-89	H.IQKEDVPSE.R	Neuropeptide, Antioxidant	Gupta et al. 2010
54.	19.89	1937.9734	-2.7	α_{s1}	83-98	Q.KEDVPSERYLGYLEQL.L	Neuropeptide, Antioxidant	Gupta et al. 2010
55.	23.81	2051.0574	0.30	α_{s1}	83-99	Q.KEDVPSERYLGYLEQLL.R	Neuropeptide, Antioxidant	Gupta et al. 2010
56.	22.85	2207.1584	-1.6	α_{s1}	83-100	Q.KEDVPSERYLGYLEQLLR.L	Neuropeptide, Antioxidant	Gupta et al. 2010
57.	24.19	1678.8929	0.9	α_{s1}	86-99	D.VPSERYLGYLEQLL.R	Neuropeptide, Antioxidant, Opioid	Loukas et al. 1983
58.	18.24	990.5498	-2.4	α_{s1}	93-100	L.GYLEQLLR.L	Opioid	Loukas et al. 1983
59.	17.48	1412.7664	0	α_{s1}	103-114	K.KYNVPQLEIVPN.L	Ace inhibitor	Yamamoto et al. 1994
60.	20.37	1725.9301	-1.1	α_{s1}	103-117	K.KYNVPQLEIVPNLAE.E	Ace inhibitor	Yamamoto et al. 1994
61.	20.21	2320.2063	-1	α_{s1}	103-122	K.KYNVPQLEIVPNLAEEQLHS.M	Ace inhibitor	Yamamoto et al. 1994
62.	21.65	2451.2468	1.1	α_{s1}	103-123	K.KYNVPQLEIVPNLAEEQLHSM.K	Ace inhibitor	Yamamoto et al. 1994
63.	20.11	2579.3418	-2.9	α_{s1}	103-124	K.KYNVPQLEIVPNLAEEQLHSMK.E	Ace inhibitor	Yamamoto et al. 1994
64.	23.10	2323.1519	1.9	α_{s1}	104-123	K.YNVPQLEIVPNLAEEQLHSM.K	Ace inhibitor	Yamamoto et al. 1994
65.	21.48	2451.2468	-3	α_{s1}	104-124	K.YNVPQLEIVPNLAEEQLHSMK.E	Ace inhibitor	Yamamoto et al. 1994
66.	20.78	2029.0480	-2.1	α_{s1}	105-122	Y.NVPQLEIVPNLAEEQLHS.M	Ace inhibitor	Yamamoto et al. 1994
67.	22.39	2160.0884	-1.5	α_{s1}	105-123	Y.NVPQLEIVPNLAEEQLHSM.K	Ace inhibitor	Yamamoto et al. 1994
68.	20.67	2288.1833	-1.2	α_{s1}	105-124	Y.NVPQLEIVPNLAEEQLHSMK.E	Ace inhibitor	Yamamoto et al. 1994
69.	22.12	2046.0455	4.3	α_{s1}	106-123	N.VPQLEIVPNLAEEQLHSM.K	Ace inhibitor	Yamamoto et al. 1994
70.	16.97	1479.7391	-2.1	α_{s1}	111-123	E.IVPNLAEEQLHSM.K	Ace inhibitor	Yamamoto et al. 1994
71.	11.63	1056.4910	-2.8	α_{s1}	115-123	N.LAEEQLHSM.K	Ace inhibitor	Yamamoto et al. 1994
72.	9.75	1184.5859	-0.1	α_{s1}	115-124	N.LAEEQLHSMK.E	Ace inhibitor	Yamamoto et al. 1994
73.	20.06	2467.2417	-1.4	α_{s1}	118-138	K.KYNVPQLEIVPNLAEEQLHSM(+15.99).K (oxidation)	Ace inhibitor	Yamamoto et al. 1994
74.	18.89	2595.3367	-1.5	α_{s1}	118-139	K.KYNVPQLEIVPNLAEEQLHSM(+15.99)K.E (oxidation)	Ace inhibitor	Yamamoto et al. 1994
75.	21.49	2339.1467	-0.3	α_{s1}	119-138	K.YNVPQLEIVPNLAEEQLHSM(+15.99).K (oxidation)	Ace inhibitor	Yamamoto et al. 1994
76.	20.65	2176.0835	-1.5	α_{s1}	120-138	Y.NVPQLEIVPNLAEEQLHSM(+15.99).K (oxidation)	Ace inhibitor	Yamamoto et al. 1994
77.	19.36	2304.1782	-2.3	α_{s1}	120-139	Y.NVPQLEIVPNLAEEQLHSM(+15.99)K.E (oxidation)	Ace inhibitor	Yamamoto et al. 1994
78.	8.95	1072.4858	0.00	α_{s1}	130-138	N.LAEEQLHSM(+15.99).K (oxidation)	Ace inhibitor	Yamamoto et al. 1994
79.	23.50	2545.2158	-2.2	α_{s1}	176-199	D.APSFSDIPNPIGSENSGKTTMPLW	Ace inhibitor	Yamamoto et al. 1994
80.	20.49	2143.0256	-0.8	α_{s1}	180-199	F.SDIPNPIGSENSGKTTMPLW	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
81.	20.19	1940.9666	1.6	α_{s1}	182-199	D.IPNPIGSENSGKTTMPLW	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
82.	6.17	1089.5302	-2.6	α_{s1}	185-195	N.PIGSENSGKTT.M	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
83.	18.04	1616.7869	-2.2	α_{s1}	185-199	N.PIGSENSGKTTMPLW	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
84.	5.20	891.4297	-1.9	α_{s1}	186-194	P.IGSENSGKT.T	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
85.	5.41	992.4774	0.4	α_{s1}	186-195	P.IGSENSGKTT.M	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
86.	16.79	1019.5110	-2.2	α_{s1}	191-199	N.SGKTTMPLW	Ace inhibitor, Antimicrobial	Yamamoto et al. 1994
87.	21.08	2561.2107	1.2	α_{s1}	191-214	D.APSFSDIPNPIGSENSGKTTM(+15.99)PLW (oxidation)	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
88.	18.66	2159.0205	1.7	α_{s1}	195-214	F.SDIPNPIGSENSGKTTM(+15.99)PLW (oxidation)	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
89.	18.30	1956.9615	2.7	α_{s1}	197-214	D.IPNPIGSENSGKTTM(+15.99)PLW (oxidation)	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
90.	15.57	1632.7817	-0.5	α_{s1}	200-214	N.PIGSENSGKTTM(+15.99)PLW (oxidation)	Ace inhibitor, Antimicrobial	Hayes et al. 2006; Yamamoto et al. 1994
91.	13.84	1035.5059	-2.4	α_{s1}	206-214	N.SGKTTM(+15.99)PLW (oxidation)	Ace inhibitor, Antimicrobial	Yamamoto et al. 1994
92.	9.82	901.4505	-1.5	β	1-7	A.RELEELN.V	Immunomodulating	Hayes et al. 2007
93.	14.10	1283.6357	-1.5	β	1-11	A.RELEELNVPGE.I	Immunomodulating	Hayes et al. 2007
94.	17.31	1624.8308	-2.7	β	1-14	A.RELEELNVPGEIVE.S	Immunomodulating	Hayes et al. 2007

95.	21.83	1824.9469	-2.5	β	1-16	A.RELEELNVPGEIVESL.S	Immunomodulating	Hayes et al. 2007
96.	21.75	2431.1602	0.3	β	1-22	A.RELEELNVPGEIVESLSSSEES.I	Immunomodulating	Hayes et al. 2007
97.	18.53	1555.7617	-3.2	β	2-15	R.ELEELNVPGEIVES.L	Immunomodulating	Hayes et al. 2007
98.	12.36	885.4080	1.2	β	4-11	L.EELNVPGE.I	Immunomodulating	Hayes et al. 2007
99.	21.26	2240.0808	2.9	β	7-27	L.NVPGEIVESLSSSEESITHIN.K	Ace inhibitor, Immunomodulating	Hayes et al. 2007
100.	20.16	2368.1758	-1.6	β	7-28	L.NVPGEIVESLSSSEESITHINK.K	Ace inhibitor, Immunomodulating	Hayes et al. 2007
101.	20.33	1904.9132	-0.8	β	16-31	A.RELEELNVPGEIVES(+79.97)L.S (phospho)	Immunomodulating	Hayes et al. 2007
102.	13.12	2509.1641	-3.2	β	29-48	K.KIEKFQSEEQQQMEDELQDK.I	Ace inhibitor	Yamamoto et al. 1994
103.	13.60	2010.8477	1.3	β	33-48	K.FQSEEQQQMEDELQDK.I	Ace inhibitor	Yamamoto et al. 1994
104.	17.95	2933.3137	-0.5	β	33-56	K.FQSEEQQQMEDELQDKIHPFAQTQ.S	Ace inhibitor	Yamamoto et al. 1994
105.	20.93	2964.5383	-1.0	β	43-68	E.DELQDKIHPFAQTQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
106.	8.66	1600.7134	0.7	β	44-55	K.KIEKFQS(+79.97)EEQQQ.M (phospho)	Ace inhibitor	Yamamoto et al. 1994
107.	10.50	2525.1592	-1.7	β	44-63	K.KIEKFQSEEQQQM(+15.99)EDELQDK.I (oxidation)	Ace inhibitor	Yamamoto et al. 1994
108.	13.95	2589.1306	0.7	β	44-63	K.KIEKFQS(+79.97)EEQQQMEDELQDK.I (phospho)	Ace inhibitor	Yamamoto et al. 1994
109.	20.32	2849.5115	-1.0	β	44-68	D.ELQDKIHPFAQTQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
110.	14.79	2461.0356	3.2	β	45-63	K.IEKFQS(+79.97)EEQQQMEDELQDK.I (phospho)	Ace inhibitor	Yamamoto et al. 1994
111.	19.88	2479.3262	-1.7	β	47-68	Q.DKIHPFAQTQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
112.	10.38	2026.8425	2.3	β	48-63	K.FQSEEQQQM(+15.99)EDELQDK.I (oxidation)	Ace inhibitor	Yamamoto et al. 1994
113.	14.37	2090.8140	-0.3	β	48-63	K.FQS(+79.97)EEQQQMEDELQDK.I (phospho)	Ace inhibitor	Yamamoto et al. 1994
114.	10.53	940.4766	-1.8	β	49-56	K.IHPFAQTQ.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
115.	10.44	1027.5087	-1.0	β	49-57	K.IHPFAQTQS.L	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
116.	20.46	2236.2043	3.2	β	49-68	K.IHPFAQTQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
117.	22.46	1995.0254	-0.9	β	50-67	I.HPFAQTQSLVYPFPGPIP.K	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
118.	19.51	2123.1204	2.9	β	50-68	I.HPFAQTQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
119.	21.02	1986.0614	3.4	β	51-68	H.PFAQTQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
120.	20.43	1889.0087	-2.1	β	52-68	P.FAQTQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
121.	7.68	646.3286	-0.5	β	53-58	F.AQTQSL.V	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
122.	19.30	1741.9402	-0.8	β	53-68	F.AQTQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
123.	19.38	1670.9031	-1.2	β	54-68	A.QTQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
124.	19.49	1542.8446	-1.1	β	55-68	Q.TQSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
125.	19.48	1441.7969	-2.6	β	56-68	T.QSLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
126.	19.65	1313.7383	0.8	β	57-68	Q.SLVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
127.	21.05	1738.9657	1.7	β	57-72	Q.SLVYPFPGPIPKSLPQ.N	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
128.	19.19	1226.7063	-1.8	β	58-68	S.LVYPFPGPIPK.S	Ace inhibitor, Opioid	Miguel et al. 2006; Yamamoto et al. 1994
129.	17.23	1113.6222	0.0	β	59-68	L.VYPFPGPIPK.S	Ace inhibitor, Opioid	Yamamoto et al. 1994
130.	16.22	1014.5538	-3.3	β	60-68	V.YPFPGPIPK.S	Ace inhibitor, Opioid	Yamamoto et al. 1994
131.	13.03	754.4377	-2.5	β	62-68	P.FPGPIPK.S	Ace inhibitor, Neuropeptide	Miguel et al. 2006; Yamamoto et al. 1994
132.	8.27	607.3693	-3.1	β	63-68	F.PGPIPK.S	Ace inhibitor, Neuropeptide	Miguel et al. 2006; Yamamoto et al. 1994
133.	18.85	1503.8297	-0.7	β	69-82	K.SLPQNIPPLTQTPV.V	Ace inhibitor	Yamamoto et al. 1994
134.	21.40	1896.0720	-1.7	β	69-86	K.SLPQNIPPLTQTPVVVPP.F	Ace inhibitor	Yamamoto et al. 1994
135.	24.41	2043.1404	2.6	β	69-87	K.SLPQNIPPLTQTPVVVPPF.L	Ace inhibitor	Yamamoto et al. 1994
136.	24.70	2510.3784	-0.1	β	69-91	K.SLPQNIPPLTQTPVVVPPFLQPE.I	Ace inhibitor	Yamamoto et al. 1994
137.	25.27	2754.5029	0.6	β	69-93	K.SLPQNIPPLTQTPVVVPPFLQPEIM.G	Ace inhibitor	Yamamoto et al. 1994
138.	25.21	2811.5244	1.1	β	69-94	K.SLPQNIPPLTQTPVVVPPFLQPEIMG.V	Ace inhibitor	Yamamoto et al. 1994
139.	25.49	2910.5928	4.5	β	69-95	K.SLPQNIPPLTQTPVVVPPFLQPEIMGV.S	Ace inhibitor	Yamamoto et al. 1994
140.	25.25	2997.6248	0.1	β	69-96	K.SLPQNIPPLTQTPVVVPPFLQPEIMGVS.K	Ace inhibitor	Yamamoto et al. 1994
141.	24.63	2085.1509	-0.4	β	73-91	Q.NIPPLTQTPVVVPPFLQPE.I	Ace inhibitor	Yamamoto et al. 1994
142.	25.33	2329.2756	3.2	β	73-93	Q.NIPPLTQTPVVVPPFLQPEIM.G	Ace inhibitor	Yamamoto et al. 1994
143.	25.42	2215.2327	2.6	β	74-93	N.IPPLTQTPVVVPPFLQPEIM.G	Ace inhibitor	Yamamoto et al. 1994

144.	22.96	1663.9185	-2.1	β	77-91	P.LTQTPVVVPPFLQPE.I	Ace inhibitor	Yamamoto et al. 1994
145.	22.21	1550.8344	-2.3	β	78-91	L.TQTPVVVPPFLQPE.I	Ace inhibitor	Yamamoto et al. 1994
146.	18.64	571.3370	-0.4	β	84-88	V.VPPFL.Q	Ace inhibitor	Yamamoto et al. 1994
147.	25.13	2926.5876	0.6	β	84-110	K.SLPQNIPPLTQTPVVVPPFLQPEIM(+15.99)GV.S (oxidation)	Ace inhibitor	Yamamoto et al. 1994
148.	23.49	1810.9539	0.30	β	93-108	L.TQTPVVVPPFLQPEIM(+15.99).G (oxidation)	Ace inhibitor	Yamamoto et al. 1994
149.	3.18	616.3908	-0.9	β	94-99	M.GVSKVK.E	Neuropeptide, Antioxidant	Yamamoto et al. 1994
150.	5.32	816.4705	-2.7	β	94-101	M.GVSKVKEA.M	Neuropeptide, Antioxidant	Yamamoto et al. 1994
151.	7.76	1243.6958	-0.07	β	94-105	M.GVSKVKEAMAPK.H	Neuropeptide, Antioxidant	Gupta et al. 2009; Yamamoto et al. 1994
152.	7.26	1380.7548	-2.1	β	94-106	M.GVSKVKEAMAPKH.K	Neuropeptide, Antioxidant	Gupta et al. 2009; Yamamoto et al. 1994
153.	6.13	1087.6060	-1.7	β	96-105	V.SKVKEAMAPK.H	Neuropeptide, Antioxidant	Gupta et al. 2009
154.	5.67	1000.5739	-0.1	β	97-105	S.KVKEAMAPK.H	Neuropeptide, Antioxidant	Gupta et al. 2009
155.	5.86	1009.5378	-0.7	β	98-106	K.VKEAMAPKH.K	Neuropeptide, Antioxidant	Gupta et al. 2009
156.	5.92	645.3156	-0.1	β	100-105	K.EAMAPK.H	Neuropeptide, Antioxidant	Gupta et al. 2009
157.	5.46	782.3745	0.00	β	100-106	K.EAMAPKH.K	Neuropeptide, Antioxidant	Gupta et al. 2009
158.	4.61	910.4694	-1.8	β	100-107	K.EAMAPKHK.E	Neuropeptide, Antioxidant	Gupta et al. 2009
159.	16.68	2277.0776	-3.0	β	106-124	K.HKEMPFPKYPVEPFTESQS.L	Ace inhibitor	Yamamoto et al. 1994
160.	18.19	2390.1616	-2.1	β	106-125	K.HKEMPFPKYPVEPFTESQSL.T	Ace inhibitor	Yamamoto et al. 1994
161.	20.59	1350.6682	-3.5	β	109-119	E.MPFPKYPVEPF.T	Ace inhibitor	Yamamoto et al. 1994
162.	6.48	1259.6907	-2.0	β	109-120	M.GVSKVKEAM(+15.99)APK.H (oxidation)	Antioxidant	Gupta et al. 2009; Yamamoto et al. 1994
163.	6.08	1396.7496	-2.9	β	109-121	M.GVSKVKEAM(+15.99)APKH.K (oxidation)	Antioxidant	Gupta et al. 2009; Yamamoto et al. 1994
164.	20.82	1995.9651	-1.8	β	109-125	E.MPFPKYPVEPFTESQSL.T	Ace inhibitor	Yamamoto et al. 1994
165.	4.93	1016.5688	0.5	β	112-120	S.KVKEAM(+15.99)APK.H (oxidation)	Antioxidant	Gupta et al. 2009
166.	4.64	798.3694	-3.0	β	115-121	K.EAM(+15.99)APKH.K (oxidation)	Antioxidant	Gupta et al. 2009
167.	2.87	926.4644	-3.2	β	115-122	K.EAM(+15.99)APKHK.E (oxidation)	Antioxidant	Gupta et al. 2009
168.	3.49	669.3268	-3.1	β	116-121	E.AM(+15.99)APKH.K (oxidation)	Antioxidant	Gupta et al. 2009
169.	9.32	763.3574	-0.4	β	123-128	K.EM(+15.99)PFPK.Y (oxidation)	Ace inhibitor	Yamamoto et al. 1994
170.	19.08	1495.7057	-1.7	β	123-134	K.EM(+15.99)PFPKYPVEPF.T (oxidation)	Ace inhibitor	Yamamoto et al. 1994
171.	23.36	1156.6968	-2.3	β	132-141	E.NLHLPLPLLQ.S	Ace inhibitor, Anthypertensive	Miguel et al. 2006; Miguel et al. 2010
172.	14.45	1281.7292	-2.0	β	164-175	L.SLSQSKVLPVPQ.K	Ace inhibitor	Yamamoto et al. 1994
173.	13.01	651.3955	-1.8	β	170-175	K.VLPVPQ.K	Ace inhibitor	Yamamoto et al. 1994
174.	15.56	1434.8235	-3.5	β	170-182	K.VLPVPQKAVPYPQ.R	Antioxidant	Hayes et al. 2007; Yamamoto et al. 1994
175.	17.01	2246.2244	-2.6	β	170-189	K.VLPVPQKAVPYPQRDMPIQA.F	Antioxidant	Hayes et al. 2007; Yamamoto et al. 1994
176.	9.15	801.4385	-1.8	β	176-182	Q.KAVPYPQ.R	Ace inhibitor, Antioxidant	Hayes et al. 2007
177.	23.95	1857.9811	-2.6	β	177-192	K.AVPYPQRDMPIQAFLL.Y	Ace inhibitor, Antioxidant	Hayes et al. 2007
178.	22.65	1202.6482	-2.1	β	183-192	Q.RDMPIQAFLL.Y	Ace inhibitor, Antioxidant	Hayes et al. 2007
179.	24.91	2106.2241	-2.0	β	191-209	F.LLYQEPVLGPVRGPFPIIV	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
180.	21.77	1667.9034	-0.8	β	193-207	L.YQEPVLGPVRGPFPI.I	Ace inhibitor	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
181.	23.52	1780.9875	-0.2	β	193-208	Y.QEPVLGPVRGPFPIIV	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
182.	24.21	1880.0559	-1.2	β	193-209	L.YQEPVLGPVRGPFPIIV	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
183.	21.19	1504.8401	-0.7	β	194-207	Y.QEPVLGPVRGPFPI.I	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
184.	18.57	1263.6975	-1.80	β	195-206	Q.EPVLGPVRGPFP.I	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
185.	21.29	1376.7815	-0.5	β	195-207	Q.EPVLGPVRGPFPI.I	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
186.	23.18	1489.8656	-2.2	β	195-208	Q.EPVLGPVRGPFPII.V	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
187.	23.99	1588.9341	-0.01	β	195-209	Q.EPVLGPVRGPFPIIV	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
188.	24.00	1459.8915	-2.4	β	196-209	E.PVLGPVRGPFPIIV	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
189.	22.81	1263.7703	-2.0	β	198-209	V.LGPVRGPFPIIV	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
190.	21.23	1150.6862	-2.4	β	199-209	L.GPVRGPFPIIV	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
191.	21.20	1093.6648	-3.4	β	200-209	G.PVRGPFPIIV	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014

192.	21.94	684.4210	-1.3	β	204-209	G.PFPIIV	Ace inhibitor, Immunomodulating	Yamamoto et al. 1994; Coste et al. 1992; Barbé et al. 2014
193.	14.55	953.5658	-1.6	κ	65-73	A.AVRSPAQIL.Q	Antimicrobial	López-Expósito et al. 2006
194.	9.41	1126.5784	-1.2	κ	96-104	T.RHPHPHLSF.M	Antioxidant	Korhonen et al. 2007
195.	8.26	897.5106	-2.3	κ	105-112	F.MAIPPKKN.Q	Antimicrobial, Antithrombotic	Jollès et al. 1986
196.	9.01	1025.5692	-2.6	κ	105-113	F.MAIPPKKNQ.D	Antimicrobial, Antithrombotic	Jollès et al. 1986
197.	12.37	2037.0928	-1.3	κ	105-122	F.MAIPPKKNQDKTEIPTIN.T	Antimicrobial, Antithrombotic	Jollès et al. 1986
198.	12.39	1157.5928	-0.9	κ	113-122	N.QDKTEIPTIN.T	Antimicrobial	Malkoski et al. 2001
199.	13.08	1258.6405	-2.2	κ	113-123	N.QDKTEIPTINT.I	Antimicrobial	Malkoski et al. 2001
200.	18.37	1470.7930	-0.8	κ	113-125	N.QDKTEIPTINTIV.S	Antimicrobial	Malkoski et al. 2001
201.	17.43	1557.8250	0.5	κ	113-126	N.QDKTEIPTINTIVS.V	Antimicrobial	Malkoski et al. 2001
202.	19.29	1984.0364	-0.4	κ	113-130	N.QDKTEIPTINTIVSVEPT.S	Antimicrobial	Malkoski et al. 2001
203.	18.71	1342.7344	-2.8	κ	114-125	Q.DKTEIPTINTIV.S	Antimicrobial	Malkoski et al. 2001
204.	19.60	1855.9778	-1.2	κ	114-130	Q.DKTEIPTINTIVSVEPT.S	Antimicrobial	Malkoski et al. 2001
205.	12.33	914.5073	-2.6	κ	115-122	D.KTEIPTIN.T	Antimicrobial	Malkoski et al. 2001
206.	18.33	1227.7074	-2.2	κ	115-125	D.KTEIPTINTIV.S	Antimicrobial	Malkoski et al. 2001
207.	19.58	998.5648	-1.3	κ	117-125	T.EIPTINTIV.S	Antimicrobial	Malkoski et al. 2001
208.	12.74	844.4542	0.7	κ	123-130	N.TIVSVEPT.S	Antimicrobial	Malkoski et al. 2001
209.	7.27	671.3676	-0.3	κ	126-131	F.M(+15.99)AIPPK.K (oxidation)	Antimicrobial, Antithrombotic	Malkoski et al. 2001
210.	6.78	913.5055	-1.9	κ	126-133	F.M(+15.99)AIPPKKN.Q (oxidation)	Antimicrobial, Antithrombotic	Malkoski et al. 2001
211.	6.35	1041.5641	-2.0	κ	126-134	F.M(+15.99)AIPPKKNQ.D (oxidation)	Antimicrobial, Antithrombotic	Malkoski et al. 2001
212.	12.17	1574.7312	4.2	κ	126-140	V.SVEPTSTPTTEAIEN.T	Antimicrobial	Malkoski et al. 2001
213.	12.21	2053.0876	-0.3	κ	126-143	F.M(+15.99)AIPPKKNQDKTEIPTIN.T (oxidation)	Antimicrobial	Malkoski et al. 2001
214.	12.11	2154.1355	-3.6	κ	126-144	F.M(+15.99)AIPPKKNQDKTEIPTINT.I (oxidation)	Antimicrobial	Malkoski et al. 2001
215.	10.17	947.4448	-1.2	κ	131-139	T.STPTTEAIE.N	Antimicrobial	Malkoski et al. 2001
216.	12.34	1119.5771	1.6	κ	158-168	E.TNTAQVTSTVV	Antithrombotic	López-Expósito et al. 2006
217.	11.55	904.4866	-1.4	κ	160-168	N.TAQVTSTVV	Antithrombotic	López-Expósito et al. 2006
218.	10.42	732.4018	-1.9	κ	162-168	A.QVTSTVV	Antithrombotic	López-Expósito et al. 2006
219.	18.70	2670.2524	0.1	κ	165-189	A.TLEASS(+79.97)EVIESVPETNTAQVTSTVV (phospho)	Antimicrobial, Antithrombotic	López-Expósito et al. 2006
220.	12.07	1055.4424	0.4	κ	166-174	T.LEAS(+79.97)SEVIE.S (phospho)	Antimicrobial	Malkoski et al. 2001
221.	9.64	942.3583	-0.4	κ	167-174	L.EAS(+79.97)SEVIE.S (phospho)	Antimicrobial	Malkoski et al. 2001
222.	13.49	1869.7881	-0.1	κ	167-183	L.EASS(+79.97)EVIESVPETNTAQ.V (phospho)	Antimicrobial	Malkoski et al. 2001
223.	14.85	2257.9839	-0.6	κ	167-187	L.EAS(+79.97)SEVIESVPETNTAQVTST.V (phospho)	Antimicrobial, Antithrombotic	López-Expósito et al. 2006
224.	12.84	1440.6021	-2.5	κ	168-180	E.AS(+79.97)SEVIESVPETN.T (phospho)	Antimicrobial	Malkoski et al. 2001
225.	17.54	2327.0781	5.3	κ	168-189	E.AS(+79.97)SEVIESVPETNTAQVTSTVV (phospho)	Antimicrobial, Antithrombotic	López-Expósito et al. 2006
226.	17.55	2256.0410	4.00	κ	169-189	A.S(+79.97)SEVIESVPETNTAQVTSTVV (phospho)	Antimicrobial, Antithrombotic	López-Expósito et al. 2006

Supplementary references

Korhonen H, & Pihlanto A. (2007) Technological options for the production of health-promoting proteins derived from milk and colostrum. Curr. Pharm. Des. 13: 829–843.

Maruyama S, Nakagomi K, Tomizuka N, Suzuki H. (1982) Angiotensin I -Converting Enzyme Inhibitor Derived from an Enzymatic Hydrolysate of Casein. II. Isolation and Bradykinin-potentiating Activity on the Uterus and the Ileum of Rats. Agric. BioI. Chem. 46: 1393–1394.

Hayes M, Stanton C, Slattery H, O'Sullivan O, Hill C, Fitzgerald G.F, Ross R. P (2007) Casein Fermentate of Lactobacillus animalis DPC6134 Contains a Range of Novel Propeptide Angiotensin-Converting Enzyme Inhibitors. Appl. Environ. Microbiol.. 73: 4658–4667.

Hayes M., Stanton C., Fitzgerald G.F., Ross R. P. (2007) Putting microbes to work: Dairy fermentation, cell factories and bioactive peptides. Part II: Bioactive peptide functions. Biotechnol. J. 2: 435–449.

Malkoski M., Dashper S. G, O'Brien-Simpson N. M, Talbo G. H, Macris M, Cross K. J, Reynolds E. C. (2001) Kappacin, a Novel Antibacterial Peptide from Bovine Milk. Antimicrob. Agents Chemoter. 45: 2309–2315

Miguel M, Recio I, Ramos M, Delgado M. A, Aleixandre M. A. (2006) Antihypertensive Effect of Peptides Obtained from Enterococcus
faecalis-Fermented Milk in Rats. J. Dairy Sci. 89: 3352–3359

Miguel M, Gòmez-Ruiz J. A, Recio I, Aleixandre A. (2010) Changes in arterial blood pressure after single oral administration of milk-casein-derived peptides in spontaneously hypertensive rats. Mol. Nutr. Food Res. 54: 1422–1427